**Experimental Engineering Section
Semiannual Progress Report
(Excluding Reactor Programs)
March 1, 1975 to August 31, 1975**

OAK RIDGE NATIONAL LABORATORY

OPERATED BY UNION CARBIDE CORPORATION FOR THE ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

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CHEMICAL TECHNOLOGY DIVISION

EXPERIMENTAL ENGINEERING SECTION SEMIANNUAL PROGRESS REPORT

(EXCLUDING REACTOR PROGRAMS)

March 1, 1975 to August 31, 1975

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SEPTEMBER 1976

OAK RIDGE NATIONAL LABORATORY
Oak Ridge, Tennessee 37830
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PUBLICATIONS

<u>Author(s)</u>	<u>Title</u>	<u>Publication</u>
Burtis, C. A., et al.	Biochemical Technology Program Progress Report for the Period July 1, 1974 to December 31, 1974	ORNL/TM-4844 (May 1975)
Burtis, C. A., et al.	Biochemical Technology Program Progress Report for the Period January 1, 1975 to June 30, 1975	ORNL/TM-5006 (August 1975)
Burtis, C. A., W. D. Bostick,	Increasing the Versatility of the Miniature Centrifugal Fast Analyzer by the Use of Multiple Optical Cnfigurations	<u>Clin. Chem.</u> 21, 1225 (1975)
Bostick, W. D., et al.	Ccagulation-Time Determination with Automatic Multivariable Analysis by Use of a Miniature Centrifugal Fast Analyzer	<u>Clin. Chem.</u> 21, 1288 (1975)
Jolley, R. L., S. Katz, J. E. Mrochek, W. W. Pitt, Jr., and W. T. Rainey, Jr.	Analyzing Organics in Dilute Acueous Solutions	<u>Chem. Technol.</u> (May) 312 (1975)
Katz, S., W. W. Pitt, Jr., and J. E. Mrochek	Comparative Serum and Urine Analysis by Dual-Detector Anion-Exchange Chromatography	<u>J. Chromatog.</u> 104, 303 (1975)
Mrochek, J. E.	Application of Integrated Gas Chromatographic and Mass Spectro- metric Methods in the Identification of Urinary Constituents Isolated by Liquid Chromatography	<u>Selected Approaches to Gas Chromatography-Mass Spectrometry in Laboratory Medicine,</u> Department of Health, Education, and Welfare, (DHEW), Publication No. (NIH)75-762 (1975)

Publications (Continued)

<u>Author(s)</u>	<u>Title</u>	<u>Publication</u>
Mrocchek, J. E., S. R. Dinsmore, and T. P. Waalkes	Liquid-Chromatographic Analysis of Neutral Carbohydrates in Serum Glycoproteins	<u>Clin. Chem.</u> <u>21</u> , 1314 (1975)
Pitt, W. W., Jr., R. L. Colley, and C. D. Scott	Determination of Trace Organics in Municipal Sewage Effluents and Natural Waters by High-Resolution Ion-Exchange Chromatography	<u>Environ. Sci. Tech.</u> <u>4</u> , 1068 (1975)
Scott, C. D., N. E. Lee, and A. W. Perkins	Automated Elution Electrophoresis: A Potential Clinical Tool	<u>Clin. Chem.</u> <u>21</u> , 1217 (1975)
Scott, C. D., and R. S. Melville	Introduction to the Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory	<u>Clin. Chem.</u> <u>21</u> , 1191 (1975)
Scott, C. D., et al.	Experimental Engineering Section Semiannual Progress Report (Excluding Reactor Programs) for the Period September 1, 1973 to February 28, 1974	ORNL/TM-4602 (December 1974)
Scott, C. D., et al.	Experimental Engineering Section Semiannual Progress Report (Excluding Reactor Programs) for the Period March 1, 1974 to August 31, 1974	ORNL/TM-4777 (July 1975)

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Scott, C. D., et al.	Experimental Engineering Section Semiannual Progress Report (Excluding Reactor Programs) for the Period September 1, 1974 to February 28, 1975	ORNL/TM-4961 (January 1976)

ORAL PRESENTATIONS

<u>Speaker and Coauthor(s)</u>	<u>Title</u>	<u>Place Presented</u>
Bostick, W. D.	Coagulation-Time Determinations with Automatic Multivariable Analysis by Use of a Miniature Centrifugal Fast Analyzer	Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge National Laboratory, Oak Ridge, Tenn., March 13 - 14, 1975
Burtis, C. A.	Development of a Multipurpose Optical System for Use with a Centrifugal Fast Analyzer	Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge National Laboratory, Oak Ridge, Tenn., March 13 - 14, 1975
Burtis, C. A.	The Effect of Sample Evaporation on Analytical Accuracy	Spring Meeting of the South-eastern Section of the American Association of Clinical Chemists, Charleston, S.C., April 3 - 4, 1975
Burtis, C. A.	Recent Advances in Centrifugal Fast Analyzers	Miami Section of the American Association of Clinical Chemists, Miami, Florida, June 3, 1975
Burtis, C. A.	The Centrifugal Fast Analyzer Program at the Oak Ridge National Laboratory	Dade Chemical Company, Miami, Fla., June 4, 1975
Burtis, C. A.	Advances in Centrifugal Analyzer Development	Invited symposium lecture at the Ninth International Congress on Clinical Chemistry, Toronto, Canada, July 19, 1975

Oral Presentations (Continued)

<u>Speaker and Coauthor(s)</u>	<u>Title</u>	<u>Place Presented</u>
Cochran, H. D.	Experimental Engineering Development of Coal Hydrocarbonization	Department of Chemical Engineering, University of Tennessee, Knoxville, Tenn., March 4, 1975
Cochran, H. D.	Experimental Engineering Development of Coal Hydrocarbonization	Tennessee Eastman Company Kingsport, Tenn., March 19, 1975
Hancher, C. W.	Unit Operations of Enzyme Purification	1975 Enzyme Engineering Conference, Reed College, Portland, Ore., August 5-8, 1975
Jolley, R. L.	Chlorination Effects on Organic Constituents in Natural and Process Waters	Grambling State University, Grambling, La., April 3, 1975
Jolley, R. L., W. W. Pitt, and	Soluble Organic Constituents in Sewage Treatment Plant Effluents as Determined by a Multicomponent Analytical Technique	169th American Chemical Society National Meeting, Philadelphia, Penn., April 6-11, 1975
Jolley, R. L.	Chlorination Effects on Organic Constituents in Natural and Process Waters	Eckard College, St. Petersburg, Fla., April 23, 1975
Jolley, R. L.	Chlorination of Cooling Water: A Source of Environmental Significant Chlorine-Containing Organic Compounds	Fourth National Symposium on Radioecology, Corvallis, Ore., May 12-14, 1975

Oral Presentations (Continued)

<u>Speaker and Coauthor(s)</u>	<u>Title</u>	<u>Place Presented</u>
Jolley, R. L., W. W. Pitt, C. D. Scott, G. Jones, and J. E. Thompson	Analysis of Soluble Organic Constituents in Natural and Process Waters by High-Pressure Liquid Chromatography	Ninth International Conference on Trace Substances in Environmental Health, Columbia, Mo., June 10-12, 1975
Lee, N. E.	Automated, High-Resolution Electrophoresis. A Fast, Efficient Technique with Potential in the Clinical Laboratory	American Chemical Society 169th National Meeting, Philadelphia, Pa., April 6-11, 1975
Mrochek, J. E.	Liquid Chromatographic Analysis of Neutral Carbohydrates in Serum Glycoproteins	Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge National Laboratory, Oak Ridge, Tenn., March 13-14, 1975
Mrochek, J. E., and C. D. Scott	Application of High-Resolution Liquid Chromatography to the Analysis of Physiologic Fluids	Symposium on Profiling of Physiologic Fluids at the 23rd Annual Conference on Mass Spectrometry and Allied Topics, Houston, Tex., May 1975
Mrochek, J. E., and T. P. Waalkes	Application of High-Resolution Liquid Chromatography to a Search for Biochemical Markers of Cancer	Symposium on Chemistry and the Cancer Problem, 170th National Meeting of the American Chemical Society, Chicago, Ill., August 1975
Perkins, A. W.	Adaptation of Enzymatic Cholesterol Procedures to the Miniature CFA	Southeast Regional Meeting, American Association of Clinical Chemists, Charleston, S.C., April 1975

Oral Presentations (Continued)

<u>Speaker and Coauthor(s)</u>	<u>Title</u>	<u>Place Presented</u>
Pitt, W. W., Jr.	Analysis of Trace Organics in Polluted Waters by High-Resolution Liquid Chromatography	Washington Chromatography Discussion Group, Gaithersburg, Md., April 24, 1975
Scott, C. D., and R. S. Melville	Introduction to the Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory	Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge National Laboratory, Oak Ridge, Tenn., March 13-14, 1975
Scott, C. D., N. E. Lee, and A. W. Perkins	Automated Elution Electrophoresis: A Potential Clinical Tool	Seventh Annual Symposium on Advanced Analytical Concepts for the Clinical Laboratory, Oak Ridge National Laboratory, Oak Ridge, Tenn., March 13-14, 1975
Scott, C. D.	Some Recent Advances in the Development of Liquid Chromatography for the Analysis of Complex Biological Mixtures	Invited Lecture at the ACS Regional Meeting, Akron, Ohio, March 20, 1975
Scott, C. D.	Some Advanced Techniques for Biochemical Indicators	Research and Development, B.F. Goodrich Company, Brecksville, Ohio, March 20, 1975

Oral Presentations (Continued)

<u>Speaker and Coauthor(s)</u>	<u>Title</u>	<u>Place Presented</u>
Scott, C. D., and S. E. Shumate, II	Affinity Chromatography with a Centrifugal Elution Chromatography	170th National Meeting of the American Chemical Society, Chicago, Ill., August 24-29, 1975
Shumate, S. E., II, and C. D. Scott	A Tapered Fluidized Bed as a Bioreactor	Third International Conference on Enzyme Engineering, Portland, Ore., August 3-8, 1975
Watson, J. S.	Technological and Environmental Aspects of Nuclear Fusion	Gzowski Society Lecture on Fusion, Ontario, Canada, March 24, 1975
Westmoreland, P. R.	Coal Pyrolysis in Underground Gasification	Southeastern Seminar on Thermal Sciences, University of Tennessee, Knoxville, Tenn., April 28-29, 1975
Westmoreland, P. R.	Coal Pyrolysis in Underground Gasification	AIChE Student Chapter Meeting, Tennessee Technological Univ., Cookeville, Tenn., May 13, 1975

SUMMARY

Centrifugal Analyzer Development

Four miniature Centrifugal Fast Analyzer (CFA) systems are being routinely operated and/or evaluated in laboratories of the ORNL Health Division; the NASA Johnson Space Center, Houston, Texas; the National Institutes of Health, Bethesda, Maryland; and the National Center for Toxicological Research, Jefferson, Arkansas. Two additional systems are currently being fabricated for subsequent evaluation by the Department of Human Genetics, at the University of Michigan, Ann Arbor, and by a blood bank laboratory. Fabrication of a third-generation prototype of the portable CFA is nearly complete, and several of its components have been evaluated on an initial basis. In addition, a versatile software package has been developed for use with the Intel 8080 microprocessor used in the portable CFA. Several environmental assays have been developed or adapted for use with the portable CFA; these include methods for determining ATP, ammonia, nitrate, nitrite, phosphate, silica, and sulfate. A new rotor which incorporates a "sedimentation with membrane filtration" concept has been designed, fabricated, and successfully evaluated. The development of rotors containing preloaded quantities of reagents is continuing; we are currently testing various reagents supplied by commercial manufacturers in both lyophilized and tablet form. A method for determining γ -glutamyl transpeptidase (GGTP) activity has been adapted for use with the miniature CFA and utilized to measure the GGTP activities of normal human subjects and cancer patients with hepatic involvement. In addition, the GGTP activities of monkeys having hematomas induced by diethylnitrosamine were also measured. To assist in the measurement of kinetic parameters, a computer program originally developed for the PDP-8/i computer was adapted for use with the PDP-8/e miniature CFA analytical system. We have also begun to adapt the Coombs test for use with the miniature CFA.

Advanced Analytical Techniques

High-resolution liquid chromatographic techniques coupled with detection by cerate oxidimetry are continuing to be used for the analysis of protein-bound carbohydrates in human serum. To speed up the analysis, isocratic elution of galactose, mannose, and fucose was tested (using a mixed eluent containing boric acid and ammonium acetate) for protein hydrolysates from human serum. Initial results were somewhat disappointing because of interference that caused difficulties in the measurement of the small fucose peak and necessitated continued use of the longer, gradient elution method to analyze serum samples. Preliminary work indicates that another protein-bound carbohydrate (sialic acid) can be successfully analyzed by anion exchange chromatography using an acetate eluent and the cerate oxidimetric

detector. Protein-bound fucose is being measured in female subjects with breast cancer, and its utility as a possible biochemical marker of this disease is being evaluated. Measurements of sialic acid will be evaluated in the same manner to determine its utility as a marker.

The processing of whole blood to separate and collect plasma, cell, and hemolysate fractions is an important aspect of programs for the monitoring of mutation rates in human populations. A third-generation prototype system to perform the necessary steps to obtain these fractions for 16 blood samples simultaneously has been designed, fabricated, and tested. Electrophoretic patterns of LDH isoenzymes in hemolysate samples prepared by the automated system were satisfactory; operational performance, while not entirely satisfactory, did indicate overall feasibility for the newly designed prototype.

A new, removable-column rotor has been developed to increase the experimental flexibility of the prototype system for centrifugal elution chromatography. Column packings consisting of inorganic alumina particles with antihuman IgG covalently attached to the surface successfully overcame the plugging problems caused by centrifugal compression of the gel packings previously tested. Three peaks (nonadsorbed protein, nonspecifically adsorbed protein, and the immunosorbed IgG) were separated from a dynamically injected sample of human serum using the removable-column rotor containing four chromatographic columns.

Bioengineering Research and Development

The practical applications of enzyme catalysis are being investigated. This work has two main objectives: (1) to establish the conditions which increase the rate of enzymic hydrogen production by hydrogenase and ferredoxin, and (2) to determine the kinetic parameters of the hydrolysis of lactose by lactase. The potential advantages to bioprocesses gained by methods of immobilizing biochemical catalysts are being investigated for several enzyme systems.

Continuous-flow bioreactors (for the abatement of organic pollutants in effluent streams of energy production facilities) and bioreactor systems (for the concentration or removal of dissolved metals including useful resource materials and radioactive waste materials from process waste streams) are being developed at the engineering bench scale.

Procedures for analyzing bioreactor feed and effluent streams for phenol, catechol, and thiocyanate have been devised, and a commercially available enzymatic assay for glucose has been adapted for use in bioprocessing studies.

Lactase enzyme immobilized on alumina particles is being used as a test system to evaluate bioreactors; some of the operating characteristics of the tapered fluidized bed bioreactor have been determined.

Environmental Studies

High-pressure, high-resolution liquid chromatographic techniques were used to detect and quantitatively separate the soluble organic constituents from a variety of waters of environmental concern. Six different natural waters were examined and several organics (principally carbohydrate-related compounds) were identified and quantified using ancillary gas chromatographic and mass spectrometric procedures. Aqueous stream samples from both atmospheric hydrocarbonization and in-situ coal-liquefaction processes were analyzed, and many uv-absorbing and cerate-oxidizable constituents (principally phenolic compounds) were separated. The high salt content of an estuarine water sample complicated the chromatographic analysis and indicated the need for additional development of methodology. Over 60 organophosphorus constituents were separated from a water sample in which ^{32}P -tagged phosphate was used to induce an algal bloom. Thirty carbohydrate-related constituents and eight uv-absorbing and/or cerate-oxidizable compounds were separated from the concentrate of a potable water sample collected from a large metropolitan city. Chlorination reaction yields of chloro-organics of 1 to 3% were determined for a chlorinated cooling water sample using chromatographic procedures. A national conference has been planned and scheduled to consider the environmental impact of water chlorination. A continuous chemical oxygen demand monitor has been developed which compares favorably with standard chemical oxygen demand measurements in the low parts-per-million range. Increased versatility for gas chromatographic detection has been obtained with the installation of two additional detectors: (1) an electrolytic conductivity detector for halogens, sulfur, and nitrogen; and (2) a plasma emission spectrometer for detection of a wide range of elements.

Chemical Engineering Research

Studies of hydrodynamics and mass transfer rates in three-phase fluidized beds were made in a 7.62-cm-ID column (53 to 206 cm long). Four different packing materials were used: (1) 6.4-mm-diam alumina beads, (2) 2.1-mm-diam alumina beads, (3) 6.35-mm-diam Plexiglas beads, and (4) 2.38-mm Plexiglas cubes. Measurements included minimum fluidization velocities, holdup of the three phases, and liquid-film-controlled mass transfer rates (CO_2 sorption into water). Accurate measurements of solid holdup could only be obtained at low gas rates and with dense particles. At higher gas rates the expanded bed height became indistinct; a different experimental technique will be required to obtain accurate data under these conditions. To interpret the mass transfer results, liquid-phase concentration profiles were fit by adjusting values of both the mass transfer coefficient and the axial-dispersion coefficient. More accurate analysis of the data will be possible after dispersion coefficients have been measured independently.

We have begun studies to determine the rates at which deuterium is oxidized after its permeation through metal surfaces. Preliminary results were obtained with 304 stainless steel and palladium permeation tubes, using very low oxygen pressures. These results indicate that, at 300 to 500°C and 10^{-6} torr oxygen pressure, the stainless steel oxidized only 40 to 50% of the permeating deuterium. More data are needed at higher oxygen pressures. Palladium at 25°C and 10^{-3} torr oxygen pressure oxidized approximately 80% of the deuterium. Conversion is expected to improve at higher temperatures.

Methods for preparing small ($< 10\text{-}\mu$) sorbent particles continued to be investigated, and 2 g of erbium oxide spheres were prepared with particle sizes ranging from 5 to 50 μ . The product was porous and friable, but firing at a higher temperature probably would have reduced the porosity and yielded a stronger material. These particles have been packed into a column to test their properties for sorption chromatography.

Adiabatic graphite oxidation studies demonstrated that burning rates are mass transfer controlled, and that the product gas temperature can be controlled by adjusting the recycle gas rate. The maximum burning rate occurs near the center of the rod and results in an "hourglass" shape for a partially burned graphite rod. The position of the maximum burning rate can be altered by changing the inlet gas temperature. Local burning rates can be predicted within 15% by a mathematical model of the burner, but more improvements in the model are needed.

An apparatus has been assembled for evaluating a perfusion-impactor for removing solid or liquid particles from gas streams. In this impaction device, the particles are collected on a porous surface through which a liquid is perfusing. The collected particles strike the liquid and are continuously removed by it.

Studies of an annular, continuous chromatograph demonstrated a separation of Blue Dextran and CoCl_2 on Sephadex G-10 resin. Separation could be observed both qualitatively (the bands could be seen visually) and quantitatively (with a photometric detector). The results were used to estimate the void fraction in the column and the distribution coefficient of CoCl_2 between the resin and the solution.

Controlled Thermonuclear Processing

Scoping studies for a Tokamak Experimental Power Reactor (EPR) operating at 150 MW(t) evaluated the environmental, vacuum pumping, and process requirements. Alternative techniques for meeting those requirements were compared, and a reference system was selected. Proposed methods for recovering tritium from liquid-lithium and molten-fluoride fusion reactor blankets were evaluated. Alternative

techniques were suggested to overcome potential difficulties in the processes, and the most promising processes were selected. For liquid-lithium blankets, a molten-chloride--salt-extraction process and metal permeation windows appeared most promising. (If the extraction process is to be used, a more suitable method must be found for removing tritium from the molten-chloride extractant.) If molten-fluoride salt blankets are operated in a reducing atmosphere, tritium removal should not be difficult in even a small spray tower.

Solid yttrium metal was shown to effectively remove tritium (up to 95% removed) from liquid potassium, but the sorption rates do not appear to be high enough for a practical process. Sorption rates increase significantly with temperature and appear to be controlled by a film resistance (probably a film on the yttrium metal itself).

An experimental apparatus has been assembled to evaluate cryo-sorption pumps for fusion reactor applications. Measurements of pumping rates and regeneration rates for hydrogen, deuterium, helium, and mixed gases are planned. Currently, equilibrium sorption isotherms and pumping rates for hydrogen on 5A molecular sieves at 77°K are being measured. Subsequent tests will be made with the sorbent at 4.2°K.

An experiment to study tritium evolution from irradiated Li-Al and Li-SAP (sintered aluminum product) has been initiated. The lithium content of each material to be tested is low (~ 0.05 wt %). Samples have been prepared by the Metals and Ceramics Division and irradiated in the HFIR. An experimental apparatus has been designed to heat the samples in a furnace with a flowing argon sweep gas. The tritium flushed from the furnace will be monitored, oxidized, and trapped.

Coal Conversion Process Development

Coal conversion development efforts included: (1) studies of methods for analyzing and characterizing liquid and vapor products and effluents from coal conversion plants, (2) hydrocarbonization process development, (3) evaluation of alternative methods for separating solids from coal-liquid products, and (4) experiments in support of in-situ gasification development. Both liquid chromatography and gas chromatography (with a number of sorbents) have been studied to develop methods for characterizing coal tars, liquid products from different coal conversion processes (COED), Synthoil, and H-Coal), and liquids from ORNL block pyrolysis studies. A number of polycyclic aromatic hydrocarbons were identified in chromatograms from these samples.

Hydrocarbonization development included ambient mock-up of a recirculating fluidized bed and a ball valve (solid coal) feeder to be used in the high-temperature experiments. Five runs were made in the atmospheric-pressure--high-temperature hydrocarbonization

system using Wyodak seam subbituminous coal. The first four runs used nitrogen as the fluidizing gas, but hydrogen was used in the fifth run. Design and fabrication of a bench-scale hydrocarbonization system capable of operating at 20 atm pressure and at temperatures up to 1250°F have been accomplished; construction of the system is nearing completion.

Studies of liquid-solid separations have included scouting investigations of solvent fractionation of coal conversion liquid products, filtration of the fractions, and the distribution of sulfur in the fractionation-filtration process. These experiments have shown that the solvent fractionation approach is a promising method for both reducing filtration problems and producing a product which meets most EPA guidelines on sulfur and ash content. Another novel approach to coal liquid-solid separations studied was the use of coal itself as a filter precoat. Illinois No. 6 crushed and sized (four size-fractions from 100 to 200 mesh) coal was tested as a filter precoat and resulted in filtration rates equal to or exceeding those obtained with standard precoats. Ash and sulfur removal (with the solids) was acceptable. Characterization of coal process liquids and solids was continued; liquid viscosities of SRC unfiltered oil were determined over a range of temperatures, and particle size distributions in SRC, COED, Synthoil, and H-Coal oil were measured. H-Coal and SRC produce very small particles with a substantial fraction less than 1 μ in diameter.

Experiments in support of in-situ coal gasification development have continued with laboratory pyrolysis of large blocks of Western subbituminous coal. Pyrolysis experiments have been conducted in an electrically heated reactor capable of maintaining programmed heating rates of 1 to 10°C/min. Maximum sample temperatures have ranged from 300 to 1000°C. Thermal profiles within the coal blocks have been measured during each experiment, and solid, liquid, and gaseous products have been sampled and analyzed.

1. INTRODUCTION

This is the fourth in a series of semiannual progress reports on many of the programs in the Experimental Engineering Section of the Chemical Technology Division.* The purpose of these reports is to document basic experimental results, many of which will be preliminary in nature. It is expected that topical reports and open-literature publications will ultimately be used to further correlate experimental results and to present conclusions based on these results.

The subject matter in these progress reports will change, of course, as the Section becomes involved in other areas; however, insofar as possible, there will be a continuity of reporting. This document, which covers the period March 1, 1975 to August 31, 1975, includes progress on all programs except those dealing with various nuclear reactors. The information and results developed by the Section in those programs (e.g., the High-Temperature Gas-Cooled Reactor, the Liquid-Metal-Cooled Fast Breeder Reactor, and the Molten Salt Reactor) are reported separately through well-established programmatic channels and, for that reason, are not presented here.

* Previous reports were Experimental Engineering Section Semiannual Progress Report (Excluding Reactor Programs), Sept. 1, 1973 to Feb. 28, 1974, ORNL/TM-4602 (December 1974), Experimental Engineering Section Semiannual Progress Report (Excluding Reactor Programs), Mar. 1, 1974 to Aug. 31, 1974, ORNL/TM-4777 (July 1975), and Experimental Engineering Section Semiannual Progress Report (Excluding Reactor Programs) Sept. 1, 1974 to Feb. 28, 1975, ORNL/TM-4961 (January 1976).

2. CENTRIFUGAL ANALYZER DEVELOPMENT

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In 1969, Dr. Norman Anderson¹ first presented his concept of a new approach to automated clinical analysis, which culminated in an instrument appropriately named the GeMSAEC[†] Centrifugal Fast Analyzer. Following this initial introduction, instrumentation based on Anderson's concept has progressed from an early prototype to second-generation commercial systems which are now well accepted and used routinely in over a thousand clinical laboratories located around the world.

The history of the Centrifugal Analyzer (Table 2.1) and the impact that it has made in the clinical field (Table 2.2) attest to its acceptance and growth. To augment this new field, we have maintained an active developmental effort whose primary objective is to develop automated analytical systems that provide rapid analyses of the constituents contained in a variety of liquids, including physiologic fluids and natural waters. Because of its broad utility, this effort has received funding from many agencies, including the Energy Research and Development Administration (ERDA), the U.S. Environmental Protection Agency (EPA), the National Aeronautics and Space Administration (NASA), the National Center for Toxicological Research (NCTR), the National Cancer Institute (NCI), and the National Institute for General Medical Sciences (NIGMS).

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** Summer participant.

[†]GeMSAEC is an acronym derived from a combination of the names of the two agencies that sponsored the work: the National Institute of General Medical Sciences and the Atomic Energy Commission.

Table 2.1. Chronological history of the development
of the Centrifugal Fast Analyzer

1966-1968	Formulation of concept and principles
1968	Fabrication of working prototype at Oak Ridge
1969	First introduction to the scientific community
1969	Commercial models introduced at FASEB
1970-1971	Clinical trials
1971	First ASCP and Oak Ridge workshops
1972-1975	Introduction and impact in the clinical laboratory

Table 2.2. Chronological summary of information disseminated on the
Centrifugal Fast Analyzer: Presentations given at national and
international clinical chemistry meetings and publications

Year	Presentations given		Number of publications
	Locality	Number	
1969	Geneva, Switzerland	1	8
1970	Buffalo, N.Y.	4	3
1971	Seattle, Wash.	5	8
1972	Cincinnati, Ohio	8	15
	Copenhagen, Denmark	8	
1973	New York, N.Y.	11	16
1974	Las Vegas, Nev.	18	29
1975	Toronto, Canada	25	13 +

During this reporting period, the development of both miniature and portable Centrifugal Fast Analyzers (CFAs) has continued. To evaluate such systems in a variety of laboratory situations, four prototypes have been fabricated and installed in laboratories in Oak Ridge, Tennessee; Bethesda, Maryland; Houston, Texas; and Jefferson, Arkansas. Two additional systems are currently being fabricated for subsequent evaluation in a blood bank and a genetic screening laboratory. A third-generation prototype of the portable analyzer has been designed and partially fabricated. A state-of-the-art microprocessor is included in this prototype, and an extensive software package has been developed for use with it. This system will be evaluated for use in on-site water analysis.

A varied applications program has continued in the general areas of chemistry, immunology, hematology, and environmental analysis. In addition, Centrifugal Analyzers are being utilized in various programs involving the use of biochemical markers for cancer and genetic screening. A previous study on the analytical effects of sample evaporation has been continued, and evaporative rates of samples have been measured as a function of cup geometry. Development of a rotor that contains preloaded reagents and is capable of receiving, processing, and analyzing the constituents of a whole-blood sample is continuing.

2.1 Evaluation and Utilization in Bioanalytical Laboratories

As part of a program to appraise the performance and broaden the utility of analytical systems which have a miniature Centrifugal Analyzer as their basic component, several prototype systems have been fabricated and are being routinely used or evaluated in a variety of analytical laboratories. These include laboratories of the ORNL Health Division;

the NASA Johnson Space Center, Houston, Texas; the National Institutes of Health (NIH), Bethesda, Maryland; and the National Center for Toxicological Research, Jefferson, Arkansas. Two additional systems are being fabricated for evaluation in a blood bank and a genetic screening laboratory.

2.1.1 ORNL evaluation

A miniature analytical system was installed in the clinical laboratory of the ORNL Health Division on April 1, 1973. After an initial evaluation, this system was subsequently utilized to routinely analyze the samples that are obtained from ORNL employees during their periodic physical examinations. During this reporting period, an improved on-line quality control (QC) protocol, described previously,² was utilized routinely and found to be satisfactory. At the request of the medical technologists, it was modified in order to obtain a listing of QC values to be plotted on QC charts. For example, for every control sample during each run, the following QC value is plotted on its respective QC chart:

$$\text{QC value} = \left(\frac{\text{observed value} - \text{mean of QC observations}}{\text{standard deviation of the mean}} \right).$$

This modification has been implemented, and the results are now being utilized by the technologists in their daily plotting of QC results.

2.1.2 NIH evaluation

A complete analytical system was installed in the clinical laboratory of the NIH, Bethesda, Maryland, on June 20, 1974. After an initial evaluation by both NIH and ORNL personnel, the system was utilized to determine the blood groups (A, B, AB, O, Rh) of more than 300 NIH patients. Comparison of these results with those obtained from the NIH Blood Bank revealed only

one discrepancy, which was later found to be a transcription error. Currently, NIH personnel are using the system to investigate reverse blood grouping as well as some of the rarer blood groups. Two manuscripts describing the concept, system, and evaluation are being prepared in a joint effort by NIH and ORNL personnel.

In addition to the blood grouping studies, the system has also been used by Harold Nishi of NIH in the adaptation of a method for the analysis of the drug diphenylhydantoin. This method is based on the EMIT enzyme immunoassay procedure as supplied by Syva Corporation, Palo Alto, California. Initial results, which are very promising, indicate that an assay for the drug can be adapted for use with the miniature system. The advantages are: (1) only 5 μ l of sample is required per assay; (2) reagent volumes are decreased, thereby reducing the reagent cost per assay; and (3) the assay is rapid since only 1 min is required to perform 16 determinations.

Downtime on the NIH system has been low, and problems have been corrected on-site as they arose, usually by NIH personnel. During this reporting period, one visit was required by ORNL personnel to correct a mechanical problem in the loader and a loose brake in the analyzer. Three rotors which had become scratched and unusable were returned to ORNL for refurbishing. Subsequently, they were disassembled and, after addition of new windows, were shipped to NIH.

2.1.3 NASA evaluation

An analytical system has been undergoing evaluation in the clinical laboratory of the NASA Johnson Space Center, Houston, Texas, since February 1, 1973. In April 1975 this system was further updated by the delivery and installation of a computerized data system that is identical to the data

systems used in the ORNL and NIH analyzers. Prior to delivery of the computer system, the NASA analyzer was returned to ORNL for incorporation of several recent electronic improvements. The analyzer was then returned and installed with the computer.

Two types of evaluation are being made with the NASA system. In one, the results of clinical assays obtained from the miniature analyzer were compared with those obtained from conventional clinical analyzers or ion-specific electrodes. In the other, the system is undergoing evaluation and consideration as an on-board analyzer in the forthcoming flights of the Space Shuttle. In September 1974 it performed satisfactorily in a certified verification test (CVT) at Houston. A second CVT was conducted at the Marshall Space Center, Huntsville, Alabama, during the week of August 11-15, 1975. In this test, three payload specialists (PSs) obtained blood samples from each other and also from a mission specialist (MS). These samples were then processed and analyzed by the PS responsible for operating the analytical system. The following tests were performed on each of the four samples: hemoglobin, LDH-L, SGOT, CPK, and SGPT. Both the performance of the system and the results obtained under the conditions of the test were quite satisfactory.

2.1.4 NCTR evaluation

One of the major objectives of the National Center for Toxicological Research, Jefferson, Arkansas, is the development of techniques and methods to determine the detrimental biological effects of various chemicals and other types of environmental insults. Since small animals will often be used as model test systems, it is imperative to have assays that require very small quantities of sample. Obviously, the analytical system should

also be highly automated and require a limited amount of sample preparation. The development of the miniature CFA has resulted in an analyzer that meets the requirements of the NCTR situation. Consequently, a collaborative program has been initiated with NCTR in which the utility of a miniature CFA system will be evaluated in the NCTR laboratory.

Description of NCTR system. The system being fabricated for NCTR consists of two miniature CFAs (NCTR-1, which will operate at 30°C, and NCTR-2, which will operate at 37°C), two automated sample-reagent loaders (modified to use microsample cups), two rotor-cleaning stations, 50 rotors, and a computer. One-half of the system (including the computer) was delivered on April 7, 1975; the other components are slated for delivery later in the year. In addition, a computer program was developed to simplify the routine operation of the system. This program includes an executive file which initiates the daily operation and, by a series of operator query:answer routines, will automatically load the specific chemistry programs into the computer core. This program was initially set up to handle 15 chemical files, which include several of the routine clinical assays plus several microsomal enzyme assays as specified by NCTR. As additional chemical procedures are developed, it can be expanded.

In a series of exchange visits, NCTR personnel were trained to operate the analytical system by ORNL staff members. The training program included instructions for performing analyses on both the ORNL analytical system, which is in routine use in the clinical laboratory of the ORNL Health Division, and the NCTR system, prior to its delivery. In an extensive evaluation, the NCTR system was demonstrated to have performance characteristics as good or better than those of the ORNL system. For example,

the NCTR automated loader would place liquids into a rotor with a precision and an accuracy of $\pm 0.5\%$ and $\pm 1-2\%$, respectively, and had a linear volume output over the 1- to 20- μ l volume range of the sample pump. The optical system of the analyzer was shown to provide a linear absorbance output at 340 nm up to 2.0 absorbance units. Its photometric precision, which was a function of absorbance, ranged from ± 0.0005 to 0.003 absorbance unit. Optical measurements made with it were shown to correlate with those obtained with the ORNL miniature analyzer and a Cary 14 spectrophotometer. The analytical in-run precision of the system ranged from 1 to 2% for four clinical assays.

Routine operation of NCTR-1. After an initial in-house evaluation, the NCTR group placed the first analyzer (NCTR-1), loader, and computer into routine operation. They are now using it to perform 19 different assays on 30 samples daily (or a throughput of 570 assays per day). These assays are for enzymes commonly found in the liver and kidney tissue of mice. They have now established the normal ranges for these enzymes for both male and female mice. Currently, they are assaying tissue samples from mice which have been challenged chemically. It is anticipated that the difference in the enzyme activities for the test and control animals will be proportional to the toxicity of the chemical challenge.

ORNL evaluation of NCTR-2. The second NCTR system (NCTR-2) consists of an analyzer which will operate at both 30°C and 37°C, a sample-reagent loader, and a cleaning station. During this reporting period, the system was fabricated and evaluated prior to delivery to NCTR.

(Optical performance). A series of experiments was performed to evaluate the photometric performance of the analyzer. For comparative

purposes, the same measurements were made on the NIH-2 and ORNL miniature CFAs, as well as on a Cary 14 spectrophotometer. The data accumulated in these studies are summarized in Tables 2.3-2.5. In general, the data obtained for all three CFAs agreed quite well; however, the results from the Cary spectrophotometer tended to be slightly higher. The optical linearity of the NCTR-2 analyzer is illustrated in Fig. 2.1; as can be seen, the unit is linear up to an absorbance of 2.0.

(Temperature control of NCTR-2). In a miniature CFA, a reference thermistor is embedded in one of the alignment pins located on the rotor holder of the analyzer. This thermistor, together with its associated circuitry, is an integral part of the control circuit that regulates the rotor temperature.³ The temperature-controlling system of the analyzer was calibrated by use of a second (calibrated) thermistor which was connected to an external monitoring circuit through a set of slip rings located above

Table 2.3. Comparison of optical measurements made at 340 nm on the NCTR-2, ORNL, and NIH-2 Centrifugal Fast Analyzers with those made on a Cary 14 spectrophotometer

NADH concentration (mg/ml)	Absorbance at 340 nm			Cary 14
	NCTR-2 MCFA	NIH-2 MCFA	ORNL MCFA	
0.1	0.4047	0.4066	0.3970	0.414
0.2	0.7983	0.8053	0.7895	0.810
0.25	1.0005	1.0149	0.9927	1.020
0.40	1.5699	1.6002	1.5680	1.638
0.50	1.9450	2.0007	1.9599	2.050

Table 2.4. Statistical correlation of optical data obtained with the NCTR-2, NIH-2, and ORNL Centrifugal Fast Analyzers and a Cary 14 spectrophotometer.

Statistical parameter	NCTR-2 MCFA	NIH-2 MCFA	ORNL MCFA	Cary 14
Slope, mg NADH per ml	3.901	3.992	3.847	4.101
Y-intercept	0.0102	0.0092	0.0281	-0.0029
Correlation coefficient, r	0.9999	0.9999	0.9999	0.9999
Relative error of r, %	0.33	0.39	0.59	0.40
Molar absorptivity	6100	6240	6010	6410

Table 2.5. Precision of NCTR-2, NIH-2, and ORNL miniature Centrifugal Analyzers in performing optical measurements at 340 nm

NADH concentration (mg/ml)	Absorbance at 340 nm					
	NCTR-2		NIH-2		ORNL	
	Mean	S.D. ^a	Mean	S.D.	Mean	S.D.
0.1	0.3970	0.0002	0.4066	0.0002	0.3970	0.0002
0.2	0.7895	0.0005	0.8053	0.0003	0.7895	0.0005
0.25	0.9927	0.0006	0.0149	0.0005	0.9927	0.0006
0.40	1.5680	0.0017	1.6002	0.0015	1.5680	0.0017
0.50	1.9599	0.0037	2.0070	0.0025	1.9599	0.0037

^aS.D. = standard deviation.

Fig. 2.1. Photometric linearity of the NCTR-2 CFA at 340 nm.

the rotor. A special test rotor was subsequently loaded with 100- μ l aliquots of H_2O in each of the sample chambers to approximate actual run conditions. A rotor loading system was used to ensure reproducibility of sample and reagent volumes in each set of chambers. The calibrated thermistor was inserted into an individual cuvet of the special test rotor, and the temperature of the rotor and its contents was brought to a preset level by rotating at 100 rpm with concurrent heating by the high-intensity heat lamp of the analyzer. Upon achieving the preset temperature, as indicated by the display meter, the contents of the reagent and sample chambers were transferred into their respective cuvetts and the temperature of the cuvet thermistor was monitored for a predetermined period of time.

A typical recorder output of the temperature control capabilities of the NCTR-2 analyzer at 30.0°C and 37.0°C, when run in a controlled-temperature environment (i.e., the ORNL Health Division laboratory with ambient temperature maintained at 22°C), is shown in Fig. 2.2. As may be seen, the output of the rotor thermistor and its display was set and calibrated to maintain the cuvet temperature at either $30.0 \pm 0.2^\circ\text{C}$ or $37.0 \pm 0.2^\circ\text{C}$.

(Summary). The evaluation of the NCTR-2 system confirmed its capability for producing accurate and precise data and for monitoring and maintaining the rotor temperature at either 30°C or 37°C. This system was delivered to and installed at the NCTR facility in Jefferson, Arkansas, on September 5, 1975.

2.1.5 NIH-2 evaluation

A second analytical system is being fabricated for evaluation at a laboratory, probably a blood bank facility, which will be chosen by NIGMS with the concurrence of ORNL. Fabrication of this system is nearing

Fig. 2.2. Temperature calibration of the NCTR-2 miniature Centrifugal Fast Analyzer.

completion, and components of it are being evaluated. In Tables 2.3-2.5, the optical data obtained from the NIH-2 analyzer are compared with other CFAs and a Cary 14 spectrophotometer. Figure 2.3 illustrates the photometric linearity of the NIH-2 analyzer at 340 nm, while Fig. 2.4 shows its correlation with results obtained from the Cary 14 instrument. These results, which are quite acceptable, demonstrate that the unit can provide precise and accurate photometric data. This system will be delivered and installed at the designated laboratory either late in 1975 or early in 1976.

2.1.6 University of Michigan evaluation

An analytical system has been fabricated for evaluation in the laboratory of the Department of Human Genetics at the University of Michigan, Ann Arbor. After the unit and its components had been evaluated, the system was delivered and installed during the week of September 29, 1975.

Photometric evaluation. A series of experiments was performed to evaluate the photometric performance of the analyzer. For comparative purposes, similar measurements were made with a Cary 14 spectrophotometer. The results from the two instruments (see Table 2.6) agree quite well and can be shown to correlate statistically (Fig. 2.5).

Evaluation of sample-reagent loader. The microvolumes required by a miniature CFA make loading of these volumes into the rotor a critical step; thus the performance of the sample-reagent loader provided with the Michigan system was evaluated. In a series of repetitive experiments, various volumes of Blue Dextran dye were individually diluted to a final volume of 0.150 ml and their absorbances measured at 620 nm. Data obtained in these experiments indicated that the delivered volumes of the sample pump were

Fig. 2.3. Photometric linearity of the NIH-2 Centrifugal Fast Analyzer at 340 nm.

ORNL DWG 75-8229

Fig. 2.4. Comparison of the optical performance of the NIH-2 Centrifugal Fast Analyzer with that of a Cary-14 spectrophotometer.

Table 2.6. Comparison of optical measurements made at 340 nm with the University of Michigan Centrifugal Fast Analyzer and a Cary 14 spectrophotometer

NADH concentration (mg/ml)	Absorbance at 340 nm	
	Michigan CFA	
	Mean $\pm$ S.D. ^a	Cary 14
0.025	0.0966 (0.0001)	0.099
0.05	0.2115 (0.0001)	0.210
0.10	0.4044 (0.0001)	0.410
0.20	0.8232 (0.0002)	0.838
0.30	1.2282 (0.0003)	1.250
0.40	1.6290 (0.0007)	1.670
0.50	2.0252 (0.0016)	2.075

^aS.D. = standard deviation.

ORNL DWG. 75-8714

Fig. 2.5. Statistical correlation of the optical performance of the University of Michigan Centrifugal Fast Analyzer with that of a Cary-14 spectrophotometer.

linear over the 1- to 20- μ l volumetric range of the pump (Fig. 2.6), and that precision ranged from 4.2% at 1 μ l to 0.42% at 20 μ l (Table 2.7). Run-to-run variation at a sample volume of 20 μ l was found to be $\pm 0.8\%$. These results, which are quite acceptable, compare favorably with those obtained in earlier evaluations of the sample-reagent loader.

Calibration of temperature control system. A typical recorder output of the temperature control capabilities of this analyzer at 30.0°C, when run in a controlled-temperature environment, is shown in Fig. 2.7. The output of the rotor thermistor and its display is set and calibrated to maintain the cuvet temperature at $30.0 \pm 0.2^\circ\text{C}$.

2.2 Engineering Development

During this reporting period, fabrication of a third-generation prototype of the portable CFA was nearly completed and several of its components were evaluated on an initial basis. The development of a rotor capable of accepting, processing, and analyzing a whole-blood sample was continued.

2.2.1 Portable Centrifugal Fast Analyzer

A portable CFA is being developed as part of a continuing effort to reduce the overall size of the analyzer and its data system. It is expected that the portable model will eventually be a small unit containing both the analyzer and the data system in a single cabinet. During this reporting period, fabrication of the analytical module of a third-generation prototype portable CFA was nearly completed, and several of its components and functions were initially evaluated. Also, work was begun on fabricating a prototype microprocessor, which will eventually be contained in the analyzer cabinet and will have the multiple functions of process control, component monitoring, and data processing. To achieve these functions, an extensive

ORNL DWG 75-8882

Fig. 2.6. Volumetric linearity of sample pump used in the Michigan sample-reagent loader.

Table 2.7. Precision of the Michigan sample-reagent loader
at various sample volumes

Diluent volumes (μ l)		Sample volumes (μ l)		Absorbance at 620 nm		
Reagent ^a	Sample ^a	Reagent ^a	Sample ^b	Mean	S.D.	R.S.D. (%)
50	49	50	1	0.0876	0.0073	4.25
50	48	50	2	0.1614	0.0037	2.30
50	45	50	5	0.3745	0.0035	0.93
50	40	50	10	0.7357	0.0037	0.51
50	45	40	15	1.0935	0.0046	0.42
50	40	40	20	1.4400	0.0059	0.42

^a Distilled water used as diluent liquid and reagent.

^b Blue Dextran used as a sample.

Fig. 2.7. Temperature calibration of the University of Michigan Centrifugal Fast Analyzer.

effort in software development is being pursued in parallel with the hardware development. Several chemical assays which are being developed for use with the portable CFA are discussed in Sect. 2.3.

Design and fabrication of advanced prototype. Assembly of the analytical module for the advanced prototype of the portable CFA is now sufficiently complete that testing of its optical, temperature control, and rapid acceleration systems can be performed under manual control. A top view of the instrument is shown in Fig. 2.8. The prototype has been interfaced with the computer system utilized by the miniature CFA to permit testing of the optical and temperature control components and circuits.

All of the electronic circuits for the control and monitoring of the various components in the analyzer have been designed and tested, where possible, in a breadboard fashion; 10 of the 15 special printed-circuit boards have been completed. Because of their commercial unavailability, we have had to make circuit boards for the new 1024x8 bit programmable, read-only memory and 4096 bit random access memory chips which could not be obtained from Control Logic Corporation, the manufacturer of the microprocessor card set that will be used in the analyzer.

The microprocessor system has been designed, and a wire list for the wire wrapping of its interconnections has been prepared; the wire wrapping of the microprocessor subsystem is almost complete. This system, along with its peripheral input and output cards, will be tested as the cards become available. The printer interface board, which is the first item of this group to be completed, has successfully passed its operational tests.

Evaluation of optical system. The optical system utilized in the portable analyzer differs from that of the miniature Centrifugal Fast Analyzer (MCFA). The MCFA utilizes a GE-1974 tungsten-halogen lamp,⁴

Fig. 2.8. Top view of advanced prototype of the portable Centrifugal Fast Analyzer.

whereas the portable system uses a subminiature GE-3027 tungsten-halogen lamp. Also, the lamp in the portable system is located closer to the multicuvet rotor, thus permitting greater signal intensity to reach the photomultiplier tube (R300, Hamamatsu Corporation, Middlesex, New Jersey). Additional intensity is achieved by operating the lamp at 7 V, rather than at the nominal rating of 6.3 V. Maximum intensity occurs in the spectral region near 520 nm, and the energy distribution is such that there is adequate source intensity for optical measurements over the range 340 to 660 nm.

Wavelength selection is achieved by the use of transmission filters located in motor-driven, six-position filter holders. One may choose filters oriented below, above, or at 90° to the multicuvet rotor, permitting either transmission, light-scatter, or luminescence measurements. The filter selection process may be performed in either of two ways: (1) by entering a selection code or (2) by utilizing the control of the micro-processor unit. Changing the filters requires 1 sec or less and may be achieved while a run is in process, making multiple-wavelength monitoring feasible. This capability may be of particular value in multiple-chemistry analyses with a single rotor.

In Fig. 2.9, the performance of the portable Analyzer is compared with that of a well-characterized MCFA for solutions of NADH monitored at 340 nm. The agreement between the two instruments is excellent. The source intensity in the portable analyzer was sufficiently high that the photomultiplier tube could be operated at a near-optimum 470 V.

Evaluation of transfer and mixing of liquids. One of the novel and potentially significant features of the portable analyzer system is the clutch-brake mechanism in the rotor drive assembly. With the clutch

ORNL DWG. 75-8713

Fig. 2.9. Optical performance at 340 nm of the portable Centrifugal Fast Analyzer compared with that of a miniature Centrifugal Fast Analyzer.

disengaged, the rotor drive mechanism may be accelerated to 4000 rpm while the rotor itself remains stationary. A fly-wheel mechanism helps maintain the momentum of the drive assembly. When the clutch is engaged, the rotor is accelerated from "rest" to 4000 rpm in 125 msec or less. Such rapid acceleration may be used to both transfer and mix samples and reagents, eliminating the necessity of a braking step to achieve mixing. This capability is most readily achieved by the use of specially developed rotors having separate sample and reagent transfer channels.^{5,6}

In the parallel mixing rotor design,⁷ aliquots of sample and reagent mix as they jet into a common cuvet. In one experiment, 65 μ l of Blue Dextran dye and 65 μ l of water were loaded by use of a Micromedic pipette into the sample and reagent chambers, respectively, of a parallel transfer rotor (Fig. 2.10). With the clutch disengaged and the rotor stationary, the rotor drive assembly was permitted to accelerate to 3500 rpm. The clutch was then engaged, accelerating the rotor to 3500 rpm in a fraction of a second. Subsequently, the rotor was allowed to coast down to 1000 rpm without braking, after which the absorbance at 620 nm of the now-diluted dye solution was measured to be 0.3519 ± 0.0063 . Next, the rotor was subjected to four complete cycles of the conventional mixing routine, consisting of acceleration to 3500 rpm followed by dynamic braking. Finally, the absorbance of the thoroughly mixed dye solutions was measured to be 0.3527 ± 0.0058 . There is no significant difference in the results of the two mixing procedures, even at the 0.6 significance level; this indicates that mixing was complete in the single, rapid transfer operation.

It should be noted that, in the experiment described above, data were taken using a PDP-8/i computer interfaced with the analyzer. Values could

FLUORESCENCE AND LIGHT SCATTER ROTORS

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Fig. 2.10. Details of a parallel transfer rotor.

not be taken at the rapid rate at which they were generated at 3500 rpm. However, the microprocessor unit, when integrated with the analyzer, will accept data at this rate. Thus, with the microprocessor unit, meaningful absorbance measurements may be made at rapid rotor speeds on well-mixed systems within the first fractional second of the reaction. The ability to observe fast kinetics with the complete portable analyzer system will approach that obtained by stopped-flow techniques. Not only may rapid reactions (e.g., chemiluminescence) be studied, but the capacity for making very early initial readings will provide automatic blanking capability for two-point, fixed-time kinetic analysis. Resolution of complex mixtures of related compounds and even isoenzymes by differential kinetics will also be feasible.

Temperature control system. The portable CFA has been designed to utilize a heat pump module to maintain and control the temperature of the rotor at either 25, 30, or 37°C. During the initial evaluation of this module, we found it necessary to utilize a better control circuit than the one originally designed for the system. Consequently, a modified three-mode controller was added to the circuit. This type of controller allows one to compensate for all but the most difficult of system dynamics and to obtain a relatively well-regulated temperature. Control and stability studies will be continued, utilizing an external thermistor assembly to measure actual cuvet temperature during a run.

Since the approach utilized to control the rotor temperature of the portable CFA is different from that in the miniature CFA,^{3,4} a mathematical model was developed in order to allow us to model and predict the thermal response of the new system.

(Model). The rotor used in the portable CFA may be approximately modeled as a composite slab, as shown in Fig. 2.11. In this model, the temperatures of interest are T_b and T_E . When T_b is known, the idealized steady-state heat flow q^* required to achieve and maintain a given T_E may be determined for the model shown from the following equation:

$$q^* = (T_b - T_E) \left(\frac{1}{hA} + \frac{L_1}{K_1 A} + \frac{L_2}{2K_2 A} \right)^{-1}, \quad (1)$$

where

h = convective heat-transfer coefficient,

K = thermal conductivity,

A = rotor surface area,

T_b = bulk phase or ambient temperature,

T_E = experimental temperature,

L = material thickness.

A positive q^* indicates heat transfer toward the rotor base, while a negative q^* indicates transfer away from the base. Thus, both heating and cooling problems may be considered.

The temperature profile in the slab may be established by use of the following equations:

$$T_0 = q^* \left(\frac{L_1}{K_1 A} + \frac{L_2}{2K_2 A} \right) + T_E, \quad (2)$$

$$T_1 = q^* \left(\frac{L_2}{2K_2 A} \right) + T_E, \quad (3)$$

$$T_2 = q^* \left(-\frac{L_2}{2K_2 A} \right) + T_E, \quad (4)$$

Fig. 2.11. Composite slab representation of rotor used in the portable Centrifugal Fast Analyzer.

$$T_3 = q^* \left(-\frac{L_3}{K_3 A} - \frac{L_2}{2K_2 A} \right) + T_E \quad (5)$$

(System behavior). Heat-transfer requirements for expected operating conditions were calculated using the information in Table 2.8; the results are given in Table 2.9. The temperature profile for a typical case ($T_b = 30^\circ\text{C}$, $T_E = 22^\circ\text{C}$, $h = 1 \text{ Btu/hr}\cdot\text{ft}^2\cdot^\circ\text{F}$) is given as follows:

T_b	T_0	T_1	T_2	T_3
30°C	22.8°C	22.2°C	21.8°C	21.2°C

These calculations, which were based on water as an assumed sample, indicate a 0.4°C temperature gradient across the center section (cuvet).

Additional insight into the behavior predicted by this model may be obtained from Eq. (1), which gives the sample temperature as

$$T_E = (-q^*/A) \left(\frac{1}{h} + \frac{L_1}{K_1} + \frac{L_2}{2K_2} \right) + T_b \quad (6)$$

and the rate of change of this temperature with respect to the heat flux as

$$\frac{dT_E}{d(-q^*/A)} = \left(\frac{1}{h} + \frac{L_1}{K_1} + \frac{L_2}{2K_2} \right). \quad (7)$$

Again, using the information in Table 2.8, results from Eq. (6) are presented graphically in Fig. 2.12. It may be seen that a large h (little resistance to heat transfer as with forced convection cooling) results in a lower sample temperature than does a low h (high resistance to heat transfer; an insulated wall in the limiting case) for a given heat flux. However, the rate of change of the sample temperature with respect to the heat flux is much higher for the low convective heat-transfer coefficient.

Table 2.8. Physical properties for heat-transfer problem

Convective heat transfer coefficient,
 $h = 0.1 \text{ to } 1.0 \text{ Btu/hr}\cdot\text{ft}^2\cdot^\circ\text{F}$

Rotor surface area, $A = 0.067 \text{ ft}^2$

Layer	L (ft)	K (Btu/hr·ft ² ·°F)	L/K
1	0.0104	0.120	0.0867
2	0.0156	0.343	0.0455
3	0.0104	0.120	0.0867

Table 2.9. Heat flow requirements^a (in Btu/hr)

h	T_b (°C)		
	25	30	37
0.1	0.036	0.095	0.179
1.0	0.326	0.870	1.631

^a($T_E = 22^\circ\text{C}$).

Fig. 2.12. Effects of heat fluxes and convective transfer coefficients on sample temperatures.

While this results in a more rapid approach to steady-state heat transfer, it also requires more accurate control over the heat flux to maintain T_E within a given range.

A complete analysis of the transient heat-transfer problem for this system is more difficult than warranted at present. However, the time required to achieve steady-state heat transfer can be estimated based on approximated solutions presented by Jaeger.⁸ Calculations made using these solutions showed that, if an h of 0.7 is assumed, approximately 4 to 5 min would be required to reach a steady-state heat transfer situation for the system described.

(Heat capacity). The performance curve for the heat pump presently in use (MELCOR thermoelectric module, CP 1.4-71-10) is reproduced in Fig. 2.13. For a heat sink temperature (equivalent to T_b) of 35°C and a cold surface temperature (equivalent to T_3) of approximately 70°F (slightly less than $T_E = 22^\circ\text{C}$, as indicated by the temperature profile given for a typical case), an input of 1.0 A causes the module to pump 18 Btu/hr. Referring to Table 2.9, it is obvious that this module will be adequate for situations presently anticipated unless the model is very inaccurate.

2.2.2 Whole-blood rotor design and development

We are continuing our efforts relative to the development of a CFA rotor which will accept, automatically process, and analyze a whole-blood sample. During the last reporting period, we discussed a rotor concept which utilized membrane filtration for the separation of cells from a whole-blood sample.² This separation concept has been explored experimentally by mounting Millipore membrane filters in Millipore filtering centrifuge tubes.

Fig. 2.13. Performance curve for thermoelectric heat pump used in the portable Centrifugal Fast Analyzer.

Membrane filtration experiments. A series of experiments was performed in which Millipore MF membrane filters (pore sizes of 1.2, 3.0, 5.0, and 8.0 μm) mounted individually in Millipore filtering centrifuge tubes were tested to determine their capability for separating cells from whole-blood specimens diluted 1:10 with a saline solution. Preliminary results showed that the filters quickly became plugged with blood cells, preventing further filtration of the samples. Serial filtration was then tested using combinations of membrane filters with "depth-type" prefilters. However, this approach resulted in limited success since only a small fraction of the sample volume could be filtered before plugging occurred.

New rotor design. Based on the results of the membrane filter experiments, it was decided that a means of preventing filter plugging must be devised. A new design was then proposed which would utilize filters mounted in the radial plane of the rotor (Fig. 2.14). A whole-blood sample injected dynamically would be partitioned into separate channels oriented radially from the center of the rotor. The pressure due to the centrifugal force acting on the contained fluid mass causes plasma to flow through the filter. At the same time, blood cells are quickly sedimented away from the membrane filter to prevent obstruction of the filter.

Test of new design. A rotor based on the design just discussed was fabricated for testing the feasibility of the "sedimentation with membrane filtration" principle for removing blood cells from a whole-blood sample. A standard 17-place CFA rotor body was machined from UVT plastic. An upper stage was machined with 17 entry and sedimentation channels. Filters were cut from Millipore RAWP 025 membrane filter (pore size, 1.2 μm) using a

Fig. 2.14. Side-sectional view of membrane filter rotor, showing one channel.

paper punch. The smallest blood cell, a platelet, is approximately $2.5\text{ }\mu\text{m}$ in diameter and therefore should be retained by this type of filter, as should erythrocytes, which are approximately $8.5\text{ }\mu\text{m}$ in diameter. The filters were sealed in the rotor using Dow Corning RTV-3145 adhesive. With the rotor spinning at 400 rpm, 2.0 ml of a sample solution prepared by diluting a whole-blood specimen 1:10 with a saline solution (0.9 w/v % NaCl), was dynamically injected into the splitting section using a 2.5-ml syringe. The rotor was then accelerated to a constant speed of 2000 rpm. By using a strobe light to observe the behavior of the sample, erythrocytes were seen to amass in the sedimentation chambers while the plasma filtrate accumulated in the cuvetts. Leakage of erythrocytes around or through membrane filters was observed in only 2 of the 17 channels. The separation was complete approximately 15 sec after the rotor speed had been increased to 2000 rpm.

We are encouraged by the results of this feasibility experiment, in which the first prototype membrane filter rotor was tested. The "sedimentation with membrane filtration" concept apparently circumvents the filter plugging problems experienced when using "straight through" filtration configurations.

2.3 Data Processing

Centrifugal Fast Analyzers produce data at a rate and in a form suitable for direct input into a small computer. An alternative to a small computer would be a microprocessor-based data system. The rapid development and low-cost features of the microprocessors make them very attractive for this application; consequently, we are developing a microprocessor-based data system for use with the portable CFA. The first such system developed, termed Microprocessor-I, allowed us to gain

valuable experience. Building on this experience, a more advanced system (Microprocessor-II) is being developed for use with the portable CFA. In addition, an extensive software development effort is also being pursued.

2.3.1 Evaluation of Microprocessor-I data system

The construction phase for the Microprocessor-I data system was completed in March 1975. Troubleshooting and modifications to the design continued into the middle of April. The first data acquisition programs were limited to 512 words of program memory and were, therefore, rather simplistic. By the middle of May, space for another 1024 words of program memory was installed. The resulting total, 1500 words, was enough to implement a tabular absorbance printout of the data from one of the CFAs. The logarithmic algorithm was accurate enough to print out absorbance to a tenth of a milliabsorbance unit. As yet, the system is not capable of using any sophisticated data acquisition schemes; however, it will soon be possible to increase the program memory capacity to over 4000 words, which makes the system potentially very useful. Because of the 14-bit analog-to-digital converter (ADC), this system has greater resolution than the PDP-8/e--based systems and can acquire data at the full speed of the analyzer (i.e., 4000 rpm).

2.3.2 Design and fabrication of Microprocessor-II

The Microprocessor-II system, which is presently in the construction phase (and should be completed by the end of 1975), will occupy the same cabinet as does the portable CFA. It will use a logic card set manufactured by the Control Logic Corporation, based on the Intel 8080 microprocessor chip. This will simplify construction and troubleshooting as compared

with the first unit, which was built from discrete components. Unlike the first unit, control of the analyzer process will be included in the capabilities of the Microprocessor-II system. Parameters such as data rate and interval, rotor speed, spectrometer wavelength, and other operating conditions can be controlled automatically. Additionally, the advanced unit will contain a feature which will allow it to monitor all the operating voltages and parameters of the analyzer and of the system itself, flagging bad conditions and warning the operator of any impending component failure.

2.3.3 Software development

A general software package has been developed for the Microprocessor-I. This package has been expanded and improved for the Microprocessor-II.

Microprocessor-I. The software development for the Microprocessor-I data system is now complete. Overall, this effort has demonstrated the feasibility of interfacing a microprocessor with a CFA.

In retrospect, one can identify two distinct phases of software development. The first phase involved the development of dedicated service-interrupt routines. In other words, one learned how to have the computer respond to the three types of interrupts - rotor, ADC, and printer interrupts. In addition to these service routines, a primitive mathematics routine was developed. The primary limitation of the mathematics was that it was strictly fixed decimal. Because of this limitation, it was possible to only print an absorbance value with a precision of 2-1/2 digits.

The second phase of software development was concerned with revamping the mathematical routines. A full floating-point package with an internal word length of 7 decimal digits was developed. In fact, the same mathematical package is to be used with the Microprocessor-II, with the exception

that its internal word length has been extended to 11 decimal digits. The modified package will allow one to print an absorbance value of 4 decimal digits.

Microprocessor-II. The software for the Microprocessor-II is characterized by two specific sections: a process control/data acquisition section, and a data analysis section.

The first section deals with regulating process variables such as speed, temperature, and optical filters. The actual data acquisition, with its associated storage, timing, and counting function, is also accomplished in this section. With the software developed, the user will have control over virtually every aspect of the data acquisition process. The variables that are to be input at the start of every experimental run include:

- (1) delay interval (in tenths of a second),
- (2) number of observations,
- (3) number of revolutions averaged per observation, and
- (4) observation interval (in tenths of a second).

The delay interval is the time elapsed from the moment the rotor clutch is energized to the time that the first data point is measured and acquired. The delay-interval dials on the instrument will give the user a range of 0 to 9.9 sec with a resolution of 0.10 sec.

The second input parameter is the number of observations acquired per cuvet during the analysis. The maximum allowable number of observations is strictly a function of random-access memory (RAM) size. The first version of the analyzer, with 2K RAM, will have a capacity of 25 sets of data points. Within a year, RAM will be expanded to 4K, and the analyzer will have a capacity of almost 80 sets of data points.

The third input parameter to be entered is the number of revolutions averaged per observation. Specifically, this function is included for signal-averaging purposes since it reduces the uncertainty of measuring an absorbance by the value $1/\sqrt{N}$, where N = the number of revolutions averaged. In practice, this parameter is given a value in the range of 10 to 20.

The last input parameter required is the time interval between observations. The sampling period dials on the instrument will give the user a range of 0 to 99.9 sec, in 0.1-sec steps.

The second section of the software package developed for the Microprocessor-II deals with data analysis. This section assumes that data from an experimental run is already stored in RAM. The task of this section is to perform mathematical analyses on the data and to format the results on the printing device.

The heart of the data analysis section is a floating-point mathematics package which was developed explicitly for the portable CFA. Square-root and log operations are included in addition to the elementary addition, subtraction, multiplication, and division capabilities.

The mantissa is carried in all mathematical operations at a precision of about 11 decimal digits. This level of precision is comparable to that of the PDP-8/e focal-based systems of the miniature Fast Analyzer. The exponent range is $10^{\pm 12}$. This is a considerably smaller exponent range than is available on PDP-8/e four-word FOCAL. However, it is felt that this exponent range is adequate for virtually any calculation that is to be done by the portable analyzer.

Using this mathematics package as a base, various subroutines that implement specialized algorithms have been developed. A brief description of each subroutine follows:

- (1) Rotor calibration. The portable analyzer will have the capacity for storing calibration data for three rotors. At any time, the user will be able to recall these factors for a particular rotor and apply them to subsequent absorbance measurements. Other parameters such as mean transmission, standard deviation of transmission, and coefficient of variation for the total number of cuvetts used in a run will also be available.
- (2) Intensity - absorbance tables. Three tables on these two parameters are accessible: (a) absorbance and change in absorbance, (b) intensity and change in intensity, and (c) absorbance and intensity. When a user requests one of the above tables, he has the option of specifying whether he wishes one specific cuvet or all 17 cuvetts to be printed. He also has the option of specifying a select group of points or time observations. These two options, which were implemented to significantly decrease the total time that the printer is operating, allows the user to obtain the desired data sooner, and prolongs the life of the analyzer batteries (if in battery mode).
- (3) Statistics tables. Four statistics tables are accessible: (a) linear variable statistics on absorbance (slope, variance of slope, Y-intercept and variance of Y-intercept, and correlation coefficient); (b) linear variable statistics on intensity [same parameters as (a)]; (c) mean statistics on absorbance (mean and variance of mean); and (d) mean statistics on intensity [same as (c)]. The user inputs the start and end points over which he wishes the statistics to be calculated.

- (4) Concentration table. Changes in absorbances, particularly end-point absorbances, are translated into concentration values through a multiplicative constant as follows:

$$\text{conc (mg/dl)} = \Delta \text{absorb} * (\text{substrate factor})$$

The constant is the substrate factor. The three important input parameters for concentration are then the start point, the end point, and the substrate factor.

- (5) Smart PM count table. This table gives the user an accurate picture of the dynamic range throughout which the ADC is operating in an experimental run. Such information is important in maintaining linearity and precision in the data. The table will also be of use in fluorescence studies where the setting of an optimal photomultiplier (PM) voltage is difficult.
- (6) Process control table. If data analysis from any subroutine is to be truly meaningful, the user must be aware of all process control variables at the time the data were collected. Answers are needed to questions such as: Was the selection of optical filters in a manual or automatic mode? If the mode was automatic, what was the value of the computer selected filter? This process table lists all the significant process control variables.
- (7) Interval timetable. In this table, time values in seconds are computed for interval numbers. Example: No. 3 interval = 10.2 sec.

This completes the listing of all the dedicated subroutines that have been written. Many of these programs are emulations of existing PDP-8/e FOCAL programs. Some of the tables, such as those for the process control and the smart PM counts, are unique to the portable analyzer.

This first version of the portable analyzer will include all of the aforementioned subroutines. In addition to these, other subroutines which have not as yet been written will probably be included.

2.4 Applications

Centrifugal Fast Analyzers are basically sophisticated optical-measuring devices which should have applications in a variety of areas. Specifically, we are utilizing them for various chemical and hematological assays.

2.4.1 Clinical analyses

During this report period, we participated in a round-robin inter-laboratory evaluation of a reference method for the assay of serum glucose and continued to adapt the Coombs test for use with the miniature CFA.

Evaluation of a glucose reference method. A reference method for determining serum glucose⁹ is currently under development under the auspices of the Food and Drug Administration, the National Bureau of Standards, and the National Center for Disease Control (CDC). This method is currently being evaluated by several independent laboratories, with the overall effort being coordinated by the Glucose Subcommittee of the Standards Committee of the American Association of Clinical Chemists.

Both manual and semiautomated procedures have been developed for the reference method, which is based on the hexokinase assay for glucose. Our laboratory was asked to participate in a round-robin evaluation of the semiautomated reference method. In this evaluation, each participant was asked to calibrate his equipment and reagents under specified conditions and then make five analytical runs plus one practice run. Analytical runs 1 through 4 consisted of duplicate analyses of five serum samples; run 5 consisted of

duplicate analyses of six serum samples. Because of an error in setting up the first run, a second batch of samples was obtained from CDC for this run. The runs were performed in the following order: practice, 2, 3, 4, 1, 5. Figures for the accuracy and precision of the Micromedic pipetting systems used in this study are summarized in Table 2.10.

The results obtained by our laboratory are summarized in Table 2.11. The raw absorbance data from which the values given in this table were obtained have been submitted to CDC. When such data have been made available by all of the participating laboratories, they will be processed and a summary of the results returned.

Adaptation of Coombs test. A desirable feature of automation in the clinical laboratory is the reduction in, or elimination of, the need to make subjective evaluations. At the same time, however, adequate controls must be included to ensure the reliability of the results indicated by the

Table 2.10. Accuracy and precision of the Micromedic pipette used in the ORNL evaluation of the semiautomated reference method for glucose

	Liquid pipetted			
	5.0 ml Ba(OH ₂)	5.0 ml ZnSO ₄	1.0 ml supernate	0.5 ml sample
Number of weighings	10	10	10	10
Mean weight, g	4.9846	4.9890	0.9982	0.4989
Coefficient of variation, %	0.01	0.02	0.05	0.20
Temperature, °C	26.1	26.0	26.1	25.4
Mean temperature- corrected volume, ml	5.0050	5.0093	1.0023	0.5009

Table 2.11. Analytical results obtained from the ORNL evaluation of the semiautomated reference method for glucose

Sample	N ^a	Results (mg/dl)		R.S.D. ^c (%)
		Mean	S.D. ^b	
Control-1	7	74.97	0.74	0.98
Control-2	7	131.20	2.41	1.83
Pool A	5	107.38	1.23	1.15
Pool B	5	204.52	2.42	1.18
Pool C	7	105.97	2.96	2.79
Sample-1	10	41.85	1.15	2.76
Sample-2	10	78.90	1.14	1.45
Sample-3	10	135.24	2.25	1.66
Sample-4	10	194.59	2.05	1.05
Sample-5	9	293.44	2.40	0.82

^aNumber of measurements.

^bStandard deviation.

^cRelative standard deviation.

instrumentation. Data which can be interpreted with "yes/no" or "on/off" types of criteria are readily adaptable to computer processing. In the field of immunohematology, controls are carried through the various procedures routinely, and the results of many of the procedures may often be interpreted as either "positive" or "negative." Thus, blood-banking protocols would appear to lend themselves to automated performance and interpretation, and, indeed, considerable effort has been expended in developing instrumentation and procedures to automate particularly those procedures resulting in hemagglutination reactions.⁹⁻¹⁹

The development of the miniature CFA system^{1,3,20,21} led to its use in studying blood grouping procedures.¹⁹ From this feasibility study, we developed a blood grouping protocol which has been evaluated extensively in the laboratories of the National Institutes of Health. During the course of Tiffany's study,¹⁹ the major factors were evaluated concerning anti-globulin testing as applied to blood grouping with the use of a rotor in which cell suspensions could be washed in situ and hemagglutination reactions performed thereafter.

The purpose of the present study was to delineate further the performance of the antiglobulin tests, especially as applied to cross-matching procedures, by use of the miniature CFA and the cell-washing rotor in an effort to extend application of the system in blood banking.

Accepted cross-matching procedures include compatibility testing of the donor cells with the recipient's serum (the major cross match), antibody screening of recipient and donor sera, and a direct antiglobulin test on the donor cells. Hence, each of these procedures at some stage involves adding antiglobulin serum to a cell suspension and checking for evidence of

agglutination. The antiglobulin reaction is a sensitive technique for detecting antigen-antibody interactions which do not otherwise lead to hemolysis or agglutination. Various experimental techniques are used to enhance the sensitivity of the antigen-antibody reaction and its detection by antiglobulin agglutination; these include the use of enzyme-treated reagent cells and the use of a medium with a high dielectric constant (i.e., albumin or serum suspensions of cells). Additional factors which affect the antigen-antibody interaction include the time and temperature of incubation.

Among other techniques, unexpected antibodies may be detected by a positive antibody screening test, an incompatible cross match, or a positive direction antiglobulin test. Consequently, the antiglobulin test is of broad utility in blood banking, and its performance with the miniature CFA represents an important step in the automation of immunohematological procedures.

(Materials). Antihuman (Coombs) serum, anti-Rh₀ (anti-D) slide test serum, Identigen* and Selectogen** reagent red blood cells (human), and Coombs control reagent red blood cells (human) were purchased from Ortho Diagnostics, Raritan, New Jersey 08869. Polyvinylpyrrolidone (PVP) was obtained from Polysciences, Inc., Warrington, Pennsylvania 18976.

* Identigen - Consists of eight separate vials, each containing red cells from different types of Group O donors. It is used to identify antibodies previously detected by screening or cross matching. Unvarying structure in every panel for the five most important Rh antigens (D, C, E, c, e) provides accuracy, simplicity, and convenience in antibody identification.

** Selectogen - Consists of two vials of human red cells for use in the detection of unexpected antibodies. Selectogen I is a 5% suspension of Group O red cells from a single donor with the Rh phenotype Rh₁ (DCE). Selectogen II is a 5% suspension of Group O red cells from another donor with the Rh phenotype Rh₂ (DcE). Together, Selectogen I and II provide a comprehensive spectrum of antigens for use in detecting unexpected, clinically significant antibodies.

Blood (anticoagulated with EDTA) and serum samples from ORNL employees were supplied by the ORNL Health Division. Cell suspensions and serum samples from other patients were donated by the East Tennessee Baptist Hospital and the Fort Sanders Presbyterian Hospital, both of Knoxville, Tennessee.

Cell washing, incubation, and agglutination reactions were performed (as described below) with a miniature CFA system. The cell washing rotor was loaded by means of an automatic sample reagent loader (SRL).²² Washing included the techniques of dynamic injection²³ of saline wash solution.

(Reagent and sample loading). The SRL used during these investigations has been described elsewhere.^{22,23} For this application, the following pumps were used and their strokes adjusted to deliver the listed volumes:

20- μ l sample pump at 30% = 6 μ l

200- μ l sample diluent pump at 12% = 24 μ l

50- μ l reagent pump at 100% = 50 μ l

200- μ l reagent diluent pump at 25% = 50 μ l

The diluent pumps were primed with and dispensed saline (0.9% saline) as the diluent. Depending on the assay, the turntable was set in either of four loading modes.²² Cups containing the samples or reagents were placed in specific positions in the carousel as dictated by the individual test (Tables 2.12-2.14).

(Cell washing). Cells were washed in situ as previously described by Tiffany.¹⁹ To verify that the supernate was removed by the aspiration step, the operator has the option of allowing the rotor to coast to a smooth stop and visually inspecting the contents of the individual cuvetts.

Table 2.12. Direct antiglobulin testing with the miniature CFA:
Loading sequence of sample and reagents

Cuvet number	Step 1	Step 2
	Sample carousel	Reagent carousel
1	Saline	Saline
2	Ortho positive control	Coombs antiserum
3	Ortho negative control	Coombs antiserum
4-17	Patient samples (whole blood)	Coombs antiserum

Table 2.13. Antibody screening with the miniature CFA:
Loading sequence of samples and reagents

Cuvet number	Step 1	Step 2	Step 3	Step 4
	Sample carousel	Reagent carousel	Reagent carousel	Reagent carousel
1	Saline	Saline	Saline	Coombs antiserum
2	Recipient's serum	Identigen-1	Saline	Coombs antiserum
3	Recipient's serum	-2	Saline	Coombs antiserum
4	Recipient's serum	-3	Saline	Coombs antiserum
5	Recipient's serum	-4	Saline	Coombs antiserum
6	Recipient's serum	-5	Saline	Coombs antiserum
7	Recipient's serum	-6	Saline	Coombs antiserum
8	Recipient's serum	-7	Saline	Coombs antiserum
9	Recipient's serum	-8	Saline	Coombs antiserum
10	Anti-D serum	-8	Saline	Coombs antiserum
11	Anti-D serum	-8	Saline	Coombs antiserum
12	Anti-D serum	Selectogen I	Saline	Coombs antiserum
13	Anti-D serum	Selectogen II	Saline	Coombs antiserum
14	Recipient's serum	Recipient's blood	Saline	Coombs antiserum
15	--	--	Coombs positive cells	Coombs antiserum
16	--	--	Coombs negative cells	Coombs antiserum
17	--	--	Recipient's blood	Coombs antiserum

Table 2.14. Cross matching with the miniature CFA:
Loading sequence of samples and reagents

Cuvet number	Step 1	Step 2	Step 3	Step 4
	Sample carousel	Reagent carousel	Sample carousel	Reagent carousel
1	Saline	Saline	Saline	Saline
2	Recipient's blood	Recipient's serum	--	Coombs antiserum
3	Donor blood-1	Recipient's serum	--	Coombs antiserum
4	-2	Recipient's serum	--	Coombs antiserum
5	-3	Recipient's serum	--	Coombs antiserum
6	-4	Recipient's serum	--	Coombs antiserum
7	-5	Recipient's serum	--	Coombs antiserum
8	-6	Recipient's serum	--	Coombs antiserum
9	--	--	Recipient's blood	Coombs antiserum
10	--	--	Donor blood-1	Coombs antiserum
11	--	--	-2	Coombs antiserum
12	--	--	-3	Coombs antiserum
13	--	--	-4	Coombs antiserum
14	--	--	-5	Coombs antiserum
15	--	--	-6	Coombs antiserum
16	--	--	Coombs negative cells	Coombs antiserum
17	--	--	Coombs positive cells	Coombs antiserum

(Direct antiglobulin test). The direct antiglobulin test is used to detect antibodies already adsorbed or coated on the red cell surface. The procedure used to perform this test on the miniature CFA is as follows:

- (1) Set the turntable of the SRL in the multiple-sample:single-reagent mode of operation.
- (2) Load aliquots of samples as indicated in step 1 of Table 2.12.
- (3) Place rotor into analyzer, transfer cells to cuvetts, and wash them in situ.
- (4) After final aspiration, place rotor back onto turntable of SRL and load aliquots of reagent as indicated in step 2 of Table 2.12.
- (5) Place rotor into analyzer, and transfer and mix the contents of the individual chambers into their respective cuvetts by: (a) acceleration to 4000 rpm followed by dynamic braking,³ (b) three cycles of acceleration to 3000 rpm followed by dynamic braking, (c) centrifugation for 60 sec at 4000 rpm followed by dynamic braking, and (d) three cycles of acceleration to 3000 rpm followed by dynamic braking. (Note: under these conditions, the nonagglutinated cells are resuspended without disturbing the agglutinated cells packed against the outer cuvet wall.)
- (6) At a rotor speed of 500 rpm, determine the absorbance of the contents of each individual cuvet at 520 nm.
- (7) If desired, incubate the rotor at room temperature and repeat analysis starting at step 5(c).
- (8) Determine the reactivity of the contents of each cuvet. An absorbance greater than 1.0 is considered negative (nonagglutination); less than 1.0 is considered positive (agglutination).

(Antibody screening - indirect antiglobulin test). The indirect test is most frequently used to detect antibodies in the serum of a pregnant woman, or to learn whether antibodies are present in the recipient of a transfusion (e.g., in the compatibility test). The procedure used to perform this test on the miniature CFA is as follows:

- (1) Set the turntable of the SRL in the multiple-sample:multiple-reagent mode of operation.
- (2) Load aliquots of sample as indicated in step 1 of Table 2.13.
- (3) Place rotor into analyzer, transfer cells to cuvetts, and wash them in situ.
- (4) Return rotor to SRL, and load reagents as indicated in step 2, Table 2.13.
- (5) Return rotor to analyzer, and transfer and mix the contents of the individual chambers into their respective cuvetts by: (a) acceleration to 4000 rpm followed by dynamic braking; (b) three cycles of acceleration to 3000 rpm and dynamic braking; and (c) centrifugation for 60 sec at 4000 rpm, and reduction of the speed to 500 rpm without braking.
- (6) Determine the absorbances of the solutions contained in the individual cuvetts at 410 nm. (Note: an absorbance greater than 1.0 is considered to be indicative of hemolysis.)
- (7) Recentrifuge the rotor for 60 sec at 4000 rpm and dynamically brake, followed by three cycles of acceleration to 3000 rpm and dynamic braking.
- (8) At a rotor speed of 500 rpm, determine the absorbances of the solutions contained in the individual cuvetts at 520 nm. (Absorbances less than 0.8 were considered to be indicative of agglutination, while absorbances greater than 1.1 were assumed to be indicative of nonagglutination.

- (9) Incubate the rotor at 37°C for 15 min. Check for evidence of hemolysis and agglutination as in steps 6 and 8.
- (10) Centrifuge the rotor at 4000 rpm for 1 min and then reduce the speed to 500 rpm without braking.
- (11) Aspirate the supernate.
- (12) Return rotor to SRL, and load aliquots of solutions as indicated in step 3 of Table 2.13.
- (13) Return rotor to analyzer, transfer aliquots into their respective cuvetts, and wash cells.
- (14) After the last supernate aspiration step, perform the direct anti-globulin test as described earlier in this report.

(Cross matching). The test between a prospective recipient of a blood transfusion and his proposed donor (or donors) is known as the cross-match, or compatibility, test. The procedure used to perform this test on the miniature CFA is as follows:

- (1) Set the turntable of the SRL in the multiple-sample:multiple-reagent mode of operation.
- (2) Load aliquots of the recipient and donor bloods as indicated in step 1, Table 2.14.
- (3) Place rotor in analyzer, and transfer aliquots into their respective cuvetts.
- (4) Wash cells in situ.
- (5) Return rotor to SRL and load aliquots of the various sera as indicated in step 2, Table 2.14.
- (6) Return rotor to analyzer and transfer aliquots into their respective cuvetts.

- (7) Check for hemolysis and agglutination as described in steps 6 through 8 of the antibody screening procedure.
- (8) Incubate the rotor for 30 min at 37°C, and recheck for hemolysis and agglutination.
- (9) Centrifuge the rotor at 4000 rpm for 1 min and then reduce the speed to 5000 rpm without braking.
- (10) Aspirate the supernate.
- (11) Return rotor to SRL, and load aliquots of blood and control cells as indicated in step 3 of Table 2.14.
- (12) Return rotor to analyzer, and transfer the aliquots of cells into their respective cuvetts.
- (13) Wash cells in situ.
- (14) Perform the direct antiglobulin test. If no hemolysis or agglutination is observed at any stage and the sensitized cells are agglutinated, the cross match is considered to be compatible.

Results. Initial experiments with the direct antiglobulin test were standardized with 10- μ l aliquots of washed cell suspensions in saline (approximately 50 ml/dl). A previous investigation¹⁹ had utilized 4.0% PVP in 0.9% saline to enhance hemagglutination reactions. Using hand-loaded rotors and washed cell suspensions, we investigated the effects of varying the amounts of antiglobulin serum and PVP on the performance of the direct antiglobulin test. As shown in Fig. 2.15, the presence of PVP made little difference in the results.

When these results were applied to samples loaded automatically with the rotor loading station, we found that the distinction between known positive and negative cells was not reproducible. We discovered that some

Fig. 2.15. Influence of the amounts of antiglobulin serum and polyvinylpyrrolidone on the performance of the direct antiglobulin test.

cells were being aspirated during the cell washing procedure, which caused the positive and negative cells to be mixed together in the cuvetts when saline was injected dynamically for the next wash. This behavior was attributed to the size of the cell mass in each cuvet. Thus, the effect of reducing the sample size on the performance of the direct antiglobulin test was investigated; concurrently, the limit of dilution of the antiglobulin serum giving an effective distinction between known positive and negative cells was determined. Figure 2.16 clearly shows that a sample size of 6 μ l is quite adequate for the performance of the direct antiglobulin test under these conditions.

Another possible factor contributing to the mixing of cells from different cuvetts was the centrifugal speed at which the supernate was aspirated. To investigate the importance of this factor, a series of experiments was conducted in which aspiration was accomplished with the rotor spinning at various rates from 500 to 800 rpm. Wash saline was loaded into the rotor with the rotor loading station to eliminate variations in the total volume added, or its distribution among the cuvetts, as might occur with dynamic injection. Blank absorbances were determined by centrifuging the rotor at 4000 rpm for 1 min and, without braking, reducing the speed to 500 rpm to determine the absorbance at 520 nm. Test absorbances were determined subsequently after accelerating to 3000 rpm and braking several times before determining the absorbance at 500 rpm and 520 nm. The data obtained in these experiments are shown in Figs. 2.17-2.20. The bars represent the 95% confidence limits of the absorbance values. Although the loss of absorbing substance was apparently reduced at the higher centrifugal speeds, it was not eliminated. Furthermore, in an experiment utilizing the

Fig. 2.1.6. Effect of sample size on the performance of the direct antiglobulin test.

Fig. 2.17. Apparent change in cell mass during washing.
Aspiration at 500 rpm.

Fig. 2.18. Apparent change in cell mass during washing.

Aspiration at 600 rpm.

Fig. 2.19. Apparent change in cell mass during washing.

Aspiration at 700 rpm.

Fig. 2.20. Apparent change in cell mass during washing.
Aspiration at 300 rpm.

same positive and negative cells, the results of the direct antiglobulin test were quite definitive even though aspiration of the wash supernate was done with the rotor spinning at 500 rpm. Therefore, all subsequent cell washings were aspirated at 500 rpm.

The effect of the length of time of centrifugation at 4000 rpm after transfer and mixing of the Coombs positive control cells with the anti-globulin serum is shown in Fig. 2.21. (Again, the bars represent the 95% confidence limits.) Since there is little to distinguish one condition from another, subsequent experiments were standardized on a 60-sec centrifugation at 4000 rpm.

Serial dilutions of a sample of human blood were made with 0.9% saline in order to evaluate the determination of hemolysis, which is a potential consequence of antigen-antibody interactions. Six-microliter aliquots of each of these dilutions were transferred into the cell washing rotor in triplicate with the rotor loading station. After the cells had been washed¹⁹ and the supernate from the final wash had been aspirated, the rotor loading station was utilized to load the rotor with saline. Subsequently, the rotor was centrifuged at 4000 rpm for 45 sec. Without braking, the speed was reduced to 500 rpm and blank absorbances were determined at three wavelengths (410, 520, and 575 nm). The saline supernate was then aspirated, and the rotor loading station was utilized to place distilled water in the rotor. Subsequently, the rotor was accelerated to 4000 rpm and braked three times. Afterwards, about 5 min was allowed for lysis to occur. Finally, the rotor was centrifuged at 4000 rpm for 45 sec and, without braking, the speed was reduced to 500 rpm. The absorbances at the same three wavelengths were determined (see Figs. 2.22-2.24).

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Fig. 2.21. Effect of time of centrifugation at 4000 rpm on results of the direct antiglobulin test.

Fig. 2.22. Detection of hemolysis at 410 nm.

Fig. 2.23. Detection of hemolysis at 520 nm.

Fig. 2.24. Detection of hemolysis at 575 nm.

The results of these experiments lead us to conclude that the Coombs test could be adapted to the miniature CFA. Consequently, we plan to obtain blood samples from patients in area hospitals, perform the assay on them, and compare the resulting data with those obtained from conventional methods.

2.4.2 Environmental analysis

A reliable, portable analyzer would be useful in several applications in the environmental sciences. However, various chemical methodologies would have to be adapted or developed for use with such an analyzer before it could be utilized in an environmental situation. A chemical development program has been initiated to supply the appropriate methodologies.

Determination of ATP in environmental samples. The production of adenosine triphosphate (ATP) is a common goal of both anaerobic and aerobic metabolic activity. ATP is a direct indication of life; indeed, the assay of ATP has been proposed as a potential tool for the detection of exo-terrestrial life.²⁴ Determination of ATP in environmental samples is frequently utilized as a measure of microbiological activity and microbial biomass.^{24,25} The exact ATP content per cell varies with the microorganism tested and with the growth stage of particular organisms, ranging from approximately 0.2 to 50×10^{-9} μg of ATP per cell.

A number of difficulties have been encountered with the assay of ATP. These include methodological problems in the techniques used to isolate ATP from various substrates and to detect and quantitate ATP, as well as the overall problem of ecological interpretation of the data obtained. Currently, the most frequently utilized assay procedure involves the luciferin (LH_2)--luciferase (E)--ATP chemiluminescent reaction:²⁴

2

where

AMP = adenosine monophosphate,

PP = pyrophosphate.

This reaction is inherently very sensitive (i.e., 0.01 to 1 pg of ATP can be detected with the most sophisticated instrumentation available²⁴). However, the reaction is dependent on magnesium concentration, temperature, oxygen availability, pH, and cationic interference. Also, measurement of the emitted radiation is a complex procedure. With conventional techniques, the time required for the mixing of sample and reagent often makes the initial reading possible only after the maximum light emission has occurred. In addition, response of the light emission is variable. Depending on several factors, such as the substrate extracted, the extraction procedure used, etc., peak emission may occur 3 to 20 sec after mixing is initiated. The lack of reproducibility of the system response requires the use of internal standards. If one employs sequential analysis systems, the period required to analyze numerous samples becomes critical, adding constraint on the stability of the ATP extracts. One of the more serious disadvantages of the chemiluminescent procedure is that ADP as well as ATP may react with the luciferin-luciferase system to produce light.

We have been working in collaboration with the Environmental Sciences Division of ORNL to develop and evaluate alternative procedures for assaying ATP. We have adapted a commercial kit (ATP Stat-Pack, Calbiochem, Los Angeles, California 90063) for use with the miniature CFA. The chemical

reaction involved is analogous to that currently in routine use in the ORNL Health Division clinical laboratory for the determination of glucose:²⁶

where NADPH is the reduced form of NADP, nicotinamide adenine dinucleotide phosphate.

The production of NADPH, monitored at 340 nm, is stoichiometrically related directly to the ATP concentration in the sample. In addition to being an absolute method, the reaction sequence is highly specific for ATP. Preliminary investigations indicated excellent linearity (Fig. 2.25) and precision (CV <1%). The sensitivity of the method appears adequate for the determination of approximately 0.2 to 8×10^{-4} M ATP (0.5 to 40 µg of ATP). Greater sensitivity, if required, can be obtained by adding the enzyme 6-phosphogluconate dehydrogenase to the reagent. This enzyme reacts with one of the products in reaction (11) to yield additional monitored species:

The multicuvet, parallel-analysis feature of the CFA will permit the simultaneous determination of numerous samples; data reduction is automatically performed with the analyzer system.

Specific assays. Several specific assays will be developed. Initially, we are concentrating on the methods to be used to assay various inorganic nutrients commonly found in water.

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Fig. 2.25. Determination of ATP via Calbiochem Stat-Pack.

(Evaluation of commercial kit procedures for adaptation to the Centrifugal Fast Analyzer). Recently, several commercial suppliers of instruments and chemicals have developed "kit" procedures for the determination of inorganic constituents in water. We have examined the utility of some of these kits from Hach Chemical Company, Ames, Iowa, for use in environmental analyses with the CFA. The kit, as supplied by the manufacturer, was evaluated (macro test), and the reagents provided were then adapted for use with the CFA (micro test). The conditions and results of the tests can be described as follows:

(1) Ammonia (Hach Ammonia Nitrogen Test, Catalog No. 12524-00)

Reagent: Nessler's reagent

Basis of test: Direct nesslerization reaction

Optimum wavelength: 400 nm (ΔA increases with decreasing wavelength, but absorbance of the blank is excessive at <300 nm)

Suitable for use with CFA? Yes

Concentration range: 0 to 3 ppm of ammonia-nitrogen ($\text{NH}_3\text{-N}$)

Comments: Macro test. Determination by comparison with permanent color standards provided in kit is semiquantitative at best. When an aliquot of macro is run on the CFA, the test is found to be linear only to 3 ppm of $\text{NH}_3\text{-N}$ at 400 nm. The chemical reaction is known to have many interferences (including many classes of organic compounds, and ions such as Mg, Mn, Fe, etc.); also, the hue (wavelength of maximum absorbance) of the reaction depends on the ammonia concentration.

(2) Nitrate (Hach Nitrate-Nitrite Test Kit, Model NL-12)

Reagent: NitraVer[®] V

Basis of test: Reduction of nitrate to nitrite by cadmium filings,
followed by reaction of nitrite

Optimum wavelength: 380 nm, with broad tailing at longer wavelengths
(high blank absorbance at 380 nm)

Suitable for use with CFA? No

Concentration range: 0 to 50 ppm of nitrate-nitrogen (claimed by kit)

Comments: Macro test. Permanent color standards are inaccurate for
concentrations of NO_3^- -N in excess of 30 ppm.

Micro test. Cadmium filings cannot be equally distributed
among cuvetts of the CFA; must perform the macro test and
take an aliquot for the CFA. At 400 nm, results are non-
linear above 20 ppm of NO_3^- -N. Results are somewhat
erratic and sensitivity is poor, suggesting incomplete and
nonreproducible reduction of nitrate.

(3) Nitrite

Reagent: NitriVer[®] III

Basis of test: Diazotization-coupling reaction

Optimum wavelength: 520 nm

Suitable for use with CFA? Yes

Concentration range: 0 to 0.5 ppm of nitrite-nitrogen

Comments: Micro test. Dissolve one reagent pack in 1.6 ml of distilled
water. For test, load rotor with 45 μl of reagent and 90 μl
of sample. Reaction is essentially complete in 500 sec.
Results are linear to 0.5 ppm of NO_2^- -N; relative standard
error of the calibration curve is <1%.

(4) Phosphate (Hach Phosphate Test Kit, Catalog No. 12522-00)

Reagent: PhosVer[®] III

Basis of test: Formation of molybdenum blue by reduction of
heteropoly acid

Optimum wavelength: 560 nm

Suitable for use with CFA? See below

Concentration range: 0 to 5 ppm of phosphate

Comments: Macro test. Permanent color standards provided with test
are wrong hue for reaction color. Five-milliliter test
cube was found to contain 5.5 ml.

Micro test. Reagent is unstable once it has been dissolved
in water, possibly due to oxidation of the reducing agent
by air. Reagent may be suitable if preloaded in rotor as
dry pellet. Color development is rapid in macro test
(complete in ≤ 3 min), and linearity is excellent over the
range of phosphate concentration 0 to 5 ppm when aliquots
of macro test are observed on the CFA at 560 nm.

(5) Silica (Hach Silica Test Kit, Model SI-3)

Reagents: Silica reagents 1, 2, and 3

Basis of test: Formation of molybdenum blue by reduction of heteropoly
acid in the presence of oxalic acid to minimize inter-
ference due to phosphate

Optimum wavelength: >600 nm

Suitable for use with CFA? See below

Concentration range: 0 to 40 ppm of SiO_2

Comments: Macro test. Color comparator of kit appears to be acceptably accurate; precision of $\pm 10\%$ appears possible with practice.

Micro test. The number and timing of the additions of reagents are somewhat awkward. It is difficult to take advantage of the greater molar absorptivity of the complex, which occurs at wavelengths > 650 nm, since the phototube of the CFA (R300, Hamamatsu Corporation) has poor response characteristics for radiation of such a long wavelength.

(6) Sulfate (Hach Sulfate Test Kit, Model SF-1)

Reagent: SulfaVer[®] IV

Basis of test: Turbidimetric determination of colloidal BaSO_4

Optimum wavelength: 340 nm, 420 nm (greatest scatter at short wavelengths)

Suitable for use with CFA? Yes, for high concentrations

Concentration range: 50 to 200 ppm of SO_4^{2-}

Comments: Macro test. The determination is performed on a simple extinction-type turbidimeter that has been calibrated for 50 to 200 ppm of sulfate. For this type of turbidimeter, the solution height for extinction ($1/D$) is reciprocally related to the SO_4^{2-} concentration (i.e., similar to a Jackson candle turbidimeter), as shown in Fig. 2.26. In practice, the accuracy and reproducibility of the method are quite modest. The volume requirement is 18 ml.

Micro test. The reagent, which is easily dissolved in a small volume of distilled water, can be conveniently loaded in the rotor. Results obtained in a micro test (see

Fig. 2.26. Turbidimetric determination of sulfate. Comparison of experimental data using the CFA with the theoretical calibration using an extinction-type turbidimeter.

Fig. 2.26) show that the experimental data are in fairly close agreement with the theoretical calibration curve for the reagent; the useful analysis range is 30 to 150 ppm of SO_4^{2-} . The precision is modest.

(Determination of sulfate by catalysis of the zirconium--methylthymol blue reaction). The analytical determination of small quantities of sulfate ion is important in many fields, especially in connection with problems of air- and water-quality measurements. Several research projects concerned with sulfate ion determination are in progress at ORNL. The Environmental Sciences Division is engaged in monitoring the sulfate ion input-output of the Walker Branch watershed. The Analytical Chemistry Division, in collaboration with the Environmental Protection Agency, is involved in the development and evaluation of novel methods and reagents for the analysis of sulfate ion. We are currently working in cooperation with both of these divisions, utilizing the broad analytical capabilities of the CFA.

Numerous difficulties are associated with existing methodologies for determining sulfate ion concentrations. The few direct methods available primarily involve turbidimetric or nephelometric determination of insoluble complexes of sulfate with barium ion (see above) or organic bases.²⁷⁻²⁹ These methods usually have only modest sensitivities and reproducibilities. The organic-based reagents, although much more sensitive than barium salts, are subject to many interferences from species such as halides and phosphate ion. Most spectrophotometric techniques, however, are indirect measurements involving the precipitation of an insoluble salt of barium and the subsequent determination of either the excess barium ion or its counterion,³⁰ or the release of counterion.³¹ These methods are also subject to many interferences.

Recently, Hems et al. introduced a direct kinetic spectrophotometric method for determining sulfate based on the catalytic effect of sulfate ion on the zirconium--methylthymol blue reaction.³² This method, which is quite sensitive (i.e., it permits detection of sub-ppm reaction concentrations of sulfate and greater), can be made relatively selective. Sulfate ion catalyzes the depolymerization of zirconyl species in acidic solution, and the free zirconium ion then reacts with methylthymol blue indicator. The age of the stock zirconium solution in weak acid determines the degree of polymerization of the zirconyl species; thus it has a significant effect on the kinetics of the reaction.

In the procedure of Hems et al.,³² the age of the stock zirconium solution is 2 to 10 days and the reaction concentration 1×10^{-4} M. The reaction is initiated, and the analytical step consists of making a single observation after a 60-min development period (at long times, the absorbance becomes independent of SO_4^{2-} concentration).

Our goals were to evaluate the potential of the method as a true kinetic assay and to take advantage of the degree of automation available with the miniature CFA system.³ Figure 2.27 shows a plot of absorbance-vs-time data for the reaction as a function of sulfate ion concentration over the range 0 to 16 ppm (0 to 320 ng of SO_4^{2-}). Conditions are established so that, after a short induction period, the reaction rate is constant over an extended time interval. At low concentrations of sulfate and zirconium, the reaction rate is pseudo-first-order with respect to sulfate ion (see Fig. 2.28). The precision of the measurement of the reaction rate is generally 1 to 2%. The linearity of the absorbance-vs-time data over such extended periods reduces the effect of reagent age on the analysis since

Fig. 2.27. Determination of sulfate by catalysis of the zirconium--methylthymol blue reaction. Age of stock zirconium, 4 days; reaction concentration of zirconium, 1.4×10^{-4} M.

Fig. 2.28. Determination of sulfate by catalysis of the zirconium--methylthymol blue reaction. Effect of zirconium reaction concentration.

there will remain a time interval over which all data are linear. We have used, in parallel, reagents prepared 4 days apart and found intervals throughout which all the data were linear; however, we confirmed that the absolute rate of the reaction does vary with reagent age, and that it is necessary to run standards with samples. The throughput advantage of the multicuvet analyzer, on the other hand, still permits a large number of samples to be processed simultaneously. Work is in progress to further evaluate the method, assess the effects of chemical interferents and procedures for their removal, and further automate the analysis.

(Enzymatic determination of ammonia). Ammonia is present in low concentrations in many raw-water sources.³³ A central species in the nitrogen cycle,³⁴ it may either be utilized as a nutrient or may accumulate as a product of microbiological activity.

We have examined the utility of an enzymatic procedure for determining the ammonia content of natural-water samples. This procedure is based on the following reaction:

The reaction is monitored by the decrease in absorbance at 340 nm due to the consumption of NADH (Fig. 2.29). In the presence of ADP as a cofactor, the reaction is rapid and highly selective for ammonia.³⁵ In contrast, the direct nesslerization procedure is known to have numerous chemical interferences, as discussed in an earlier subsection.

Da Fonseca-Wollheim³⁵ has examined the effects of pH, cofactors, ionic strength, and substrate concentration on the determination of ammonia by L-glutamate dehydrogenase. He has found this enzyme to be the most stable

Fig. 2.29. Absorbance-vs-time data for the enzymatic determination of ammonia via L-glutamate dehydrogenase.

and to exhibit the highest activity under conditions of relatively high ionic strength and a pH of 8.6 in the presence of ADP and NADH. In contrast, many other workers have been using the less-favorable conditions of lower pH and no stabilizing cofactor ADP in their reagent.^{36,37} At lower pH levels (e.g., 7.0 to 7.5), high concentrations of NADH as well as the product NAD inhibit the reaction and cause denaturation of the enzyme.³⁸ Under these conditions, we have found that NADPH provides greater enzyme stability and results in greater reaction rates than does NADH. The presence of ADP, however, stabilizes the enzyme and permits analysis at the pH that is optimum with either coenzyme.

We have approximated the conditions specified by Da Fonseca-Wollheim (Table 2.15). The enzymic reagent is stabilized sufficiently to be concentrated for use with the automatic loading station, thus simplifying the procedure. The analysis may be performed using either an initial-rate

Table 2.15. Initial reaction concentrations for determination of $\text{NH}_3\text{-N}$ in the sample concentration range of 0 to 10 ppm

Constituent	Initial reaction concentration
NADH	$3.5 \times 10^{-4} \text{ M}$
ADP	$1.6 \times 10^{-3} \text{ M}$
α -ketoglutarate	$1.6 \times 10^{-2} \text{ M}$
L-glutamate dehydrogenase	80 mg of protein (~4600 IU per liter)
NH_3	0 to 3.2 ppm (0 to 0.5 μg) of $\text{NH}_3\text{-N}$
pH	8.6

(Fig. 2.30) or a two-point, fixed-time kinetic approach²⁶ (Fig. 2.31). The latter has the advantage of being an absolute method, not requiring the continuous use of standards (see Table 2.16). The precision of the two-point, fixed-time analysis is <1%; the relative standard error of the data (estimated from linear least-squares regression analysis) is similar.

(Simultaneous determination of silica and phosphate in water from a single experiment). Silica and orthophosphate, two of the major inorganic nutrients in natural waters,³⁹ must frequently be determined in the presence of each other. The colorimetric determination of these species is usually achieved through the formation of heteropolymolybdates. Often, to enhance the sensitivity of the assays, the (yellow) heteropolymolybdates are subsequently chemically reduced to yield a "heteropoly blue" or "molybdenum blue" complex.^{40,41} Since any of a number of species [including P(V), Si(IV), As(V), Ge(IV), Ti(IV), and Zr(IV)] may be the central ion of the hetero-12-molybdates,⁴² several procedures have been developed to increase the selectivity of the analytical reaction.

Under the usual conditions for determining phosphate as the molybdenum blue complex, soluble silica is generally not a serious interferent.⁴³ Arsenate, on the other hand, does interfere when present at a sufficient concentration. Such interference may be eliminated by reduction of arsenate to arsenite⁴⁴ or by selective extraction techniques.⁴⁵ Likewise, phosphate may be a serious interferent in the determination of silica; however, the addition of tartaric acid almost completely prevents formation of the arsenic and phosphorus complexes while leaving the silicon complex largely unaffected.⁴⁶ Kinetic differentiation between silica and phosphorus is also possible.^{47,48} Ingle and Crouch⁴⁸ have determined silica by

Fig. 2.30. Enzymatic determination of ammonia by an initial rate approach.

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Fig. 2.31. Enzymatic determination of ammonia by two-point kinetic analysis.

Table 2.16. Enzymatic determination of ammonia-nitrogen

NH ₃ -N taken (ppm)	NH ₃ -N found ^a (ppm)	Percent error
2.0	1.983 ± 0.015 ^b	-0.85
4.0	4.017 ± 0.045	+0.43
6.0	5.967 ± 0.050	-0.55
8.0	7.967 ± 0.029	-0.41
10.0	10.06 ± 0.029	+0.60

^aFrom the formula:

$$C, \text{ ppm} = (\text{SF})(\Delta A_{340}) ,$$

where SF is the theoretical substrate factor.

^bMean ± standard deviation of three replicates.

measuring the rate of formation of the unreduced heteropoly acid and have subsequently determined phosphate in the same sample by measuring the rate of reduction to the molybdenum blue complex.

Conditions are present here that will permit both a kinetic analysis for molybdate-reactive silicon and an estimation of phosphate concentration from data obtained in a single experiment. These analyses are discussed in the context of a possible "nutrient rotor" concept, whereby several important inorganic constituents could be determined simultaneously by use of a miniature CFA.^{4,49}

Centrifugal Fast Analyzers have demonstrated considerable utility for equilibrium (end-point) as well as kinetic photometric or luminescence analyses.^{26,50-52} Fast Analyzers are multicuvvet instruments capable of analyzing a number of samples and standards in parallel. Since all reactions are initiated and observed essentially simultaneously under similar reaction conditions, including temperature, many of the sources of variability in the relationship between reaction rate and analyte concentration are eliminated.⁵² Relatively rapid mixing (3 to 6 sec) and real-time computer control of the analyzer make it possible to obtain a series of accurately timed, early analog measurements during the first few seconds of the reaction. Reaction rates approaching the initial rate can then be computed by using the data processing unit and an extrapolation subroutine. Standards and samples can be processed simultaneously.

To date, most applications of kinetic analysis by CFAs have been in the field of clinical chemistry and have largely been concerned with enzymatic reactions. Recently, however, nonequilibrium fixed-time analyses for such inorganic species as sulfur dioxide⁵³ and ozone⁵⁴ have been reported. In

addition, Goldstein et al. have successfully used the variable-time kinetic analysis for selenium.⁵⁵

Our procedure for determining both silica and phosphate in water from a single experiment is detailed in the paragraphs that follow.

A. Experimental

(1) Apparatus:

The miniature CFA, as modified to give a multipurpose optical system, is described elsewhere.⁵⁶

(2) Reagents:

The reagent used in our work was prepared by dissolving 9.35 g of $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ in distilled water, adding 8 ml of concentrated H_2SO_4 , and diluting to 1 liter with distilled water.

A stock silicon solution, containing approximately 100 ppm of silicon, was prepared by dissolving 0.508 g of $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$ in distilled water, adjusting the pH to approximately 3 [for maximum stability of monomeric (hence, molybdate-reactive) silicon units⁴²], and diluting to 500 ml. For accurate work, the solution should be standardized gravimetrically.⁴⁸ However, the molybdate-reactive silicon concentration of the solution was also checked via a kit procedure (Silica test kit model SI-3, Hach Chemical Company, Ames, Iowa 50010) based on the production of a heteropoly blue complex in the presence of oxalic acid to reduce phosphate interference. Using the permanent color standard comparator provided with the kit, the mean estimate of the stock silicon content was found to be 102 ppm ($\pm 10\%$).

A stock phosphate solution, containing 500 ppm of phosphate, was prepared by dissolving 0.7165 g of KH_2PO_4 in distilled water and diluting to 1000 ml.

(3) Procedure:

Fifty microliters of sample and 80 microliters of reagent were loaded into the inner and outer loading ports, respectively, of a standard design rotor.^{4,49} The temperature of the rotor and its contents was brought to $30.0 \pm 0.1^\circ\text{C}$; then the contents were transferred and mixed via rapid acceleration of the rotor to 4000 rpm, followed by dynamic braking. After a 10-sec delay, the reaction was monitored at 1-sec intervals for up to 48 intervals. At each interval, data from five successive passes of the cuvet over the stationary phototube (one pass every 80 msec) were averaged and stored by the computer. The absorbance of each cuvet at each time interval was computed, and the data acquisition system was linked with a linear least-squares regression analysis routine to automatically compute the slope and intercept of the absorbance-vs-time data.

B. Results and Discussion

(1) Determination of reactive silica:

Our procedure, which is a modification of that described by Ingle and Crouch,⁴⁸ is different in several important respects. For example, we modified the reagent to permit a more rapid analysis. Also, we found that the sensitivity of the procedure was approximately four times as great when monitored at 340 nm; therefore, we used this wavelength instead of the 400 nm stipulated by Ingle and Crouch. With the modified reagent, we found that reactive silica could be determined either by an initial-rate kinetic approach (rate estimated by the slope of the progress curve over the time interval 10 to 15 sec after initiation of the reaction) or by an end-point absorbance measurement. Each approach yielded a calibration curve that was linear for a broad range of silica concentrations and had a

near-zero intercept. However, when the effect of phosphate interference was investigated, the superiority of the initial-rate procedure was clearly established. Hargis⁴⁷ has studied the effect of extraneous ions on the determination of silicon by an initial-rate approach and has found PO_4^{3-} , AsO_4^{3-} , Fe^{3+} , and F^- to be the most serious interferents.

Table 2.17 presents data showing the effect of selected chemical interferents on the determination of silicon by our initial-rate procedure. Phosphate, which reacts very rapidly with the modified reagent and thus contributes little to the slope of the data used to determine silicon (see Fig. 2.32), produces very little interference. Arsenate, which reacts very slowly with the reagent, does not interfere significantly at levels likely to be encountered in environmental water samples. In contrast, when the absorbance at equilibrium is used to compute silicon content, 10 ppm of PO_4^{3-} produces an error of approximately 75% in the determination of 5 ppm of silicon.

The linearity of the initial-rate (slope) calibration curve extends to at least 40 ppm of silicon. The sensitivity of the assay (estimated as twice the standard deviation of the measurement of the initial rate of a 5-ppm sample, divided by the linear least-squares slope of the calibration curve) is 0.2 to 0.4 ppm. Thus the assay appears to be adequate for the determination of silicon in a variety of raw-water sources.⁵⁷ The within-run reproducibility of the measurement of the reaction rate is 1 to 3%. The relative standard error of the calibration curve (as computed by linear least-squares regression analysis) averages about 2.5%. The day-to-day reproducibility of the least-squares slope of the calibration curve (determined over a two-week interval) is about 3%.

Table 2.17. Effect of chemical interferences on the determination of silicon by the initial-rate approach, using modified Ingle and Crouch reagent

Concentration taken (ppm)				Concentration found (ppm)	Percent error
Si	PO_4^{3-}	AsO_4^{3-}	Cl^-	Si	
10.0	3.5	--	--	9.97	-0.3
10.0	5.0	--	--	10.06	+0.6
10.0	7.5	--	--	10.05	+0.5
10.0	10.0	--	--	10.02	+0.2
10.0	12.5	--	--	9.87	-1.3
10.0	--	5.0	--	10.10	+1.0
10.0	--	--	550	10.03	+0.3
0.0	50.0	--	--	0.07	--
0.0	--	20.0	--	0.40	--

Fig. 2.32. Simultaneous determination of silicon and phosphate. The slope of the absorbance-vs-time data is a measure of the silicon content of the sample, and the absorbance intercept is a measure of the phosphate content.

We obtained a sample of raw Mississippi River water and passed it through a 0.45- μ filter. This sample, as well as a "spiked" sample (10 ppm of silicon added), were analyzed; after correction for dilution, the recovery was calculated to be 99.6%.

(2) Simultaneous determination of phosphate and silica in the same reaction:

Under the conditions selected, both silicon and phosphate react rapidly; however, it is known that the reaction with phosphate proceeds more rapidly.^{48,58} In investigating the possibility of using a differential kinetic assay for determining silicon and PO_4^{3-} , it was noted that the PO_4^{3-} reaction was essentially complete prior to the measurement of the initial slope for the determination of silicon (see Fig. 2.32). From the phosphate interference study, it was noted that the absorbance-intercept from the linear least-squares analysis of the initial slope varied precisely with the phosphate content of the sample. In the presence of silicon only, the absorbance-intercept is relatively small (see Fig. 2.32). In the absence of silicon, the absorbance-intercept of the slope data for phosphate standards over the concentration range 2.5 to 12.5 ppm corresponded to 96 to 100% of the mean absorbance, indicating that the intercept is a measure of the equilibrium absorbance for the phosphate reaction. For PO_4^{3-} in the range 2.5 to 12.5 ppm, the within-run reproducibility of the intercept is 2 to 6%, and the relative standard error estimated from the least-squares regression analysis of absorbance-intercept vs PO_4^{3-} concentration is approximately 2.5% (correlation coefficient ~ 0.998 , $p < 0.005$). The calibration curve is linear to at least 50 ppm (highest concentration investigated). In the presence of extraneous ions (see Table 2.18), results are most reliable for phosphate concentrations greater than about 2 to 3 ppm.

Table 2.18. Effect of chemical interferents on the determination of PO_4^{3-} by the absorbance intercept of the initial-slope procedure

PO_4^{3-}	Concentration taken (ppm)		Concentration found (ppm)
	Si	AsO_4^{3-}	PO_4^{3-}
2.5	10.0	--	2.73
5.0	10.0	--	5.26
7.5	10.0	--	7.47
--	20.0	--	0.49
--	40.0	--	3.31
--	--	20.0	2.82

The literature contains references to several other methods for the determination of phosphate by use of a CFA. Daly and Ertingshausen⁵⁹ introduced a direct method for determining phosphate at 340 nm as the unreduced heteropoly acid. Their molybdate reagent contained a surfactant, Tween-80 (a polyoxethylene sorbitan monooleate). These researchers were investigating phosphate in serum, and the surfactant was reported to serve as a solubilizing agent for serum protein. They also found it necessary to employ an end-point analysis since they had noted that protein had an accelerating effect on the reaction, thus precluding an initial-rate approach.⁵⁹ More fundamentally, other researchers⁶⁰ have reported that, under the conditions used by Daly and Ertingshausen, no reaction occurs when Tween-80 is omitted from the reaction mixture. This fact has been confirmed in our laboratory; in addition, we have noted that increasing the amount of Tween-80 appears to enhance the rate of color development for both the phosphate and the arsenate reactions. The presence of the nonionic detergent in minute quantities [$\sim 0.3\%$ (v/v)] does not affect the pH of the reaction medium, and no explanation has been offered for its role in the reaction.

Another phosphate reaction which may be monitored at 340 nm is the enzymatic method of Pesce et al.,⁶¹ which utilizes a combined enzyme-substrate system linked to the oxidation-reduction reaction of NADP. The final products of the sequence of reactions are 6-phosphogluconate and NADPH. The amount of NADPH formed is monitored at 340 nm and is proportional to the concentration of inorganic phosphate. Since the reagent mixture is complex and somewhat expensive and the sensitivity of the method is approximately the same as that for the unreduced heteropoly acid method, the method

as described in the literature would have no particular advantage for determining phosphate in environmental samples. However, it should be possible to increase the sensitivity of the method by a factor of 2 via addition of the enzyme 6-phosphogluconate dehydrogenase; this would effectively double the production of the monitored species (NADPH) and should make the sensitivity of the enzymatic method comparable to that of the heteropoly blue method (as measured at 650 nm).

Coleman et al.⁶² have reported an adaptation of the heteropoly blue procedure for orthophosphate to a centrifugal photometric analyzer. The data are linear over the PO_4^{3-} concentration range 0 to 4 ppm; a usable calibration curve may be obtained for up to 14 ppm by employing special data processing techniques.

Our procedure has the advantage of rapid, automatic determination of both silicon and phosphate in a single experiment. In the case of silicon determination, the rapid measurement of the 12-molybdosilicic acid may have certain advantages (e.g., in water with high salinity). Large amounts of electrolytes can adversely affect the molar absorptivity of the unreduced molybdosilicic acid by accelerating the conversion of the β -isomer to the α -isomer.⁶³ This effect, which can produce substantial negative errors, is greatly minimized by rapid formation and measurement of the silicon complex⁶³ (see Table 2.17).

(3) The "nutrient rotor" -- a concept:

We have demonstrated that both silica and phosphate may be determined by monitoring the formation of the heteropolymolybdates at 340 nm. The procedure is based on measurement of the rate of the reaction and extrapolation of the data to obtain a measure of the "initial" absorbance. [In the

case of the phosphate reaction, we have seen that the extrapolated "initial" absorbance may, in fact, represent the end-point absorbance for a (relatively) very fast reaction.] This extrapolation procedure is analogous to that which has been previously employed with CFAs for the two-point, fixed-time kinetic analysis of glucose.^{26,64} Similarly, we have used this procedure for the enzymatic determination of ATP and of ammonia. The substrate is enzymatically linked with the production of NADPH, and the reaction is monitored at 340 nm. The first few seconds of the reaction are monitored to obtain (by extrapolation) an estimate of the initial absorbance (A_0); then a final reading is made to obtain the final absorbance (A_f) after the reaction is complete. The change in absorbance ($A_f - A_0$) is directly related to substrate concentration.

Several of the inorganic nutrients may also be enzymatically linked to the oxidation-reduction reaction of NADH or NADPH. We have previously mentioned such a procedure for phosphate.⁶¹ Another example is the determination of ammonia using L-glutamate dehydrogenase. In addition, the work of Garrett and Nason⁶⁵ suggests that it may be possible to develop an assay for nitrate utilizing the enzyme NADPH: nitrate oxidoreductase (EC 1.6.6.2).

We are currently investigating the possibility of combining our procedure for determining silicon and phosphate with several of the enzymatic assays for inorganic constituents to permit multiple chemical analyses of the major inorganic nutrients in a single rotor under a single set of observation conditions (including radiation wavelength). The analytical capabilities of the CFA, combined with the concept of a portable analyzer,⁶⁶ may yield a powerful tool for use in environmental analysis. Further developments in this concept will be reported in future communications.

2.4.3 Factors influencing evaporation from sample cups* and the assessment of their effect on analytical error

An initial study² indicated that evaporative loss from sample cups was appreciable and had a significant effect on analytical results. During this reporting period, we continued this study and developed a model which allows evaporation rate to be predicted.

Quantitative measurement of evaporative losses. We measured the effects of evaporation both chemically and gravimetrically. In the chemical studies, the miniature CFA was used to analyze either control sera or an aqueous standard solution for glucose²⁶ as a function of time under several different experimental conditions. In the gravimetric studies, various volumes of water or serum were dispensed into several different types of tared sample cups, and the weight loss of each was determined as a function of time by weighing the cup and its contents at 1-hr intervals for up to 8 hr. We used an analytical balance that had a repeatability of ± 0.1 mg and had been calibrated against NBS certified weights. Table 2.19 lists the identification, source, and critical dimensions of the various cups we used.

Estimation of evaporative losses. Results of the evaporation studies led to the development of a mathematical model that allows one to estimate the quantity of a particular liquid lost by evaporation over a specific period of time from a cup of known geometry (Fig. 2.33). The theoretical

* A volatile solute can escape from the solvent, thereby decreasing its concentration in the sample, or the solvent (usually water) can evaporate, thereby increasing the concentration of the nonvolatile solutes in the sample, or both. Although the subsequent discussion deals primarily with the second effect of evaporation, most of the observations and discussion pertain to both.

Table 2.19. Sample cups used in evaporation studies

Code number	Sample cup and source	Code and volume tested ^a	Dimensions		
			Diameter (cm)	Area (cm ²)	Depth ^b (cm)
1	Micro-Tube Hruder Laboratory Products Ann Arbor, Michigan	MT-50	0.357	0.1001	2.18
		-100	0.380	0.1134	1.75
		-150	0.391	0.1207	1.35
		-200	0.422	0.1399	0.97
		-250	0.439	0.1513	0.60
		-300	0.439	0.1513	0.27
2	Eppendorf Tube Brinkman Instruments Westbury, New York	EP-1500	0.91	0.6503	0.39
3	Centrifichem Sample Cup (0.25 ml) Union Carbide Corporation Tarrytown, New York	UC-100	0.69	0.3739	0.98
		-200	0.69	0.3739	0.73
		-400	1.00	0.7853	0.30
4	Nilab Sample Cup National Instrument Laboratory Co. Silver Spring, Maryland	NI-1000	1.21	1.1499	0.72
5	0.5-ml AutoAnalyzer Cup Technichor Corporation Tarrytown, New York	TC-500	0.70	0.3848	0.48
6	5-ml SMAC Tube Technichor Corporation Tarrytown, New York	SM-1000	1.25	1.2271	3.43
		-2000	1.25	1.2271	1.28
		-3000	1.25	1.2271	0.58
		-4000	1.25	1.2271	0.24
		-5000	1.25	1.2271	
7	ABS-100 Micro-Cup Abbott Diagnostic Division South Pasadena, California	AB-50	0.45	0.1590	0.40

^aCup code followed by volume (μl) tested.^bDistance from top of cup to surface of liquid.

TIME = t_0

TIME = t_f

Fig. 2.33. Cross-sectional view of a typical sample cup from which evaporation has occurred during the time t_f .

basis for the model was diffusion through a stagnant gas film.⁶⁷ The following assumptions were made: (a) water diffuses upward through a stagnant film of air; (b) the mole fraction of water at the gas/liquid interface can be calculated from the vapor pressure of water at room temperature; (c) the mole fraction of water in air at the top of the cup is the same as in the laboratory atmosphere; (d) the air/water mixture is an ideal gas; (e) the solubility of air in water is negligible; (f) the entire system is at constant temperature and pressure; and (g) the part of the cup that is above the liquid level can be approximated by a cylinder with the same diameter as the liquid interface. The applicable equation is, then:

$$N_{H_2O} \bigg|_{Z=Z_1} = \frac{pD_{H_2O-air}}{RT(Z_2 - Z_1)} \cdot \ln \frac{p_{air_2}}{p_{air_1}}, \quad (14)$$

where*

$$N_{H_2O} \bigg|_{Z=Z_1} = \text{rate of water evaporation at the interface, } \frac{\text{mole}}{\text{cm}^2 \cdot \text{sec}} ;$$

Z_1 = gas/liquid interface, cm;

Z_2 = top of cup, cm;

p = atmospheric pressure, atm;

* Variables and constants included in the model are given in metric units since most handbooks list them in this manner. In the SI system, the following units should be used:

$$\text{pressure} = \text{N/m}^2 \quad (1 \text{ atm} = 101,325 \text{ N/m}^2)$$

$$\text{gas constant} = 8.31433 \times 10^6 \frac{\text{cm}^3 \cdot \text{N}}{\text{K} \cdot \text{m}^2 \cdot \text{mole}} .$$

All other units are as listed in the text.

D_{H_2O-air} = diffusivity of water in air, cm^2/sec ;

R = gas constant = $82.05 \text{ atm} \cdot cm^3/mole \cdot K$;

T = temperature, K ;

$p_{air_1} = p - p_{H_2O}$ (p_{H_2O} = vapor pressure of water);

$p_{air_2} = p - \frac{(RH)(p_{H_2O})}{100}$ (RH = relative humidity, %).

With the incorporation of the appropriate terms, Eq. (14) can be expressed in terms of the volume lost at the interface, as follows:

$$\frac{dv}{dt}, \frac{cm^3}{sec} = \left(N_{H_2O} \Big|_{Z=Z_1}, \frac{mole}{cm^2 \cdot sec} \right) \left(M_{H_2O}, \frac{g}{mole} \right) \left(\frac{1}{\rho_{H_2O}}, \frac{cm^3}{g} \right) (A_1, cm^2) . \quad (15)$$

The expression then becomes:

$$\frac{dv}{dt} = \frac{p M_{H_2O} (D_{H_2O-air}) A_1}{\rho_{H_2O} \cdot RTh} \cdot \ln \frac{p_{air_2}}{p_{air_1}} , \quad (16)$$

where

$\frac{dv}{dt}$ = rate of water evaporation at the interface, cm^3/sec ;

M_{H_2O} = molecular weight of water, $g/mole$;

ρ_{H_2O} = density of water, g/cm^3 ;

A_1 = area of sample cup at the liquid-air interface, cm^2 ;

$h = Z_2 - Z_1$ = height of stagnant air mass, cm (i.e., the vertical distance from the surface of the air-liquid interface to the top of the sample cup).

In addition, the relationship between the rate of evaporation, dv/dt , and the rate of change in the height of the stagnant air mass can be expressed as follows:

$$\frac{dv}{dt} = A_1 \frac{dh}{dt} . \quad (17)$$

Substituting the expression for dv/dt from Eq. (16) into Eq. (17), we have:

$$\frac{dh}{dt} = \left[\frac{p_{H_2O}^M D_{H_2O \text{ air}}}{\rho_{H_2O} RT} \cdot \ln \frac{p_{air_2}}{p_{air_1}} \right] \cdot \frac{1}{h} . \quad (18)$$

The term in brackets is a constant (C) for a particular set of conditions and can be readily calculated.

Integrating Eq. (18) between the initial height, h_1 , and the final height after evaporation, h_f , gives

$$\frac{(h_f)^2 - (h_1)^2}{2} = C \cdot t . \quad (19)$$

From the geometry of the cup, one may calculate the volume loss corresponding to the change in height of the stagnant air mass ($h_f - h_1$) for a given time t .

This model can then be used to predict evaporative loss from a given cup containing a specific volume of liquid under a selected set of environmental conditions. It should be noted, however, that losses due to convection are not included in the model.

Analytical effects of evaporation. The primary effect of evaporation is on analytical accuracy because the concentration of dissolved solutes in a sample will increase as solvent evaporation continues. This is demonstrated in Fig. 2.34, which shows the glucose concentrations of duplicate aliquots of two control sera as a function of time. To minimize evaporation,

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Fig. 2.34. Analytical effect of sample evaporation as a function of time.

75 μ l of silicone oil (Sera-Seal; Abbott Diagnostics Div., South Pasadena, California 91030) was layered over one of the aliquots. The results very clearly indicated that evaporation had occurred in the uncovered samples since their glucose concentrations increased as a function of time, while the glucose concentrations of the covered samples remained nearly constant throughout the 5-hr experiment.

Factors affecting evaporative loss. Many factors influence the magnitude of evaporative loss; several of the more obvious ones are listed in Table 2.20. Using either actual measurements of the evaporative loss or the experimental model to estimate it, we have attempted to quantitate the effects of the more important factors.

(Time). Evaporation is a time-dependent phenomenon; thus time is a very critical factor in determining the magnitude of evaporative loss. From the moment the sample is withdrawn from the patient until the final quantitative measurement is made, evaporation can occur throughout the analytical process and cause an appreciable analytical error. For example, the data which will be subsequently presented and discussed will demonstrate that this error can be as high as 40 to 50% over an 8-hr period.

(Environmental factors). As would be expected, environmental factors have a pronounced influence on the evaporative loss of a liquid from a sample cup. The mathematical model discussed earlier [Eq. (16)] can be used to demonstrate the effect of environmental factors on evaporative loss. Figures 2.35-2.38 show evaporative loss from the Micro-Tube (MT), Union Carbide (UC), SMAC, and Abbott (AB) sample cups as a function of ambient temperature and relative humidity; as may be seen, it is directly proportional to ambient temperature and inversely proportional to relative humidity. The

Table 2.20. Factors affecting evaporative loss

-
- I. Time
 - II. Environmental factors
 - A. Ambient temperature
 - B. Relative humidity
 - C. Air flow
 - III. Sample cup properties
 - A. Design
 - B. Volume capacity
 - IV. Sample properties
 - A. Nature of solvent
 - B. Solutes
 - C. Surface effects
 - V. Instrumental factors
 - A. Mode of operation
 - B. Sample cup environment
 - C. Sample protection
 - VI. Operator technique
-

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Fig. 2.35. Effects of ambient temperature and relative humidity as a function of time on evaporative loss from the Micro-Tube sample cup.

Fig. 2.36. Effects of ambient temperature and relative humidity as a function of time on evaporative loss from the Union Carbide sample cup.

Fig. 2.37. Effects of ambient temperature and relative humidity as a function of time on evaporative loss from the SMAC sample cup.

Fig. 2.38. Effects of ambient temperature and relative humidity as a function of time on evaporative loss from the Abbott sample cup.

strong influence that these two environmental factors exert on evaporation may be partially responsible for the seasonal fluctuations some laboratories experience in their quality control data.

A third environmental factor which potentially can cause the greatest evaporative loss is the magnitude of air flow within the laboratory. As the air flow increases across the surface of a liquid-air interface, vapor is swept with it, providing a driving force for evaporation. For this reason, sample covers are provided with most instruments. However, as will be discussed later, appreciable evaporative loss can occur even when such covers are employed.

(Sample cup properties). Although the evaporation of liquid from a sample cup is basically a function of the interaction of several environmental factors, its magnitude is influenced by the geometry, design, and physical properties of the cup.

The effect of sample cup geometry was determined by placing selected volumes of distilled water into various types of tared sample cups and then weighing them at designated times to determine evaporative losses. Between weighings, the sample cups were covered unless specified. The cups that were studied and their critical dimensions and volumes are listed in Table 2.19. Cross-sectional views of the cups are illustrated in Fig. 2.39.

The evaporation rates obtained for these cups and the environmental conditions under which they were measured are summarized in Table 2.21. In general, the rate of evaporation was proportional to the surface area of the liquid and inversely proportional to the vertical height of the stagnant air mass that is located between the liquid surface and the top of the sample cup.

Fig. 2.39. Cross-sectional views and diameters for seven sample cups used in evaporation studies.

Table 2.21. Environmental conditions and estimated vs measured evaporation rates for several sample cups

Cup ^a	Ambient temperature (°C)	Relative humidity (%)	Barometric pressure (mm Hg)	Diffusivity ^b (cm ² /sec)	Vapor pressure ^c (mm Hg)	Evaporation rate (μl/hr)		Relative loss (%/hr)
						Predicted	Measured	
AB-50	25.4	53	736.6	0.2660	24.294	3.7	5.5	11.0
EP-1500	22.3	38	752.9	0.2547	20.149	16.0	16.2	1.1
MT-50	25.1	56	736.6	0.2642	23.977	0.5	0.5	1.0
-100						0.6	0.8	0.8
-150						0.9	1.1	0.7
-200						1.4	1.5	0.8
-250						2.3	2.2	0.9
-300						4.0	3.2	1.1
NI-1000	22.3	38	752.9	0.2547	20.149	17.2	22.7	2.3
SM-1000	23.9	63	746.6	0.2593	22.243	2.7	2.9	0.3
-2000						3.7	3.7	0.2
-3000						5.4	5.3	0.2
-4000						9.7	8.2	0.2
TC-500	22.3	38	752.9	0.2547	20.149	12.9	12.9	2.6
UC-100	22.3	36	744.5	0.2601	20.149	4.4	4.5	4.9
-200						5.8	5.9	3.0
-300						8.0	8.2	2.8
-400						11.5	11.1	3.1
UC-300 ^d	22.3	38	752.9	0.2547	20.149	-	14.6	4.9

^aSee Table 2.19 for identification and sources of the individual sample cups.

^bDiffusivity calculated from the relationship

$$D = (0.220) \left(\frac{T}{T_0} \right)^{1.75} \left(\frac{P_0}{P} \right),$$

where T = ambient temperature, K; T₀ = 273 K; P₀ = 760 mm Hg; P = barometric pressure, mm Hg (1 mm Hg = 133 Pa).

Reference: W. P. Boynton and W. H. Bralton, Interdiffusion of Gases and Vapors.

^cValues obtained from R. H. Perry and C. H. Chilton, Chemical Engineers Handbook, 5th ed., p. 3-45, McGraw-Hill, New York, 1973.

^dSample cups were uncovered during the entire experiment.

Results of the experiments summarized in Table 2.21 indicated that evaporative loss is dependent on the type of sample cup used. Since a gravimetric study is laborious and time-consuming and there are literally hundreds of different types of sample cups, it was desirable to develop a model which could be used to predict evaporative loss for any cup of known geometry under specific environmental conditions. Consequently, the model described previously was developed to serve this purpose.

The predictive capabilities of the model were evaluated by comparing the evaporative losses measured gravimetrically for the seven cups studied with those calculated by the model. These comparisons are listed in Table 2.21. In general, the agreement is good, indicating that the model is approximately correct. Note that the discrepancies encountered always occurred when the liquid level was near the top of the cup. Under these conditions, differences between the predicted and measured values can be attributed to convective air currents and measurement errors. When the liquid level is near the top of the cup, convective air currents may sweep into the cups. Since the model is based on diffusion of a vapor through a stagnant air mass, air convection would be expected to decrease the predictive power of the model, which is consistent with the data obtained in our study. In addition, when the liquid level is near the top of the cup, it is more difficult to measure the depth of the stagnant air mass; hence the predictive value of the model is more subject to error and uncertainties under these conditions.

The results of these experiments and those predicted by the model indicated a direct relationship between the volume of liquid placed in a sample cup of a particular design and the absolute quantity of liquid lost

by evaporation. For example, when the evaporation data obtained from the UC cup are plotted as a function of time and sample volume (Fig. 2.40), the largest quantitative loss was observed with the 400- μ l sample, with lesser losses observed for the 300-, 200-, and 100- μ l samples. Similar results were also obtained with the various volumes placed in the other types of sample cups (Table 2.21, Figs. 2.41-2.43).

Evaporative loss, when related to its effect on analytical error, can be demonstrated to be proportional to the relative loss of liquid from the sample. For example, the initial concentration of a given solute can be expressed as:

$$C_o = \frac{q}{V_o} , \quad (20)$$

where

C_o = initial concentration of solute,

q = quantity of substance,

V_o = initial volume of sample.

After a finite time (t) in which an evaporative volume loss of V_e has occurred, the concentration of the solute then becomes:

$$C_f = \frac{q}{V_o - V_e} . \quad (21)$$

Relating the initial to the final concentration, it can be shown that:

$$\frac{C_o}{C_f} = \frac{V_o}{V_o - V_e} . \quad (22)$$

Thus the change in concentration of the substance is proportional to the relative loss of liquid from the sample.

Fig. 2.40. Evaporative loss as a function of cup volume and time for the Union Carbide sample cup.

Fig. 2.41. Evaporative loss as a function of cup volume and time for several different types of sample cups.

Fig. 2.42. Evaporative loss as a function of cup volume and time for the Micro-Tube sample cup.

Fig. 2.43. Evaporative loss as a function of cup volume and time for the SMAC sample cup.

When the data shown in Fig. 2.40 are replotted as a relative evaporative loss vs time and sample volume (Fig. 2.44), the 100- μ l sample was found to have the greatest percentage loss, followed by the 400-, 200-, and 300- μ l samples. Thus one would expect the analytical error due to evaporation to be greater in the 100- μ l sample than in the 400-, 200-, and 300- μ l samples. This expectation was confirmed experimentally by analyzing various volumes of an aqueous glucose solution for glucose content as a function of time. The data obtained in this experiment (Table 2.22) are consistent with the above observation and indicate a greater analytical error due to evaporation for the 100- μ l sample; the smallest error was observed for the 300- μ l sample. Therefore, when the problem of evaporation is considered, one should relate the quantitative evaporative loss to the starting volume since the analytical error is proportional to the relative loss instead of the quantitative loss. Consequently, the relative evaporative losses determined for the various sample cups are also listed in Table 2.21 and shown graphically in Figs. 2.45-2.47.

The experiments discussed above demonstrated that evaporative loss should be expressed on a percentage or fractional basis in order to relate it to analytical error. This fractional loss can also be expressed in terms of the change in depth of the liquid sample as evaporation occurs. For example, for a cup of constant diameter (Fig. 2.33), the fractional loss (F) can be expressed as:

$$F = \frac{h_f - h_l}{h_o}, \quad (23)$$

Fig. 2.44. Relative evaporative loss as a function of cup volume and time for the Union Carbide sample cup.

Table 2.22. Effect of sample cup volume on the magnitude of analytical error in glucose measurements^a due to evaporation

Time (hr)	Sample cup volume							
	100 μ l		200 μ l		300 μ l		400 μ l	
	Conc. (mg/l)	Error (%)	Conc. (mg/l)	Error (%)	Conc. (mg/l)	Error (%)	Conc. (mg/l)	Error (%)
0	1911	0.0	1907	0.0	1907	0.0	1904	0.0
0.9	1970	3.1	1936	1.5	1928	1.1	1940	1.9
1.7	2040	6.8	1982	3.9	1972	3.4	1990	4.5
3.5	2187	14.4	2058	7.9	2023	6.1	2055	7.9
6.0	2405	25.9	2176	14.1	2080	9.1	2102	10.4

^aGlucose concentration measured under the following analytical conditions: sample assay volume = 2 μ l; reaction volume = 122 μ l; wavelength = 340 nm; temperature = 30.0°C; reaction time = 6 min; type of assay = equilibrium, with sample blank correction.

Fig. 2.45. Relative evaporative loss as a function of cup volume and time for several different types of sample cups.

Fig. 2.46. Relative evaporative loss as a function of cup volume and time for the Micro-Tube sample cup.

Fig. 2.47. Relative evaporative loss as a function of cup volume and time for the SMAC sample cup.

where

F = fraction of liquid lost;

h_o = initial depth of liquid;*

h_f = height of stagnant air mass after evaporation;

h_l = initial height of stagnant air mass.

As shown earlier in Eq. (19) of the evaporation model, the final height of the stagnant air mass is given, after rearrangement, by the equation

$$h_f = \sqrt{h_l^2 + 2Ct} = h_l \sqrt{1 + \frac{2Ct}{h_l^2}}. \quad (24)$$

The square-root term for this expression can be expanded into a power series, and for small evaporative losses (e.g., when $\frac{2Ct}{h_l^2} \ll 1$) the final height of the stagnant air mass is approximately:

$$h_f = h_l \left(1 + \frac{Ct}{h_l^2} + \dots \right). \quad (25)$$

Substituting this expression for h_f into Eq. (23), the fractional loss of liquid becomes:

*The initial depth of the liquid (h_o) can be measured directly or calculated using the relationship:

$$h_o = \frac{4V_o}{\pi d^2},$$

where

V_o = initial volume of sample,

d = diameter of the cylindrical section of the cup.

It should also be noted that this expression can be used to obtain an effective depth of liquid in the event the cup should contain a conical bottom.

$$F \approx \frac{h_1 \left(1 + \frac{Ct}{h_1^2} \right) - h_1}{h_o}, \quad (26)$$

which reduces to

$$F \approx \frac{Ct}{h_1 h_o}. \quad (27)$$

Note that Eq. (27) predicts that the fraction of liquid loss will increase linearly with time, and for a given cup will depend on the product of the initial depth of the liquid in the cup and the height of the stagnant air mass located over it as well as the atmospheric parameters included in the constant C. The height of the stagnant air mass can also be related to the depth of liquid by the expression:

$$h = h_o + h_1, \quad (28)$$

where

h = the height of the sample cup.

Then the rate of fractional liquid loss, R , for short times (small losses) is:

$$R = \frac{dF}{dt} = \frac{C}{(h - h_o)h_o} = \frac{pM_{H_2O}^D \cdot D_{H_2O-air}}{(h - h_o)h_o \rho_{H_2O} RT} \cdot \ln \frac{p_{air_2}}{p_{air_1}} \quad (29)$$

In addition to predicting the evaporation rate for a given cup, Eq. (29) also suggests an optimum depth for filling the cup with liquid to reduce the effects of evaporation. As noted, R is a function of h_o and is a minimum when the denominator of Eq. (29) is a maximum. This occurs when:

$$\frac{d[(h - h_o)h_o]}{dh_o} = 0 = h - 2h_o ; \quad (30)$$

that is, when

$$h_o = \frac{h}{2} . \quad (31)$$

Thus, to minimize the evaporative loss from a sample cup, the cup initially should be only half full. This somewhat surprising relationship has been confirmed experimentally as shown by the data listed in Table 2.21. For example, when the fractional evaporative losses obtained from the MT sample cups were plotted as a function of cup capacity (Fig. 2.48), the minimum loss occurred when the cups were approximately 50% full. Similar curves were also obtained for the UC (Fig. 2.49) and SMAC (Fig. 2.50) cups. The data also suggest that, for a given sample cup, a useful volumetric range to minimize evaporation would be from 25 to 75% of the cup capacity. Sample volumes below or above this range would be severely affected by evaporation.

(Sample properties). The chemical and physical properties of a sample will affect evaporative loss. For example, the properties of the solvent in which the solutes of the sample are dissolved or suspended are important in determining the magnitude of the loss. In biological samples, this solvent is water; therefore, evaporation from such samples is a function of the physical properties of water, including vapor pressure, diffusivity, density, molecular weight, etc. When a liquid other than water is used as the solvent (e.g., an organic solvent from an extractive process), its physical properties will be different and the rate of evaporation will be affected accordingly. In most cases, the organic solvent will have a greater vapor pressure than water and a greater evaporative loss.

Fig. 2.48. Relative evaporative loss as a function of cup capacity for the Micro-Tube sample cup.

Fig. 2.49. Relative evaporative loss as a function of cup capacity for the Union Carbide sample cup.

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Fig. 2.50. Relative evaporative loss as a function of cup capacity for the SMAC sample cup.

The inter- and intramolecular forces exerted between the solutes and solvent of a liquid sample will also affect evaporative loss. For example, it has been reported that evaporation of water was more rapid from buffered solutions or ovalbumin or hemoglobin than from solutions of various surface-active agents or from buffer alone.⁶⁸ In a gravimetric study of the comparative evaporative losses of water vs serum samples as a function of volume size, we found the evaporative loss from serum samples to be consistently higher than those from water samples (Fig. 2.51).

There is also an interaction between the surface properties of the sample and the material used to fabricate the sample cup. If the surface of the cup is wet by the sample, the resulting larger meniscus may provide additional surface for evaporation. If the surface of the cup is non-wettable, the sample will have a smaller meniscus. This will result in relatively less evaporative loss than from a similar cup fabricated from a wettable material. Thus, one means by which evaporative loss can be decreased is to use cups fabricated from nonwettable material.

(Instrumental factors). Several instrumental factors, for example, the type of instrument used and the method of operation, will influence the analytical error due to evaporation. In a continuous-flow or discrete-type analyzer which is electronically calibrated against the chemical responses obtained from reference standards, evaporative loss may not be a problem, especially if the reference and patient samples are treated exactly the same. Under these conditions, a similar volume of liquid is lost from each as evaporation occurs; thus the analytical error due to evaporation would be masked due to the ratioing mode in which the machine is calibrated. One should be aware that since evaporation will occur at different rates for serum and aqueous samples (see Fig. 2.51), evaporation

Fig. 2.51. Evaporative loss as a function of cup volume and time for both serum and water samples.

may cause analytical problems when aqueous solutions of standards are used for calibration and sera obtained from patients are the samples being analyzed. In addition, to minimize the effects of evaporation, similar volumes of standards and of sera from patient should be placed into the sample cups.

The instruments in which evaporation can potentially cause the greatest analytical error are the discrete-type analyzers which are operated in the batch mode, offer microvolume capabilities, and use stoichiometric factors instead of chemical calibration factors. For example, with these instruments, small volumes of samples (usually 500 μl or less) are poured into cups which are subsequently placed into a carousel. During the course of the day, aliquots of sample are repetitively sampled from these cups as the technologist performs several different chemical analyses on them, sequentially and batchwise. Hence, the samples may remain in the cups for periods up to several hours. Under these conditions, progressive evaporation of solvent from the small volumes involved will significantly increase the concentration of the dissolved analytes, especially if preventive measures are not taken.

Previously we have discussed the role that environmental factors have on the magnitude of evaporative loss. The manner in which an instrument maintains temperature control can also influence evaporative loss if it changes the immediate or proximal environment of the samples. For example, evaporative loss will be increased in a machine which maintains the samples at an elevated temperature such as 30°C or 37°C. In addition, if an open water bath is used to maintain temperature, it may increase the relative humidity and thus decrease evaporative loss.

A fourth factor in governing the evaporative loss that might be expected from a particular instrument is the technique or means used to prevent or minimize evaporation. In most instruments, this consists of a cover which fits over the sample carousel and thereby protects the surface of the sample. However, one should be aware that appreciable evaporative loss can occur even if the sample cup is covered and convective air currents are eliminated. This loss is predicted by the evaporation model and confirmed experimentally by measuring the evaporative loss from both covered and uncovered UC cups which contained 300- μ l aliquots of water. In this study, the covered and uncovered sample cups had evaporative losses of 2.8% and 4.9% per hour, respectively (Table 2.21). Thus evaporative loss was retarded by covering the sample; however, the loss even from the covered cup was appreciable.

(Technique). Another factor that influences the magnitude of evaporative loss is the technique of the operator. For the most part, technique involves the use of common sense and good laboratory practice. For example, if the system requires sample covers, the operator should make a conscientious effort to use them, even though it may delay his work schedule slightly. In addition, he should not place a loaded sample carousel in an area where a draft exists or in a sunny location near a window. In general, the evaporative loss due to technique can be held at a minimum if the operator is aware of the problem and exercises care in his work.

Minimizing evaporative loss. These studies have shown that evaporative loss is a serious analytical problem and must be minimized. Several solutions to achieve this goal are possible:

- (1) Control and maintenance of the laboratory environment. The three factors that may be controlled to minimize evaporative loss are temperature, relative humidity, and air flow. As in most situations, a compromise must be made since the environmental conditions that would result in decreased evaporative loss (i.e., low ambient temperature, high relative humidity, and decreased air flow) would be very uncomfortable for the operator and might also result in operating problems with the system. It becomes obvious, then, that a compromise must be made between comfort and minimized evaporative loss, which, under these conditions, might mean an air-conditioned laboratory having an ambient temperature of 21°C (70°F) and 50% relative humidity.

If fluctuations in the laboratory environment become a problem, one possible solution* is the use of a chamber in which the samples are maintained at a reduced temperature and at an increased relative humidity during the time they are not actively in the loading process. However, care should be taken that water does not actually diffuse into the sample, or that condensation, which would collect on the walls and ceiling of the chamber, does not fall into the sample cups and dilute their contents. A simple alternative to the use of an environmental chamber would be to place the sample carousel in a refrigerator between loadings.

- (2) Covering of the sample. Theoretically, evaporation could be minimized by keeping the sample covered at all times and by minimizing the

* Suggested by Dr. James Penton, Institute for Health Research, San Francisco, California.

volume of the stagnant air mass located between the liquid level of the sample and the cover. However, since aliquots of the sample must be taken for analysis, this is often not a practical solution unless a means of sampling through the cover is available. Two options are available for this: (a) the cover could be of a rigid, semirigid, or permeable membrane that is punctured by the sampling probe; or (b) the cover could be an immiscible liquid such as a silicone fluid which is layered over the top of the sample and through which the probe can easily pass. Both of these options have disadvantages; the first requires a strong and inflexible probe, the availability of which is limited especially for 1- to 10- μ l volumes of sample, while the second risks contaminating the sample probe with the silicone fluid. Also, as shown in Fig. 2.52, care must be taken to ensure that an adequate volume of silicone fluid is used; otherwise, part of the surface of the sample will be exposed and evaporative loss will still occur.

- (3) Selection of sample cup with desired features. As demonstrated earlier in the data listed in Table 2.21, evaporative loss occurs at different rates from cups having different cross-sectional designs. In general, evaporative loss is less for tall cups (cups 1, 2, and 6) and greater for short cups (cups 3, 4, 5, and 7). Thus evaporative loss can be minimized by the use of a particular cup design having a relatively large height-to-diameter ratio. This condition is fulfilled by the 0.2-ml Micro-Tube (cup 1, Fig. 2.39). The validity of this selection is predicted by the mathematical model and has also been confirmed experimentally (Fig. 2.48, Table 2.21).

Fig. 2.52. Evaporative loss and percent error as a function of surface treatment and time for the Abbott ABA-100 sample cup.

The minimal evaporative loss from the Micro-Tube led us to consider using this type of cup in the sample-reagent loader which has been developed for use with the miniature Centrifugal Fast Analyzer. When selecting a sample cup, one has to consider both theoretical and practical aspects. For example, our experimental results, as well as the mathematical model, indicated that evaporative loss can be minimized by decreasing the diameter of a cup relative to its height. However, there is a practical limitation to the benefit gained by this approach since filling of the cup with sample becomes a problem as the diameter of the cup is decreased. In addition, the sampling probe that is inserted into the cup may also impose a practical limitation on the minimum diameter of the cup. These problems were considered specifically with regard to the dimensions of the Micro-Tube cup. It was found that samples could be very easily introduced into this cup via a disposable pipette or a similar type of pipetting device. In addition, we found that it was possible, with the assistance of a probe guide, to position a sampling probe over the cup and then direct the probe into its contents for sampling. Consequently, the turntable of the sample-reagent loader was modified to accept a carousel which holds 17 of the Micro-Tubes (Fig. 2.53).

To demonstrate that the use of Micro-Tubes will minimize the analytical effects of evaporation, an experiment was conducted in which various volumes of an aqueous standard solution of glucose were placed into Micro-Tubes and their glucose content measured as a function of time. The volumes studied were 50, 100, and 200 μ l, with four replicate samples being assayed for each volume. For reference purposes, four tubes were filled with 200 μ l each and capped between loadings. The other tubes remained open during

Fig. 2.53. Modification of carousel of sample-reagent loader to accept Micro-Tube sample cups. A. Micro-Tube sample cup used in modified loader. Height = ~ 2.5 cm; working capacity = 20 to 200 μ l; may be capped between samplings. B. Carousel of sample-reagent loader which has been modified to hold 17 Micro-Tube sample cups. C. Guide which is placed over the sample carousel containing the Micro-Tubes. Note the beveled edge of each aperture which serves to guide the sampling probe downward into the contents of each Micro-Tube.

the entire experiment. The results, which are summarized in Table 2.23, indicate that very little evaporation occurred. For example, the 8-hr loss was only 3.5% for the 50- μ l sample, while the losses for the 100- and 200- μ l samples were hardly detectable. The samples were also assayed 24 hr after the experiment had been initiated. At this time, evaporation had begun to have a noticeable effect on the glucose concentration of the uncapped samples; however, this would not present a problem in routine analyses since samples are not normally allowed to remain in their cups that long.

This study with the Micro-Tubes pointed out an additional advantage which was gained by using these cups; that is, sample volumes as small as 50 μ l could be used and processed. In fact, by positioning the sample probe to withdraw sample from the bottom of the tube, a 10- μ l aliquot can be obtained from a total volume as small as 20 μ l. This low-volume capability should prove very useful in pediatric and small-animal clinical studies.

In summary, current studies have demonstrated that the loss of liquid from a sample due to evaporation can be a serious problem, especially when microliter volumes are involved. The magnitude of this loss is determined by the interrelationship of a complex set of variables which include environmental, instrumental, and operational factors as well as the chemical and physical properties of the sample and its container. Since the error due to evaporation can, in some instances, be as high as 25 to 50%, steps must be undertaken to minimize its effect. Evaporative loss can be minimized by controlling the laboratory environment, selecting a sample cup of a favorable design, filling the cup only one-half full of sample,

Table 2.23. Effect of evaporation on the glucose content of samples placed in Micro-Tube sample cups

Time (hr)	Sample volume							
	50 μ l		100 μ l		200 μ l		200 μ l + cap	
	Mean ^a	RSD (%)	Mean ^a	RSD (%)	Mean ^a	RSD (%)	Mean ^a	RSD (%)
0	2002	0.7	1997	0.9	1999	0.6	2000	0.6
1	1996	0.5	2008	0.4	1999	0.5	1997	1.1
2	1997	0.4	1996	0.7	1998	0.6	2002	0.6
3	1989	1.3	2008	0.6	1994	0.9	1990	0.7
4	2039	0.7	2026	0.8	2025	0.7	2005	0.5
5	2030	0.1	2010	0.3	2037	0.5	2008	0.6
6	2031	0.9	2030	0.5	2033	0.5	1999	1.5
7	2057	0.4	2007	0.6	2022	0.9	1995	0.4
8	2070	0.2	2017	0.6	2027	0.6	1985	0.8
24	2311	0.6	2176	0.9	2180	0.6	2019	1.1

^a Glucose concentration in mg/liter; mean of four measurements at each time.

using effective sample covers, and following good laboratory practice. In this way, the analytical error, even with microliter volumes of sample, can be held to 1 to 2% or less.

2.4.4 Biochemical markers of cancer - kinetic analysis for serum γ -glutamyl transpeptidase

Batsakis et al.⁶⁹ have defined a group of enzymes [γ -glutamyl transpeptidase (GGTP), leucine aminopeptidase (LAP), and 5'-nucleotidase] as "biliary tract enzymes," whose elevated activities in serum reflect, to varying degrees, obstruction, proliferation, inflammation, and malignancy involving the bile duct system of the liver.⁷⁰ Each of these enzymes parallels, usually with greater sensitivity and specificity, the activity of nonspecific alkaline phosphatase (AP) in hepatobiliary disease. Several variant isoenzymes of serum AP, including the Nagao,⁷¹ the Regan,⁷² and the Regan Variant⁷³ have been associated with neoplasia; and, at least for hepatic malignancy, GGTP is the most frequent and sensitive indicator of an abnormality.⁷⁴

The sensitivity of the enzyme to liver disorders, its marked storage stability, and the simplicity of its determination make plasma measurements of GGTP the best available means of screening for liver disease.⁷⁴ The availability of a reagent kit* for kinetic measurement of GGTP activity and its ready adaptability to use on the Centrifugal Fast Analyzer made it highly desirable to develop a kinetic measurement of GGTP and to evaluate this enzyme as a potential biochemical marker of hepatic cancer.

*Worthington Statzyme γ -GTP, marketed by the Worthington Biochemical Company, Freehold, New Jersey.

Introduction. The enzyme GGTP (EC 2.3.2.1) catalyzes a reaction between glutathione and any one of a number of amino acids to form the corresponding γ -glutamyl-amino acid and cysteinyl glycine. The enzyme can also catalyze transpeptidation reactions involving γ -glutamyl-amino acids other than glutathione. Its activity toward γ -glutamyl-p-nitroanilide forms the basis for the currently used clinical estimations of serum GGTP activity;⁷⁵ the reaction involved is as follows: γ -glutamyl-p-nitroanilide + glycylglycine $\xrightarrow{\text{GGTP}}$ p-nitroaniline + γ -glutamylglycylglycine. Released p-nitroaniline absorbs at 400 nm, and the enzyme activity is proportional to the increase in absorbance at this wavelength.

Absorbance linearity on the CFA. The response of the CFA optical system to p-nitroaniline using the 400-nm filter is illustrated in Fig. 2.54. A linear response was observed for absorbances from 0.1 to 1.9, covering a range of concentrations from 5×10^{-6} to 2.05×10^{-4} M. The nonzero intercept resulted from the use of a water reference rather than a reagent blank. The molar absorptivity of p-nitroaniline was determined to be 8900 using the 400-nm filter on the CFA.

Assay method. The Worthington kit contains the lyophilized reagents as listed in Table 2.24. Reconstitution is achieved by the addition of 3.0 ml of distilled water, and dissolution is effected by placing the reconstituted reagent in a constant-temperature bath at 37°C for 10 min. Glycylglycine in the reagent kit serves as the acceptor of the γ -glutamyl group upon enzymatic hydrolysis. Glutamate is present at approximately 86 times the concentration which normal serum would contribute (free glutamate) in order to mask any enhancing effect⁷⁶ of extraneous glutamate on apparent GGTP activity.

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Fig. 2.54. Linearity of absorbance measurements for p-nitroaniline at 400 nm on the Centrifugal Fast Analyzer.

Table 2.24. Reagents contained in the
Worthington Statzyme γ -GTP Kit

Reagent	Concentration ^a (micromoles/ml)
γ -Glutamyl-p-nitroanilide	4.14
Glycylglycine	41.33
Glutamate	1.03
Tris, pH 8.1	191.1

^aAfter the reagent has been reconstituted by the addition of 3.0 ml of distilled water.

The procedure devised for use with the automated loading station of the CFA employs a reagent volume of 120 μ l with no reagent diluent and a serum volume of 5 μ l with 15 μ l of water diluent. Analytical precision could be improved by making the reagent more concentrated and adding reagent diluent; however, difficulties encountered with reagent solubility at higher concentrations made this impossible.

After the samples and reagents had been mixed by accelerating and braking the rotor, optical density measurements were taken every 15 sec during the interval 2 to 6 min after initiation of the reaction. Each 15-sec measurement was the average of absorbances taken over 20 successive revolutions of the CFA rotor. A linear least-squares analysis of the data (16 points per determination) was performed to determine the slope of the data ($\Delta A/\text{min}$). The slope, when multiplied by the appropriate enzyme factor (determined individually for each rotor), yielded the GGT activity in IU/liter at 30°C.

Four serum samples were analyzed on two successive days in the same rotor using different reagents. The samples were stored at refrigerator temperatures between successive analyses. Although the data indicated a slight gain in activity on the second day (i.e., increases in activity of 2.8, 1.3, 6.0, and 1.0%), all the results were well within the standard deviation of the method.

Under the indicated conditions, the cost of reagent per serum sample (analyzed in duplicate) is 33.8¢ with each rotor (17-place) containing a reagent blank and a standard serum sample in duplicate. In the absence of a standard and with only one analysis per serum sample, the reagent cost would be 15.8¢ per analysis. This compares with a reagent cost of \$3.15 for one analysis using the suggested analytical procedure with the kit.

Effect of freezing and thawing on GGTP activity. A brief study was made of the effect of sample freezing and thawing on GGTP activity. The results (see Table 2.25) indicate that the activity of the sample increases after the first freeze-thaw cycle and decreases (but not quite to the original value) after the second cycle.

Table 2.25. Effect of freezing and thawing on GGTP activity

	Serum sample		
	As collected	Frozen once	Frozen twice
Number of analyses	16	8	8
GGTP, IU/liter	6.14	7.79	6.69
C.V., ^a %	5.0	4.4	5.5
95% range	5.53 to 6.76	7.11 to 8.48	5.95 to 7.43

^aCoefficient of variation.

Linearity of the enzyme method. An experimental serum control* sample having a relatively high GGTP activity (257 IU/liter at 30°C) was analyzed at several dilutions to evaluate the linearity of response over a relatively wide range of enzyme activities. Figure 2.55 shows that the enzyme activity is linear over the range 16.9 to 257.3 IU/liter at 30°C. The Worthington Biochemical Company recommends dilution of samples with activities greater than 313 IU/liter when their reagent kit is used with a final substrate concentration in the reaction mixture of 4.0 millimoles/liter; however, the final concentration used in our studies was 3.55 millimoles/liter.

Evaluation of precision and accuracy of kinetic GGTP analysis. The precision of the kinetic analysis of GGTP was evaluated by using analytical data for the Statzyme[®] control sera** obtained over a period of two days in which 26 analyses were performed in 13 rotor loadings. The mean activity was 9.27 IU/liter with a C.V. of 12.5%. Such a C.V. is not impressive; however, a low GGTP activity and an extremely low $V_{\max}$ (0.0088 absorbance unit/min for this control serum) are contributing factors to the high value obtained. The mean activity measured by the CFA compares very well with the activity measured by Worthington and reported on the control sheet (9.2 ± 1.4).

The analyses of 178 serum samples were performed in triplicate and the C.V. of each determined. The mean C.V. for this sample population was

* Samples obtained through the courtesy of Dr. J. Giegel, Dade Division of the American Hospital Supply Corporation, Miami, Florida.

** Obtained from the Worthington Biochemical Company.

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Fig. 2.55. Linearity of γ -glutamyl transpeptidase activity with dilution, using a high-activity (257-IU/liter) control serum.

3.27%, with a standard deviation of 3.16. The samples were drawn from the ORNL population and, in general, were representative of normal subjects.

Analytical results for apparently normal subjects. Serum samples from apparently normal subjects (male and female) from the ORNL population were analyzed for GGTP activity. The results, classified according to sex and age, are listed in Table 2.26. They suggest a slightly skewed distribution, as evidenced by the difference between the mean and median for all groups.

There appeared to be no significant change in GGTP activity with age. Although men 20 to 29 years old had slightly lower activities and those older than 60 had slightly higher activities, the statistical significance of these differences is questionable because of the small population in these two groups. Women had lower GGTP activities than men, as has been reported by others,^{77,78} and no significant change was noted for women 20 to 49 years old; however, women over 50 showed increased GGTP activities comparable to those of men in the combined age groups 30 to 59. Here again the statistical significance of these data is questionable because of the relatively small population examined.

Frequency distributions for GGTP activities of all subjects are shown in Fig. 2.56. Fourteen of 243 apparently normal subjects had serum GGTP activities greater than 45 IU/liter; six of these exceeded 85. The latter subjects had various abnormalities, including diabetes (or a family history of diabetes), but no known liver dysfunction. No significant difference in average GGTP activity was observed with respect to the day of the week on which samples were collected.

Table 2.26. Serum γ -glutamyl transpeptidase activities
for apparently normal human subjects

Group description	Number of subjects	GGTP activity (IU/liter at 30°C)		
		Mean	Median	Range
All	237	17.1	14.6	6.6-38.4
Males	177	17.9	16.0	8.2-38.4
20-29 years	6	14.2	10.8	8.2-28.1
30-39 years	30	18.2	14.0	8.6-36.8
40-49 years	58	17.7	15.6	8.5-32.2
50-59 years	60	17.1	15.1	8.4-38.4
>60 years	14	19.8	17.4	12.9-29.6
Females	49	13.3	11.5	8.2-38.3
20-29 years	10	11.4	9.8	6.9-27.6
30-39 years	10	11.9	11.5	6.6-23.2
40-49 years	10	10.9	9.2	6.8-19.7
50-59 years	13	17.4	13.3	8.8-38.3
>60 years	3	17.0	15.7	15.6-19.5

Fig. 2.56. Histogram showing frequency distribution of γ -glutamyl transpeptidase activities for men and women.

GGTP activities in cancer patients with hepatic involvement. Serum samples from 139 cancer patients with hepatic involvement were analyzed for GGTP activity on the CFA. Ninety-two of these patients had activities greater than 100 IU/liter, with 25 exceeding 500 IU/liter and 9 exceeding 1000 IU/liter. Also, 20 patients had GGTP activities less than 40 IU/liter, which might be considered at the upper end ($+2\sigma$) of the normal range. The frequency distribution of activities for these individuals is shown in Fig. 2.57. More complete data regarding clinical diagnoses and conditions of the patients are being collected.

Serum GGTP activities for monkeys with hepatomas induced by diethylnitrosamine. Serum samples* from 11 monkeys were analyzed for GGTP activity on the CFA. Each of the monkeys had been sampled over a 1- to 2-year period. Ten of them had been given dosages of carcinogenic diethylnitrosamine (DENA) ranging from 1 to 40 mg. Paired sets of two monkeys, born within a few days of each other, were given 1, 5, 10, and 40 mg of DENA. One monkey, Lco, whose age was not specified, served as a control. Two other monkeys (913L and 877K), not paired, also had received 40 mg of DENA; however, samples obtained for these monkeys did not cover the period prior to neoplastic development as measured by a positive α -fetoprotein test (see Table 2.27).

Figures 2.58-2.62 depict the variation in GGTP activity with time for Leo (the control) and the four sets of paired monkeys. The control monkey appears to be manifesting a gradual increase in activity with time (see Fig. 2.58). The reason for this is unknown; however, it might be an aging phenomenon.

* Obtained from the National Cancer Institute.

Fig. 2.57. Frequency distribution of γ -glutamyl transpeptidase activities for cancer patients with known hepatic involvement.

Table 2.27. Analysis of serum γ -glutamyl transpeptidase in monkeys treated with diethylnitrosamine

Control (birth date, unknown)		1 mg DENA		5 mg DENA		10 mg DENA		40 mg DENA		40 mg DENA		40 mg DENA		40 mg DENA	
		902L (birth date, 5/10/72)	903L (birth date, 5/12/72)	951M (birth date, 4/15/73)	952M (birth date, 4/22/73)	960M (birth date, 7/25/73)	962M (birth date, 7/27/73)	993M (birth date, 12/28/73)	994M (birth date, 12/28/73)	913L ^a (birth date, 6/12/72)	877K ^b (birth date, 9/27/71)				
Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)	Sample date	GGTP (IU/L)
<u>1974</u>		<u>1973</u>		<u>1973</u>		<u>1973</u>		<u>1974</u>		<u>1974</u>		<u>1974</u>		<u>1974</u>	
2-18	62.2	3-31	32.9	3-31	17.4	8-17	93.1	3-31	80.6	8-17	74.3	6-20	64.0	6-20	94.3
3-15	71.0	9-14	36.1	10-25	22.8	9-14	81.6	9-14	77.1	10-26	68.3	8-28	74.1	8-28	101.5
6-7	73.0	10-25	27.9			10-26	93.6					9-26	72.3	9-26	97.4
6-14	58.9			<u>1974</u>				<u>1974</u>		<u>1974</u>		10-24	77.4	10-24	99.2
6-21	78.5	<u>1974</u>		<u>1974</u>		<u>1974</u>		<u>1974</u>		11-21	28.8	11-21	95.4	10-24	49.3
7-12	69.2			6-20	24.0			5-20	65.5	6-20	66.2	12-5	54.1	12-5	89.7
7-19	64.8	5-20	32.1	3-1	24.3	6-20	77.6	8-11	72.0	8-1	62.0				
8-9	68.6	3-1	27.7	7-26	24.8	7-3	83.9	9-26	53.2	8-28	64.2	<u>1975</u>		<u>1975</u>	
10-25	72.9	3-28	30.3	10-24	25.8	8-28	87.9	10-24	57.2	9-26	53.8			<u>1975</u>	
		9-26	28.3	11-21	22.7	9-26	63.1	11-21	55.5	10-24	76.7	2-6	60.1	1-23	86.0
<u>1975</u>		10-24	33.9	12-5	29.8	10-24	97.3	12-5	52.4	11-21	60.6	3-20	67.0	2-6	115.9 ^c
		11-21	38.7							12-5	63.9	4-4	65.8	3-6	111.0
3-21	64.3	12-5	31.8	<u>1975</u>		<u>1975</u>		<u>1975</u>						3-26	102.8
3-28	80.0									<u>1975</u>				5-28	107.4
5-9	92.0	<u>1975</u>		2-6	22.2	4-4	74.6	3-6	35.2					5-25	197.1
5-21	80.1			3-20	24.3			4-4	50.0	2-5	50.1				
5-28	79.7	2-6	38.1	4-4	28.7			6-25	47.3	3-5	62.7				
6-4	94.6	3-20	29.1	5-28	26.6					3-20	58.6				
6-25	90.7	5-28	27.1	5-25	26.1					5-28	78.6				
		5-25	26.5	7-23	27.2					6-25	74.6				

^a α -Fetoprotein became positive on October 12, 1973.^b α -Fetoprotein became positive on November 9, 1972.^c α -Fetoprotein positive.

Fig. 2.58. Variation of γ -glutamyl transpeptidase activity with time for an untreated control monkey.

Fig. 2.59. Variation of γ -glutamyl transpeptidase activity with time for two monkeys treated with 1 mg of diethylnitrosamine.

Fig. 2.60. Variation of γ -glutamyl transpeptidase activity with time for two monkeys treated with 5 mg of diethylnitrosamine.

Fig. 2.61. Variation of γ -glutamyl transpeptidase activity with time for two monkeys treated with 10 mg of diethylnitrosamine.

Fig. 2.62. Variation of γ -glutamyl transpeptidase activity with time for two monkeys treated with 40 mg of diethylnitrosamine. Note the dates when α -fetoprotein tests became positive.

The two animals (902L and 903L, see Fig. 2.59) who were treated with 1 mg of DENA show relatively constant activities throughout the entire sampling period. It is interesting that the activity levels of GGTP for these animals are much lower than those for any of the other animals. Possible species differences have not been documented at this time. α -Fetoprotein assays have remained negative for 902L and 903L.

The activities of the two animals (951M and 952M) who received 5 mg of DENA have shown a slight downward trend with time (see Fig. 2.60). This trend seems to be somewhat stronger for 952M than for 951M. α -Fetoprotein assays have remained negative.

Results for the monkeys receiving 10 mg of DENA show some rather abrupt variations in activity beginning in about August 1974 (see Fig. 2.61). Unfortunately, early samples for 962M were not available to determine whether the base-line GGTP activity was as constant as that for 960M. Again, the α -fetoprotein assays have remained negative.

Both of the two monkeys (993M and 994M) who received 40 mg of DENA manifested a positive response for α -fetoprotein, one in February and the other in March of 1975 (see Fig. 2.62). In general, the GGTP activity for 994M went through some rather steep changes; a rather large increase was observed after the positive α -fetoprotein assay. An increase from an activity of 86 IU/liter to 116 IU/liter was observed for 993M (see Fig. 2.62); however, the activity diminished slightly thereafter.

The remaining two animals (913L and 877K) had manifested a positive α -fetoprotein response prior to any of the samples we analyzed (see Table 2.27). Both animals had elevated GGTP activities, and 877K (earliest positive α -fetoprotein) had very high activities (ranging from 885 to 2093 IU/liter) which were a factor of 10 to 20 times the normal value.

More information will be forthcoming with regard to the times at which DENA was administered to these monkeys and the general clinical symptoms that were observed.

Evaluation of K_m and V_{max} for GGTP. Both K_m (Michaelis-Menton constants) and V_{max} (maximum enzyme velocity) values were determined for GGTP in sera from a human subject with malignant liver involvement, from monkeys with chemically induced hepatoma, and from two enzyme control preparations.* The data (K_m and V_{max}) were measured only as a function of substrate concentration (γ -glutamyl-p-nitroanilide) at pH 8.1. The results, shown in Table 2.28, were obtained with all dilutions of substrate performed on the automated rotor-loading system and show reasonably good precision.

Differences in the constants and maximum reaction velocities shown in Table 2.28 apparently reflect differences in enzyme sources. The experimental control sera from Dade contain GGTP activity isolated from beef kidney, whereas the Statzyme control sera from Worthington contain GGTP from human serum.

Azzopardi and Jayle⁷⁹ have separated five zones of GGTP activity from normal human sera by acrylamide gel electrophoresis. Sephadex gel filtration has enabled the separation of at least three components, indicating that GGTP isoenzymes of serum may reflect differences not only in charge but also in molecular weight.^{74,80,81} Miyazaki and Okumura⁸² reported three zones of activity for serum GGTP by agar gel electrophoresis and indicated that the middle zone (GT-II) was enhanced in all malignant lesions

* Statzyme enzyme control from the Worthington Biochemical Company and an experimental enzyme control preparation from Dade Division of American Hospital Supply Corporation.

Table 2.28. Michaelis constants and maximum velocities for GGTP from several sources at 30°C

GGTP source	K _m	C.V. (%)	V _{max}	C.V. (%)	GGTP activity (IU/liter)
Human serum ^a	0.845 0.838	20.0 11.3	0.213 0.192	8.2 1.6	2023 ^b
Monkey serum (994M) ^c	0.616	4.8	0.225	1.5	197.1
Monkey serum (913L) ^c	0.650	10.9	0.237	3.5	188.7
Dade control	0.671	7.1	0.303	2.7	257.0
Statzyme control	0.164	41.6	0.0088	9.1	9.3 ^d

^a Malignancy with hepatic involvement; two different measurements.

^b Diluted 1:10 with normal saline.

^c Chemically induced hepatoma.

^d Average of 26 analyses in 13 different rotors; listed as 9.2 ± 1.4 on Worthington control sheet.

involving the hepatobiliary and/or pancreatic areas. These literature data and a comparison of the K_m and V_{max} values for a human with cancer with those for other human sera (compare Statzyme control sera with sera from a patient with a malignancy, Table 2.28) may indicate exploitable differences in GGTP isoenzymes between sera from normal subjects and sera from cancer patients. Further studies of K_m and V_{max} for normal sera are needed since preparation of the standard control serum (lyophilization) may have perturbed the structure of this enzyme in some manner to yield K_m and V_{max} values as different as are shown in Table 2.28 for the human sources of GGTP.

2.4.5 Measurement of genetic variation: adaptation and development of a computer program for enzyme parameter measurement

In a program funded by the Energy Research and Development Administration and conducted in collaboration with the Department of Human Genetics of the University of Michigan, Ann Arbor, we have been investigating kinetic spectrophotometric procedures that can be adapted to a miniature CFA for genetic monitoring of isoenzymes on variant hemoglobins. (As described in Sect. 2.1 of this report, an analytical system to transfer the technology generated by this program is being fabricated for use at the University of Michigan.)

In an earlier project,⁵⁰ a computer program was developed for the GEMSAEC CFA:PDP-8/i analytical system to use in the measurements of kinetic enzyme parameters such as K_m and V_{max} values. Since these types of measurements will be advantageous in the genetic screening program, this computer program has been adapted for use with the miniature CFA:PDP-8/e system.

The computer program originally was contained in five program files and has provisions for displaying the data on a programmable display scope. Since the miniature CFA:PDP-8/e system does not contain this type of scope, the program can be condensed into three files. The program texts for each of these files are listed in Tables 2.29-2.31. The sequence of operation and the functions contained in each of these files are summarized in the paragraphs that follow.

File 1. In this file, information relative to general identification is entered (Fig. 2.63) and the experimental matrix for a particular analytical run is set by entering the desired variables (Fig. 2.64). After the experimental matrix has been set, the millimolar substrate concentrations are entered for the designated cuvetts (Fig. 2.65). In the example shown in Figs. 2.63-2.65, one substrate was analyzed at eight different substrate concentrations in duplicate. Next, a known substrate factor can be entered or one can be calculated by entering the necessary information (Fig. 2.66). File 2 is then automatically entered into the computer.

File 2. In this file, the variables are entered for acquiring data at a specified rate and at designated intervals (Fig. 2.67). Data are then acquired when the initiation signal is received from the analyzer. The resulting data are subsequently processed by a quadratic curve-fitting algorithm, summarized, and printed in a cuvet--substrate-concentration--reaction-rate format (Fig. 2.68). File 3 is then automatically entered into the computer.

File 3. In this file the data generated by file 2 are processed using an algorithm developed by Wilkinson⁸³ to statistically evaluate kinetic steady-state data. The resulting data are then printed in the format shown

Table 2.29. Text of kinetic enzyme parameter program - Part 1

```

01.01 C-ENZYME KINETIC PROGRAM-PART 1
01.02 T !!!! "ENZYME KINETIC PROGRAM FOR THE EVALUATION OF KM AND VMAX"
01.03 E
01.04 S DY=FDAY(0); S T=FTIM(0); S HR=FITR(T/3600)
01.06 S MN=FITR((T-3600*HR)/60); S SC=FITR(T-3600*HR-60*MN)
01.03 T !!!! "DAY" %3, DY, " TIME" %2, HR, " : "MN, " : "SC
01.09 T !! "ENTER I.D. INFORMATION"
01.10 A ! " ENZYME      ", P, !, " BUFFER      ", P, !, " MOLARITY    " P
01.12 A !, " PH          " P, ! " OPERATOR    " P
01.14 A ! " WAVELENGTH " P
01.23 D 9
01.30 D 5
01.32 D 2
01.40 A !! "IS ENZYME FACTOR KNOWN (YES OR NO)" A
01.42 I (A-0YES) 1.44, 1.50, 1.44
01.44 I (A-0NO) 1.40, 1.60, 1.40
01.50 A ! "ENTER ENZYME FACTOR" SF
01.52 L G 12, G 1.01
01.60 D 10
01.62 G 1.52

02.01 C -OBTAIN SUBSTRATE CONCENTRATIONS
02.02 F I=1, VS; T !! "EXPERIMENT #", %2, I; D 2.04
02.03 R
02.04 S I1=2+(I-1)*NS*NR; S I2=I1+NR*NS-NR; D 31
02.90 R

05.01 C-SET EXPERIMENTAL MATRIX
05.10 T !! "ENTER VARIABLES THAT SET EXPERIMENTAL MATRIX"
05.12 A ! " NUMBER OF EXPERIMENTS PER ROTOR "VS
05.14 A ! " NUMBER OF SUBSTRATE CONCENTRATIONS PER EXPERIMENT "NS
05.16 A ! " NUMBER OF REPLICATES ASSAYED PER SUBSTRATE CONCENTRATION "NR
05.20 I (NC-NR*NS) 5.22, 5.24, 5.24
05.22 T ! "ILLEGAL EXPERIMENT MATRIX"; G 5.14
05.24 I (NC-NR*NS*VS) 5.26, 5.90, 5.90
05.26 T ! "ILLEGAL RUN MATRIX"; G 5.12
05.90 R

09.01 C-UNIT SELECTION
09.24 A ! "GEMSAEC UNIT" UN; S NC=FITR(UN); I (NC-17) 9.24, 9.90, 9.24
09.25 S ST=(FITR((UN-NC)*10+0.5)-1)*64
09.26 I (ST) 9.24; I (512-ST) 9.24; I (NC-17) 9.24
09.90 R

10.01 C-CALCULATION OF ENZYME FACTOR
10.02 T ! "CALCULATION OF ENZYME FACTOR"
10.10 A ! "MOLAR ABSORPTIVITY      ", MA
10.15 A ! "ENZYME VOLUME (UL)      ", EV
10.20 A ! "TOTAL REACTION VOLUME (UL) " RV
10.30 A ! "CUVET PATHLENGTH (CM)   " PL
10.40 S SF=(RV*1000)/(MA*EV*PL)
10.50 T 1%6.04, "MILLIMOLAR ENZYME FACTOR      : ", SF, !
10.99 R

31.04 F J=I1, NR, I2; D 31.06; D 31.08
31.05 R
31.06 T !! "SUBS. CONC. (MMOL) FOR CUVETS"; S XL=J; F L=XL, XL+NR-1; T %2, L, "
31.08 A S(J); S XL=J; F L=XL, XL+NR-1; S S(L)=S(J); R
31.09 R

```

Table 2.30. Text of kinetic enzyme parameter program - Part 2

```

01.01 C-ENZYME KINETIC PROGRAM -PART 2;3/E ADAPTATION-CAB(3-22-75)
01.03 D 6

04.01 C - SET UP AND TAKE DATA
04.06 S D=FCLK(,,,);S D=FTAK(,,NC);S D=FTAK(SC,NI,NC);D 27
04.11 S ZZ=FCNI(2);T !"COMPUTER IS READY!"
04.16 I (FCNI(2))4.16,4.26;Q
04.26 S D=FCLK(MD,CD,MI,CY,1)

05.01 C-CALCULATION OF REACTION RATE
05.10 D 8
05.20 F XL=1,VS;D 5.35;D 25.10;D 5.4
05.22 F J=2,VS*NR*NS+1;D 25.2
05.25 L G 13,G 1.1
05.35 S I1=2+(XL-1)*NS*NR;S I2=I1+NS*NR-NR
05.40 F J=I1,NR,NR*NS*XL+1;D 26
05.90 Q

06.01 C-SET DATA ACQUISITION MATRX
06.10 T !"ENTER VARIABLES FOR ACQUIRING DATA"
06.12 A !" DELAY INTERVAL--SEC"DI;I (600-DI)6.12
06.14 A !" OBSERVATION INTERVAL-SEC"SE;I (60-SE)6.14;I (SE-1)6.14
06.16 S SI=SE;S MD=FITR(DI/60);S CD=60*(DI-60*MD);S MI=0;S CY=60*SE
06.17 S IM=FITR(963/[2*FITR(NC/2+1)])
06.18 T !" NUMBER OF OBSERVATIONS (NOTE ";T %3,IM
06.20 T " MAXIMUM)"A NI
06.24 I (NI-1)6.18;I (IM-NI)6.18
06.26 A !" NUMBER OF REVOLUTIONS AVERAGED"SC;I (100-SC)6.26
06.28 S DT=CY/3600
06.30 D 27
06.90 R

07.01 C-ABSORBANCE CALCULATION
07.10 S S1=FGET(N,0);S S2=FGET(N,1)-S1;I (-S2)7.3;T !"BLANK ERROR"Q
07.30 S SN=FGET(N,J)-S1;I (-SN)7.5;S AB(J)=-9.99;R
07.50 S AB(J)=.4343*FLOG(S2/(SN*FSTR(ST+J)))
07.60 R

08.01 C-HEADING FOR DATA OUTPUT
08.15 T !"CUVET SUBSTRATE CONC. REACTION RATE"
08.20 T !" # (MMOL) (ABS./MIN.) (MMOL/MIN.)"
08.99 R

25.10 F J=I1,XL*NR*NS+1;S Y1=0;S Y2=0;S Y3=0;D 29;D 28
25.12 G 1.5
25.16 R
25.20 T !,X2,J," ",X3.04,S(J)," ",X6.04,A1(J)," ";D 25.21
25.21 T X3.04,AV(J)

26.01 C-CALCULATION OF AVE. RATE PER [S]
26.10 S I=J;S VV=0
26.20 F L=I,1+NR-1;D 26.5
26.30 S VV(J)=VV/NR
26.40 R
26.50 S VV=VV+AV(L);R

27.01 C-DL FOR DETERMINANT
27.06 S X0=NI;S X1=NI*(NI+1)/2;S X2=X1*(2*NI+1)/3
27.11 S X3=X1^2;S X4=X2*(3*NI^2+3*NI-1)/5
27.16 S DL=X0*(X2*X4-X3*X3)-X1*(X1*X4-X2*X3)+X2*(X1*X3-X2*X2)
27.21 R

28.01 C-CALCULATION OF SLOPE AND ACTIVITY
28.11 S A1(J)=(X0*(Y2*X4-X3*Y3)-X1*(Y1*X4-X2*Y3)+X2*(Y1*X3-X2*Y2))/(DL*DT
28.12 S AV(J)=A1(J)*5F;R
28.29 R

29.91 F N=1,NI;D 7;S Y1=Y1+AB(J);S Y2=Y2+AB(J)*N;S Y3=Y3+AB(J)*N^2

```


Table 2.31. Text of kinetic enzyme parameter program - Part 3

```

01.01 C-ENZYME KINETIC PROGRAM-PART 3-PARAMETER EVALUATION-FILE 13
01.10 F XL=1,VS;D 6;D 3;D 8
01.23 T !!!;Q

02.10 S AA=AA+VV(J)^3;S BB=BB+VV(J)^4;S CC=CC+(VV(J)^3)/S(J);
02.20 S DD=DD+(VV(J)^4)/S(J);S KK=KK+[(VV(J)^2)/S(J)]^2
02.22 R

03.01 C -ITERATIVE ADJUSTMENT OF KM AND VMAX
03.10 F I=1,10;D 4
03.12 S SE=FSQT((LL-B1*CC-B2*KK)/(NS-2));S SK=(SE/B1)*FSQT(AA/DE)
03.14 S SV=(VO*SE)*FSQT(DD/DE)
03.16 T %6.04,!"OPTIMIZED KM = ",KO
03.17 T " OPTIMIZED VMAX = ",VO
03.18 T !"STD. ERROR FOR KM = ",SK," STD. ERROR FOR VMAX = ",SV
03.20 T !"REL. STD. ERROR(%) = ",%5.02,(SK/KO)*100
03.22 T " REL.STD. ERROR(%) = ",(SV/VO)*100
03.40 T !!!# OF ITERATIONS = ",%2,DU
03.44 T !"B2 = ",%3.06,B2

04.10 S AA=0;S BB=0;S CC=0;S DD=0;S KK=0;S LL=0;F J=11,NR,12;D 5
04.12 S DE=AA*DD-BB*BB;S B1=(DD*CC-BB*KK)/DE
04.14 S B2=(AA*KK-BB*CC)/DE;I (.0000100-FABS(B2))4.16,4.20,4.20
04.16 S VO=B1*VO;S KO=KO+B2/B1
04.18 R
04.20 S DU=I;S I=11;R

05.10 S AA=AA+(VO*S(J)/(S(J)+KO))^2
05.12 S BB=BB+(VO*S(J)/(S(J)+KO))*[-(VO*S(J)/(S(J)+KO))^2]
05.14 S CC=CC+VV(J)*(VO*S(J)/(S(J)+KO))
05.16 S DD=DD+[-(VO*S(J)/(S(J)+KO))^2]^2
05.18 S KK=KK+VV(J)*[-(VO*S(J)/(S(J)+KO))^2];S LL=LL+(VV(J)^2)

06.01 C-CALCULATION OF PROVISIONAL KM AND VMAX
06.05 T !!!EXPERIMENT # ",%2,XL
06.12 S I1=2+(XL-1)*NS*NR;S I2=I1+NR*NS-NR
06.14 S AA=0;S BB=0;S CC=0;S DD=0;S KK=0;S DE=0
06.16 F J=11,NR,12;D 2
06.18 S DE=AA*KK-CC*DD
06.20 S KO=(BB*CC-AA*DD)/DE
06.22 S VO=(BB*KK-DD*DD)/DE
06.24 T !!!PROVISIONAL KM = ",%6.04,KO
06.25 T " PROVISIONAL VMAX = ",VO
06.99 R

08.01 C-SUMMARY OF DATA FOR LATER PLOTTING
08.10 T !!!DATA SUMMARY"
08.20 T !!! [S] V 1/V 1/[S] "
08.21 T " [S]/V V/[S]"
08.40 F J=11,NR,XL*NR*NS+1;D 8.80
08.50 R
08.90 T 1,%8.04,,S(J), " ",VV(J), " ",1/VV(J), " ",1/S(J), " ";D 8.92
08.92 T S(J)/VV(J), " ",VV(J)/S(J)

```

ORNL DWG 75-8925

ENZYME KINETIC PROGRAM FOR THE EVALUATION OF KM AND VMAX.

DAY 3 TIME 23 : 41 : 47

ENTER I.D. INFORMATION

ENZYME : GGTP
RUFFER : TRIS
MOLARITY : .191
PH : 8.1
OPERATOR : JM
WAVELENGTH : 400
GEMSAEC UNIT : 17.1

Fig. 2.63. Format for entering identification information for the statistical evaluation of K_m and V_{max} . Underlined values are those entered by the operator.

ORNL DWG 75-8926

ENTER VARIABLES THAT SET EXPERIMENTAL MATRIX

NUMBER OF EXPERIMENTS PER ROTOR :1

NUMBER OF SUBSTRATE CONCENTRATIONS PER EXPERIMENT :8

NUMBER OF REPLICATES ASSAYED PER SUBSTRATE CONCENTRATION :2

Fig. 2.64. Format used to set experimental matrix in the
statistical evaluation of K_m and V_{max} program. Underlined values are
those entered by the operator.

EXPERIMENT # 1

SUBS. CONC. (MMØL) FOR CUVETS 2, 3, : .2957
SUBS. CONC. (MMØL) FOR CUVETS 4, 5, : .5175
SUBS. CONC. (MMØL) FOR CUVETS 6, 7, : .7393
SUBS. CONC. (MMØL) FOR CUVETS 8, 9, : 1.0350
SUBS. CONC. (MMØL) FOR CUVETS 10, 11, : 1.4786
SUBS. CONC. (MMØL) FOR CUVETS 12, 13, : 2.0700
SUBS. CONC. (MMØL) FOR CUVETS 14, 15, : 2.6614
SUBS. CONC. (MMØL) FOR CUVETS 16, 17, : 3.2528

Fig. 2.65. Format used to enter substrate concentrations in the statistical evaluation of K_m and V_{max} program. Underlined values are those entered by the operator.

ORNL DWG 75-8928

IS ENZYME FACTOR KNOWN (YES OR NO): NO
CALCULATION OF ENZYME FACTOR
MOLAR ABSORPTIVITY : 8900
ENZYME VOLUME (UL) : 5
TOTAL REACTION VOLUME (UL) : 140
CUVET PATHLENGTH (CM) : .5096
MILLIMOLAR ENZYME FACTOR : 6.1736

Fig. 2.66. Format used to enter enzyme factor in the statistical
evaluation of K_m and V_{max} program. Underlined values are those entered
by the operator.

ORNL DWG 75-8929

ENTER VARIABLES FOR ACQUIRING DATA
DELAY INTERVAL--SEC:10
OBSERVATION INTERVAL-SEC:15
NUMBER OF OBSERVATIONS (NOTE 53 MAXIMUM):24
NUMBER OF REVOLUTIONS AVERAGED:20
COMPUTER IS READY!

Fig. 2.67. Format used to set data acquisition matrix in the
statistical evaluation of K_{II} and V_{max} program. Underlined values are
those entered by the operator.

CUVET #	SUBSTRATE CONC. (MMOL)	REACTION RATE	
		(ABS./MIN.)	(MMOL/MIN.)
2	0.2957	0.0110	0.0678
3	0.2957	0.0111	0.0683
4	0.5175	0.0170	0.1051
5	0.5175	0.0168	0.1035
6	0.7393	0.0197	0.1213
7	0.7393	0.0205	0.1263
8	1.0350	0.0231	0.1426
9	1.0350	0.0228	0.1408
10	1.4786	0.0266	0.1641
11	1.4786	0.0251	0.1551
12	2.0700	0.0277	0.1709
13	2.0700	0.0292	0.1803
14	2.6614	0.0292	0.1800
15	2.6614	0.0292	0.1804
16	3.2528	0.0304	0.1877
17	3.2528	0.0303	0.1870

Fig. 2.68. Typical summary of data produced by the statistical evaluation of K_m and V_{max} program.

in Fig. 2.69. The substrate concentrations and velocities are also printed in the forms commonly used by kineticists to plot such data (Fig. 2.70). If necessary, the operator can call an additional file to list in a tabular form all of the absorbance data acquired in the experiment. For convenience, file 3 (Table 2.32) has also been modified to allow the manual entry of kinetic data followed by its processing with the Wilkinson algorithm.

2.4.6 Development of rotors preloaded with reagent

One of the ultimate objectives of the CFA Program is to develop rotors that contain preloaded quantities of reagent in each cuvet. With this type of rotor, the reagents would be dissolved and reconstituted at the time of analysis by the dynamic introduction of water or buffer. Results of a series of feasibility studies^{2,23} made previously demonstrated that reagents can be successfully lyophilized within the cuvetts of the rotor and then dynamically reconstituted and used for assay.

With the successful conclusion of the feasibility studies, our next step was to pursue a collaborative study with one or more of the commercial reagent manufacturers. Such an arrangement would couple the ORNL Fast Analyzer technology with the in-house reagent technology of the commercial firm(s). Consequently, a report describing our objectives, past experiences, and future plans was prepared and submitted to seven reagent manufacturers. All of them expressed interest in such a project; therefore, a proposal for a subcontract calling for delivery of several rotors containing preloaded reagents was supplied to each of the firms. Subsequent subcontracts were awarded to the Worthington Biochemical Corporation, Freehold, New Jersey, and to Smith Kline Instruments, Inc., Sunnyvale, California.

ORNL DWG 75-8931

EXPERIMENT # 1

PROVISIONAL KM	=	0.6105	PROVISIONAL VMAX	=	0.2242
OPTIMIZED KM	=	0.6159	OPTIMIZED VMAX	=	0.2246
STD. ERROR FOR KM	=	0.0298	STD. ERROR FOR VMAX	=	0.0034
REL. STD. ERROR(%)	=	4.83	REL. STD. ERROR(%)	=	1.52
# OF ITERATIONS	=	3			
B2	=	0.000005			

Fig. 2.69. Typical data obtained by the statistical evaluation of K_m and V_{max} program.

ORNL DWG 75-8932

DATA SUMMARY

[S]	V	1/V	1/[S]	[S]/V	V/[S]
0.2957	0.0680	14.6991	3.3818	4.3465	0.2301
0.5175	0.1043	9.5855	1.9324	4.9605	0.2016
0.7393	0.1238	8.0785	1.3526	5.9725	0.1674
1.0350	0.1417	7.0575	0.9662	7.3045	0.1369
1.4786	0.1596	6.2655	0.6763	9.2642	0.1080
2.0700	0.1756	5.6960	0.4831	11.7908	0.0848
2.6614	0.1802	5.5493	0.3758	14.7690	0.0677
3.2528	0.1873	5.3387	0.3074	17.3657	0.0576

Fig. 2.70. Typical substrate concentration and velocity data produced by the statistical evaluation of K_m and V_{max} program for plotting purposes.

Table 2.32. Text of kinetic enzyme parameter program:
Manual entry of data

```

01.01 E; C-MANUAL ENTRY OF DATA FOR KM CALCULATION
01.02 T !! "MANUAL ENTRY OF DATA FOR KM CALCULATION"
01.03 A ! "NUMBER OF SUBSTRATE CONCENTRATIONS ASSAYED" NS
01.09 S VS=1; S NR=1
01.11 F J=1, NS; T ! "[SUBSTRATE](MMOL)", %3, J; A S(J+1); D 1.14
01.12 F XL=1; D 6; D 3; D 8; T !!!!; Q
01.14 T "ACTIVITY (MMOL/MIN)"; A VV(J+1)
01.23 T !!!!; Q

02.10 S AA=AA+VV(J)^3; S BB=BB+VV(J)^4; S CC=CC+(VV(J)^3)/S(J);
02.20 S DD=DD+(VV(J)^4)/S(J); S KK=KK+[(VV(J)^2)/S(J)]^2
02.22 R

03.01 C -ITERATIVE ADJUSTMENT OF KM AND VMAX
03.10 F I=1, 10; D 4
03.12 S SE=FSQTC(LL-B1*CC-B2*KK)/(NS-2); S SK=(SE/B1)*FSQTC(AA/DE)
03.14 S SV=(VO*SE)*FSQTC(DD/DE)
03.16 T %6.04, ! "OPTIMIZED KM = ", KO
03.17 T " OPTIMIZED VMAX = ", VO
03.18 T ! "STD. ERROR FOR KM = ", SK, " STD. ERROR FOR VMAX = ", SV
03.20 T ! "REL. STD. ERROR(%) = ", %5.02, (SK/KO)*100
03.22 T " REL. STD. ERROR(%) = ", (SV/VO)*100
03.40 T ! "# OF ITERATIONS = ", %2, DU
03.44 T ! "B2 = ", %3.06, B2

04.10 S AA=0; S BB=0; S CC=0; S DD=0; S KK=0; S LL=0; F J=11, NR, 12; D 5
04.12 S DE=AA*DD-BB*BB; S B1=(DD*CC-BB*KK)/DE
04.14 S B2=(AA*KK-BB*CC)/DE; I (.0000100-FABS(B2))4.16, 4.20, 4.20
04.16 S VO=B1*VO; S KO=KO+B2/B1
04.18 R
04.20 S DU=1; S I=11; R

05.10 S AA=AA+(VO*S(J)/(S(J)+KO))^2
05.12 S BB=BB+(VO*S(J)/(S(J)+KO))*[-(VO*S(J)/(S(J)+KO))^2]
05.14 S CC=CC+VV(J)*(VO*S(J)/(S(J)+KO))
05.16 S DD=DD+[-(VO*S(J)/(S(J)+KO))^2]^2
05.18 S KK=KK+VV(J)*[-(VO*S(J)/(S(J)+KO))^2]; S LL=LL+(VV(J)^2)

06.01 C-CALCULATION OF PROVISIONAL KM AND VMAX
06.05 T ! "EXPERIMENT # ", %2, XL
06.12 S I1=2+(XL-1)*NS*NR; S I2=I1+NR*NS-NR
06.14 S AA=0; S BB=0; S CC=0; S DD=0; S KK=0; S DE=0
06.16 F J=11, NR, 12; D 2
06.18 S DE=AA*KK-CC*DD
06.20 S KO=(BB*CC-AA*DD)/DE
06.22 S VO=(BB*KK-DD*DD)/DE
06.24 T ! ! "PROVISIONAL KM = ", %6.04, KO
06.25 T " PROVISIONAL VMAX = ", VO
06.99 R

08.01 C-SUMMARY OF DATA FOR LATER PLOTTING
08.10 T ! "DATA SUMMARY"
08.20 T ! " [S] V !/V 1/[S]
08.21 T " [S]/V V/[S]"
08.40 F J=11, NR, XL*NR*NS+1; D 8.80
08.50 R
08.80 T !, %3.04, S(J), " ", VV(J), " ", 1/VV(J), " ", 1/S(J), " "; D 8.92
08.82 T S(J)/VV(J), " ", VV(J)/S(J)

```

Each firm will utilize a different approach in loading the reagents into the rotors. For example, Worthington will first load the reagents in liquid form into the cuvetts and then lyophilize them in situ. Smith Kline, on the other hand, will provide the reagents in the form of tablets, one of which will be added to and sealed within an individual cuvet.

These collaborative efforts will be conducted in two phases. In phase 1, which is already under way, we are interested in demonstrating multiple-sample:single-test rotors; in phase 2, multiple-sample:multiple-test rotors will be demonstrated.

Lyophilized reagents. Worthington has supplied us with a total of 40 loaded rotors, 10 for each of four chemical assays (i.e., glucose, SGOT, alkaline phosphatase, and LDH-L), as a part of the phase 1 program. The rotors to be loaded were shipped from ORNL to Worthington in partially assembled fashion (i.e., the rotor body plus the bottom window as one component, and the top cover window as the second component). Worthington then prepared the reagents in liquid form and loaded them into the rotors by pipetting an aliquot of the appropriate reagent, containing the necessary quantities of reactants, into cuvetts 2 through 17. The reagents were subsequently lyophilized in situ, and a top cover window was placed onto each rotor and fastened with a rubber band. After each individual rotor had been inserted into a plastic bag containing a small bag of desiccant, the entire batch of rotors was shipped to ORNL. On arrival, they were assembled by bonding a cover window to the rotor body--bottom window component and then placed into individual bags which were stored in desiccators at 0°C. At desired intervals, the rotors were removed from the desiccators and evaluated by comparing the values obtained with the

lyophilized reagents with those for freshly prepared Worthington reagents (Statzyme kits).

(Glucose). The rotors containing the hexokinase-glucose reagent were initially evaluated by comparing results obtained with them with those obtained by using freshly prepared reagent. In each case, the lyophilized reagent was reconstituted by loading 70 μ l of distilled water into each reagent chamber by means of the SRL. The rotor was subsequently placed on the analyzer and the aliquots of water transferred into their respective cuvetts by acceleration of the rotor to 4000 rpm. Using a strobe light, the dissolution of the reagent was observed to be almost instantaneous. The rotor was then allowed to coast to a stop, was repositioned into the SRL, and loaded with sample. After loading was complete, the rotor was placed on the analyzer again and the glucose content of the sample measured using a combined kinetic-equilibrium procedure.²⁶ The results from these comparison studies are summarized in Table 2.33. As may be seen, the data for the lyophilized and the freshly prepared reagents are generally in good agreement.

To obtain the dynamic range of the lyophilized glucose reagent contained within the rotors, a second series of analyses was performed in which aqueous glucose solutions of increasing concentration were assayed for glucose content using both lyophilized and freshly prepared reagents. The results, which are summarized in Table 2.34, show that the lyophilized and the freshly prepared reagents gave equivalent results up to a glucose concentration of 200 mg per 100 ml. At a concentration of 400 mg per 100 ml, both the lyophilized and the freshly prepared Worthington reagents gave significantly lower values as compared with the Calbiochem reagent.

Table 2.33. Comparison of glucose data obtained from preloaded and freshly prepared Worthington glucose reagents

Rotor number	Run number	Reagent	Glucose concentration (mg/100 ml)			
			N ^a	Mean	S.D.	R.S.D.
252	1	L ^b	15	98.6	1.62	1.64
265	2	L	16	96.8	2.09	2.16
274	3	L	16	97.7	2.03	2.08
286	4	L	14	99.5	2.49	2.51
290	5	L	14	96.9	1.77	1.83
Total		L	75	97.9	2.21	2.26
252	6	F ^c	16	102.5	1.26	1.22
265	7	F	16	99.6	2.70	2.71
274	8	F	13	98.1	1.82	1.85
286	9	F	16	98.7	2.05	2.07
290	10	F	16	96.8	3.42	3.52
Total		F	75	99.3	2.64	2.66

^aN = number of observations.

^bPreloaded lyophilized reagent.

^cFreshly prepared reagent.

Table 2.34. Dynamic range of lyophilized (L) vs freshly prepared (F) Worthington glucose reagent

Run number	Type of reagent	Reagent absorbance		Glucose concentration (mg/100 ml)					
				100		200		400	
		$\bar{X}$	C.V.	$\bar{X}$	C.V.	$\bar{X}$	C.V.	$\bar{X}$	C.V.
1	L	0.0401	10.91	97.9	2.03	198.3	1.74	318.8	7.29
2	L	0.0353	5.82	99.7	0.58	197.9	1.02	323.7	2.42
3	L	0.0272	21.40	105.5	0.22	208.8	0.60	316.2	4.17
4	F1 ^a	0.0236	6.92	102.4	0.80	197.7	2.80	206.9	0.42
5	F1 ^b	0.0452	1.53	102.2	0.77	195.6	0.30	202.2	0.20
6	F2 ^c	0.0239	5.89	103.7	0.40	204.5	1.73	395.1	0.91

^aReagent was prepared by dissolving the contents of a Worthington reagent vial (Statzyme-15) in 5 ml of distilled water. Reaction conditions: 20 μ l of reagent, 2 μ l of sample, 100 μ l of diluent.

^bReagent was prepared by dissolving the contents of a Worthington reagent vial (Statzyme-15) in 5 ml of distilled water. Also 2.25 millimoles of NADP was added. Reaction conditions were the same as those for run 4.

^cReagent was prepared by dissolving the contents of a Calbiochem Glucose Stat-Pack in 2 ml of distilled water. Reaction conditions were the same as those for run 4.

This difference is obviously due to the quantity of reactants in the Worthington reagent, and additional quantities of this reagent will be requested in phase 2 of the program.

(SGOT). To evaluate the rotors filled with lyophilized reagents for the SGOT assay (Henry method), the reagents were reconstituted as described in the previous subsection. For comparison values, Worthington SGOT-Statzyme 15 reagent was prepared fresh at approximately the same concentration as the lyophilized reagent. As a representative sample, Worthington Statzyme with a stipulated value of 58 ± 3.5 IU/liter at 30°C and an acceptable range of 51 to 65 IU/liter was assayed by both types of reagents. The results are summarized in Table 2.35. When prepared at approximately the same concentration, the lyophilized (runs 1-3) and the freshly prepared reagents (run 4) gave equivalent results. However, the mean of the resulting values (i.e., 49) was slightly outside the accepted range (51-65). When the reagent was freshly prepared at a higher concentration (run 5), the mean value fell within the accepted range.

(Alkaline phosphatase). To evaluate the rotors filled with lyophilized reagents for the alkaline phosphatase assay (Bowers and McComb method), the reagents were reconstituted by adding 40 μl of MAP (2-amino-2-methyl-1-propanol) buffer (as supplied by Worthington) and 30 μl of diluent to each cuvet as described in the subsection on glucose. Then the Worthington Statzyme control, which had a labeled value of 44 ± 4.2 units/liter, with a range of 35 to 52 IU/liter at 37°C , was repetitively assayed using both the lyophilized and freshly prepared reagents. The results are summarized in Table 2.36. Although the mean values obtained with the two types of reagents were generally comparable, each gave higher values than the listed

Table 2.35. Comparison of SGOT data obtained from preloaded (L) and freshly prepared (F) Worthington SGOT reagents

Run number	Type of reagent	Reagent absorbance			Activity (IU/liter at 30°C)		
		N ^a	$\bar{X}$	C.V.	N ^a	$\bar{X}$	C.V.
1	L	-	-	-	16	49.09	1.37
2	L	4	0.3633	12.38	10	49.89	2.09
3	L	4	0.3526	0.55	12	49.09	1.32
4	F1 ^b	4	0.3079	2.11	12	49.88	1.33
5	F2 ^c	3	0.5621	0.60	13	52.58	1.60

^a Number of observations.

^b Reagent was prepared by dissolving the contents of a Worthington Statzyme-15 vial in 3.5 ml of water. Reaction conditions: 20 μ l of reagent, 10 μ l of sample, 100 μ l of diluent.

^c Reagent was prepared by dissolving the contents of a Worthington Statzyme-15 vial in 2 ml of water. Reaction conditions were the same as those for run 4.

Table 2.36. Comparison of alkaline phosphatase data obtained from preloaded (L) and freshly prepared (F) Worthington alkaline phosphatase reagents

Run number	Type of reagent	Reagent absorbance			Activity (IU/liter at 30°C)		
		N ^a	$\bar{X}$	C.V.	N ^a	$\bar{X}$	C.V.
1	L	5	0.4226	5.94	11	51.40	1.16
2	L	4	0.5608	5.05	12	57.26	1.35
3	L	4	0.5192	8.06	12	57.84	1.94
4	F1 ^b	4	0.3038	5.11	10	52.59	0.87
5	F2 ^c	4	0.1733	0.72	10	56.34	1.23

^aNumber of observations.

^bReagent was prepared by dissolving the contents of a Worthington Statzyme-15 vial in 2 ml of Worthington buffer. Reaction conditions: 40 μ l of reagent, 10 μ l of sample, 80 μ l of diluent.

^cReagent was prepared by dissolving the contents of a Worthington Statzyme-15 vial in 4 ml of Worthington buffer. Reaction conditions were the same as those for run 4.

range (35 to 52 IU/liter) for the control serum; again, however, this may be related to reactant concentration. It was also observed that the lyophilized alkaline phosphatase reagent was very powdery and tended to sift out of the cuvetts into other chambers of the rotor. This could cause a serious analytical problem, especially if some of the reagent "dust" should fall into the sample chamber.

(LDH-L). In evaluating the rotors filled with reagents for the LDH-L assay, we had planned to follow the same procedure used for the glucose, SGOT, and alkaline phosphatase evaluations. However, when the rotors were removed from the freezer, the reagents showed evidence of deterioration (i.e., the contents of the cuvetts had a yellowish-brown to brown appearance). This deterioration was confirmed analytically since a very low enzyme activity was obtained when these rotors were used to assay for LDH-L activity. For example, when these reagents were used to assay a sample known to have an LDH-L activity of 170 IU/liter at 30°C, an activity of less than 20 IU/liter was obtained.

After consultation with Worthington, ten rotors were returned to them for reloading with LDH-L reagent. When the reloaded rotors were returned to Oak Ridge, they were immediately stored in a desiccator which was placed in a freezer at 0°C. To complete the assembly of the rotors, two rotors were removed from the 0°C desiccator and placed in a 25°C desiccator, where they were allowed to remain until they reached ambient temperature. At this point, the rotors were removed from the desiccator, adhesive was applied to the top surface of the rotor-body:bottom-window components, and a top cover window was added to each rotor; the resulting assembled rotors were then placed in individual presses. Each press was placed in a

desiccator and stored for approximately 18 hr at 4°C. At the end of this period, the rotors were removed from the presses, placed in individual plastic bags, and stored in a desiccator at 0°C.

After the rotors had been stored for three days, their contents were reconstituted by adding 70 μ l of diluent to each cuvet as described in the subsection on glucose. Worthington Statzyme Control (labeled LDH-L, activity of 168 ± 12.6 IU/liter, acceptable range 143 to 193 units/liter) was used as the reference sample. For comparative purposes, this reference sample was also assayed using freshly prepared Worthington Statzyme-15 LDH-L reagent. The results, which are summarized in Table 2.37, show that low values were obtained from both rotors (runs 1 and 2) containing the lyophilized reagents. The rotors that contained freshly prepared reagent (runs 3-5) gave acceptable results.

To determine whether the low values obtained with the lyophilized reagent was due to a loss in NAD or lactate, the two major reactants in this assay, an experiment was performed in which the lyophilized reagent was fortified with either freshly prepared NAD or lactate. Two rotors containing the LDH-L reagent were assembled as previously described. Subsequently, the reagents of one rotor were reconstituted by adding 20 μ l (0.5 micromole) of a freshly prepared NAD solution plus 50 μ l of diluent to each reagent chamber and then transferring the contents as previously described in the glucose section. The same procedure was followed with the second rotor, except that 20 μ l (10.8 micromoles) of freshly prepared solution of lactate was used instead of the NAD solution; Worthington Statzyme was used as the reference sample. The results are summarized in Table 2.38. Lower values were again obtained from the rotors in which

Table 2.37. Comparison of LDH-L data obtained from preloaded (L) and freshly prepared (F) Worthington LDH-L reagents

Run number	Type of reagent	Reagent absorbance			Activity (IU/liter at 30°C)		
		N ^a	$\bar{X}$	C.V.	N ^a	$\bar{X}$	C.V.
1	L	4	0.2061	7.55	12	82.4	10.85
2	L	4	0.2429	7.51	12	56.2	9.48
3	F ^b	4	0.1887	2.34	10	171.1	1.04
4	F ^c	4	0.1347	3.01	12	164.5	1.30
5	F ^b	4	0.1895	3.07	12	171.6	1.51

^aNumber of observations.

^bReagent was prepared by dissolving the contents of an LDH-L Statzyme-15 vial in 3 ml of water. Reaction conditions: 40 μ l of reagent, 10 μ l of sample, 80 μ l of diluent.

^cReagent was prepared by dissolving the contents of an LDH-L Statzyme-15 vial in 4 ml of water. Reaction conditions were the same as those for runs 3 and 5.

Table 2.38. Comparison of LDH-L data obtained from fortified preloaded reagent (L) and freshly prepared (F) Worthington LDH-L reagents

Run number	Type of reagent	Reagent absorbance		Activity (IU/liter at 30°C)	
		$\bar{X}$	C.V.	$\bar{X}$	C.V.
1	L	0.2061	7.55	82.4	10.85
2	L	0.2429	7.51	56.2	9.48
3	L + NAD	0.2909	3.07	70.0	8.41
4	L + lactate	0.1716	10.73	94.5	7.81
5	F ^a	0.0806	3.41	183.9	0.52
6	F ^b	0.1283	3.52	172.6	0.71

^aReagent was prepared by dissolving the contents of a Calbiochem LDH-L Statpack in 2 ml of water. Reaction conditions: 20 μ l of reagent, 10 μ l of sample, 100 μ l of diluent.

^bReagent was prepared by dissolving the contents of a Worthington LDH-L Statzyme-15 vial in 3 ml of water. Reaction conditions: 40 μ l of reagent, 10 μ l of sample, 80 μ l of diluent.

the reagents were lyophilized, and fortification with either NAD or lactate failed to return the activities to levels observed for freshly prepared reagents. These results indicate that the LDH-L reagent is very unstable and that a different approach may have to be taken in loading the reagents for this assay. We had also experienced this problem with the LDH-L reagent in our feasibility studies,⁷ but were able to circumvent it by lyophilizing the LDH-L reactants separately (i.e., the NAD was lyophilized in the cuvetts and the lactate in the reagent chambers). This approach will be discussed with Worthington.

(Summary). These studies indicate that reagents can be lyophilized in the cuvetts of a rotor in situ by the reagent manufacturer and that they can be subsequently easily reconstituted and used for analysis. Some difficulties were encountered with regard to the quantities of reagent used for the various assays, but they can probably be eliminated by increasing the concentration of each reagent prior to lyophilization. The instability of the LDH-L reagent also poses a problem; however, it is hoped that this problem can be minimized by lyophilizing the reactants for this reagent in separate chambers of the rotor.

Reagents in tablet form. An alternative to in-situ lyophilization of the reagents is the use of reagent tablets. These tablets would be sealed into designated cuvetts and reconstituted prior to analysis. Ease and flexibility in loading are the attractive features of this approach. In addition to the general chemical problems expected to be encountered, potential physical problem areas with this approach would include dissolution time, difficulties associated with the manufacture and quality control of the small tablets (10 mg), and the extremely small quantities of

reactants that they would contain. Smith Kline instruments chose to follow this approach because they routinely manufacture and market the Eskalab line of reagent tablets and thus have had considerable experience with reagent tablet technology.

Smith Kline has produced two types of placebo tablets, containing different buffer formulations, for use in determining some of the physical problems we might encounter with small tablets. In addition, each tablet contains a given quantity of NADH, which will act as an optical marker to allow the properties of the tablet to be studied. The first type of tablet supplied was an effervescent (E) formulation (lot 30-3-89) which incorporated NaHCO_3 to effect effervescence to enhance dissolution of the tablet. Each tablet, which weighed approximately 10 mg, was formulated to give an absorbance of 1.0 at 340 nm when dissolved in 130 μl of water. The second type of tablet was a noneffervescent (NE) formulation (lot 30-3-90) having the same specifications as the E tablets.

To evaluate these tablets, four rotors were filled with each type of formulation by using tweezers to place individual tablets into cuvetts 2 through 17 of the rotors. The top cover windows were then bonded to the rotor body (the bottom window had previously been bonded to the rotor body), the entire rotor was placed in a press, and the adhesive was allowed to cure overnight at ambient conditions. Subsequently, the rotors were placed in plastic bags, which, in turn, were placed in desiccators at 0°C until used.

The tablets were reconstituted by using the SRL to load a total of 130 μl of water into the sample and reagent chambers (i.e., 60 μl in the sample chamber and 70 μl in the reagent chamber). The rotor was then placed on the analyzer and the contents of the chambers transferred into

their respective cuvetts by increasing the rotor speed. Both the transfer operation and the subsequent dissolution were visibly observed with the use of a stroboscope. The dissolution time was found to be a minimum when the liquid was transferred by increasing the rotor speed to 700 rpm, followed by a decrease to 100 rpm or less until the tablets were dissolved. If the rotor speed used in the transfer or subsequent dissolution was too fast, any particulate matter present in the cuvetts would sediment and pack against the peripheral walls and hence retard dissolution. After the tablets had dissolved, the solutions were mixed by one or more dynamic acceleration and braking cycles (i.e., rest - 4000 rpm - 0 rpm - 1000 rpm). The absorbances of the solutions were then measured at 340 nm at a rotor speed of 1000 rpm. For comparative purposes, fresh tablets were obtained from newly opened vials, dissolved in an appropriate quantity of water (20 tablets/2.6 ml), and the absorbance of the resulting solution measured at 340 nm. The data obtained in the initial study are summarized in Table 2.39.

As shown in the table, the E tablets dissolved much faster than the NE tablets (2 min vs 15 min). However, each type of tablet had a significantly lower absorbance than expected; decreases of 83% and 60% were obtained for the E and NE tablets, respectively, when compared with fresh tablets. In addition, even the tablets that were stored in the vials exhibited a loss in absorbance when compared with Smith Kline values. Thus the data obtained in this initial evaluation indicate that both types of the NADH-containing tablets are unstable. The results also indicate that most of the NADH is lost during the rotor assembly step or during storage, or both.

Table 2.39. Initial evaluation of placebo reagent tablets

Run number	Formulation	Dissolution time (min)	Absorbance (340 nm, 0.5 cm)		
			Mean	S.D.	R.S.D.
1	E	2.0	0.0695	0.0088	12.62
2	E	2.0	0.0767	0.0152	19.84
3	E	2.0	0.0576	0.0081	14.07
4	E	2.0	0.0684	0.0058	8.46
5	E ^a	-	0.3709	0.0039	1.05
	E ^b	-	0.436	0.003	0.70
6	NE	15.0	0.1349	0.0352	26.09
7	NE	5.0	0.1652	0.0181	10.97
		15.0	0.1701	0.0191	11.25
		30.0	0.1705	0.0193	11.25
8	NE	15.0	0.1622	0.0121	7.45
9	NE ^a	-	0.4155	0.0046	1.10
	NE ^b	-	0.436	0.0080	1.83

^aTwenty tablets of either E or NE formulation were dissolved in 2.6 ml of water. Absorbances of 16 aliquots were measured at 340 nm at a path-length of 0.5 cm.

^bData supplied by Smith Kline.

To minimize loss of NADH during assembly and storage of the rotors, a new technique was developed in which fresh tablets were removed from newly opened vials and immediately placed in their respective cuvetts. The adhesive was quickly applied to the rotor body, a top cover window placed upon it, and the assembled rotor put into a press. Each press and rotor were then immediately stored in a desiccator at 4°C for approximately 18 hr to allow the adhesive to cure. After curing, the rotors were removed from the presses and each placed in a plastic bag, which was then stored in a desiccator at 0°C until needed. At specified times, the rotors were removed from the freezer, the tablets dissolved as previously described, and the absorbances of the resulting solutions measured. The results from the second series of experiments are summarized in Table 2.40. The data indicate that less NADH was lost with the new assembly and storage procedure. Again, the E formulation was found to be less stable than the

Table 2.40. Second evaluation of placebo reagent tablets

Formulation	Storage time (days)	Absorbance (340 nm, 0.5 cm)			Percent decrease
		Mean	S.D.	R.S.D.	
E	0	0.3695	0.0088	2.39	-
	1	0.3310	0.0194	5.85	10.4
	27	0.2326	0.0144	6.19	37.0
NE	0	0.4041	0.0076	1.88	-
	1	0.3943	0.0202	5.12	2.4
	27	0.3782	0.0141	3.71	7.4

NE; it lost 37% of its NADH activity when stored 27 days, while the NE formulation lost only 7% during the same period.

On discussion of these data with Smith Kline personnel, it was mutually agreed that two additional types of placebo formulations should be tried. One type of tablet was a noneffervescent, placebo-PIPES (NEP) formulation (lot 30-3-104), with each 10-mg tablet formulated to yield an initial absorbance of 1.0 at 340 nm (1.0-cm cell) when dissolved in 130 μ l of water. The buffer used is PIPES (piperazine-N,N-bis-2-ethane sulfonic acid) monosodium salt, which eventually, when mixed with active raw materials, will constitute the glucose tablet. The second tablet was an effervescent (E-2) formulation (lot 30-3-103) with specifications similar to those of the NEP tablets. On receiving the new tablets, we loaded two rotors from each formulation using the new assembly and storage technique described previously. To evaluate the tablets, they were reconstituted as described, and the absorbances of the resulting solutions measured at 340 nm. Fresh tablets from newly opened vials served as controls. The results are summarized in Table 2.41. In general, the data obtained indicated that the NEP tablets were stable, while the E tablets were less stable and lost approximately 24% of their NADH content after only two days of storage. A large vial-to-vial variation was observed with the E tablets; a mean of 0.5901 ± 0.0449 (7.6%) was obtained when the data for runs 5-8 were statistically processed. A large within-rotor, tablet-to-tablet variation was observed for both types of tablets (runs 3, 4, 9, and 10). Upon inspection of the raw data, it was obvious that the random high values (which were periodically obtained) were approximately twice or three times the mean value for a given rotor. This suggests that two or

Table 2.41. Initial evaluation of second batch
of placebo reagent tablets

Run number	Formulation	Storage time (days)	Absorbance (340 nm, 0.5 cm)		
			Mean	S.D.	R.S.D.
1	NEP	0	0.5794	0.0016	0.28
2	NEP	0	0.5558	0.0039	0.70
3	NEP ^a	2	0.6104	0.1613	26.43
4	NEP ^a	2	0.5710	0.1036	18.14
5	E-2	0	0.6440	0.0038	0.58
6	E-2	0	0.5741	0.0208	3.62
7	E-2	0	0.6039	0.0032	0.53
8	E-2	0	0.5383	0.0021	0.40
9	E-2 ^b	2	0.4610	0.1315	28.51
10	E-2 ^b	2	0.4491	0.0796	17.73

^aDissolution time = approximately 15 min.^bDissolution time = approximately 2 min.

more tablets were inadvertently loaded per cuvet instead of the one tablet intended. Unfortunately, the small size of the individual tablets makes such an error difficult to detect and prevent when loading the rotors.

The high relative standard deviations observed in runs 3, 4, 9, and 10 led us to perform an experiment to directly measure the tablet-to-tablet variation for fresh tablets. In this experiment, individual, fresh tablets were dissolved in 1.30 ml of water and the absorbances of the resulting solutions measured at 340 nm. The experiment was repeated on the following day. The results show (see Table 2.42) relatively high tablet-to-tablet variations of $\pm 19.4\%$ and $\pm 30.9\%$ for the E-2 and NEP formulations, respectively. Upon inspection of the raw data, several tablets gave absorbances that were a factor of 2 or 3 higher than the mean absorbance for a given rotor. Again, this is probably due to the inadvertent placing of more than one tablet in each vial; however, the technique used to manufacture the tablets may also be involved.

The data obtained from the second batch of tablets were summarized and compared with specification data provided by Smith Kline (Table 2.43). In general, the measured absorbances of the tablets and vials measured by both groups agreed very well. The higher within-tablet variation observed in the ORNL evaluation was probably due to a loading error (as previously discussed).

In summary, four different types of placebo tablet formulations have been provided by Smith Kline Instruments. In general, the effervescent formulations will dissolve in 2 min or less, while the noneffervescent one may require up to 15 min for complete dissolution. Reagent stability is a problem with the effervescent formulation since tablets of this type

Table 2.42. Within-tablet variation of the second batch of placebo reagent tablets

Date	Run number	Formulation	N ^a	Absorbance (340 nm, 0.5 cm)		
				Mean	S.D.	R.S.D.
9-11-75	1	E-2	16	0.0583	0.0077	13.4
	2	E-2	16	0.0575	0.0165	28.7
9-12-75	1	E-2	16	0.0556	0.0080	14.4
	2	E-2	16	0.0586	0.0092	15.7
Combined		E-2	64	0.0575	0.0112	19.4
9-11-75	3	NEP	16	0.0597	0.0068	11.43
	4	NEP	16	0.0730	0.0315	43.1
9-12-75	3	NEP	16	0.0539	0.0089	16.6
	4	NEP	16	0.0562	0.0067	11.9
Combined		NEP	64	0.0608	0.0188	30.9

^aNumber of observations.

Table 2.43. Comparison of ORNL and Smith Kline evaluations of second batch of reagent tablets

Formulation		ORNL			SK		
		Mean	S.D.	R.S.D.	Mean	S.D.	R.S.D.
E-2	Within-tablet	0.0608	0.0188	30.89	0.059	0.009	15.2
	Within-vial	0.5901	0.0449	7.61	0.587	0.035	6.00
NEP	Within-tablet	0.0575	0.0112	19.37	0.059	0.0042	7.03
	Within-vial	0.5676	0.0030	2.6	0.557	0.0175	3.14

lost from 20 to 80% of their optical activity when stored in the rotors for two days or more. An improved technique in the assembly and storage of the rotors that contain tablets has decreased the loss of optical activity, especially in the case of the noneffervescent tablets. On the basis of this initial evaluation, a noneffervescent formulation is probably the preferred choice for future reagent tablets. This type of formulation requires a longer time for dissolution; however, the constituent reagent appears to be more stable. We are currently awaiting the shipment and arrival of glucose tablets in a noneffervescent formulation.

2.5 References for Section 2

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3. ADVANCED ANALYTICAL TECHNIQUES

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New analytical concepts are being considered for use in clinical and biomedical research laboratories. Some of these concepts, after initial testing proves their feasibility, will be developed into prototype systems which can be evaluated under realistic laboratory conditions. Ultimately it is hoped that successful developments will be produced by commercial firms to aid in the process of technology transfer.

3.1 High-Resolution Liquid Chromatographic Systems

Various disease states may give early warning of their onset or their continuing presence by subtle changes in excretion levels of molecular constituents in physiologic body fluids. Prompt medical treatment based on these early symptoms may prevent the disease from developing, retard the progress of the disease, or at the very least, minimize its debilitating effects. Detection and monitoring of these early symptoms will require that research and clinical laboratories be equipped with automated high-resolution analytical systems that will identify and quantitate many specific molecular constituents. To attempt to fill the need for specificity and high sensitivity, liquid chromatographic (LC) systems are being developed. These high-resolution systems, having the advantages of unattended operation and minimal sample preparation, enable a large number of important nonvolatile metabolites to be monitored with minimal use of skilled technical labor using automated data processing methods.

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3.1.1 Glycoprotein carbohydrate analyzer

We have continued routine use of gradient elution chromatography for the analysis of the protein-bound carbohydrates mannose, galactose, and fucose. Further studies have been performed on a LC system using a shorter column and employing isocratic elution to attempt to analyze the protein-bound carbohydrates in about 4 hr.

Shortened analysis time for protein-bound neutral carbohydrates.

Preliminary information published previously^{1,2} suggested the possibility of analyzing serum protein-bound mannose, galactose, and fucose by isocratic elution using a 0.22- by 50-cm column. While separation had been demonstrated using reference standard sugars in about the same relative amounts as expected in serum samples,^{1,2} analysis of a serum sample had not been attempted. Illustrated in Fig. 3.1 is the separation of a hydrolyzed serum sample using isocratic elution (0.168 M boric acid, 0.0075 M ammonium acetate, pH 7.7). Note the large peak at the beginning of the chromatogram representing breakthrough of nonadsorbed materials. The massive size of this peak relative to the three carbohydrate peaks results in considerable overlap of this peak with those of mannose and fucose, making quantitation of both carbohydrates difficult because of baseline uncertainties. Ethanol, used in precipitation of serum protein, was found to contribute to the size of the first peak, and resulted in a decrease in the size of the first peak when it was removed completely. However, baseline slope was still excessive.

In addition to excessive slope of the baseline, there are at least two peaks whose elution positions overlap that of fucose (see Fig. 3.1). This causes additional uncertainties in measurement of the small peak area

Fig. 3.1. Chromatographic separation of protein-bound mannose, fucose, and galactose in a hydrolyzed serum sample using isocratic elution. Note the baseline slope due to the massive peak at the front of the chromatogram.

for fucose. The origin and identity of these interfering peaks have not yet been determined; however, reversion of mannose, galactose, or fucose under these elution conditions has not been eliminated as a possibility, although not observed for standard carbohydrates. Accurate quantification of the relatively small fucose peak is not feasible with the peak overlap and the baseline slope shown in Fig. 3.1.

Measurement of sialic acid. The methyl pentose fucose is one of the terminal sugars of glycoproteins. The second terminal compound found in carbohydrate side chains of glycoproteins is n-acetylneuraminic or sialic acid. The former compound, with its terminal methyl group, is hydrophobic in nature, whereas the latter, with its terminal polar carboxyl group, is hydrophilic.

Evaluation of the efficacy of measurements for both fucose and sialic acid as biochemical markers of cancer is desirable. However, hydrolysis and chromatographic conditions for these two compounds differ substantially. Sialic acid is destroyed under hydrolysis conditions required to liberate fucose (1 N HCl for 4 hr), whereas fucose is not released under hydrolysis conditions which liberate sialic acid nondestructively (0.1 N H₂SO₄ at 80°C for 1 hr).^{3,4} This makes it virtually impossible to analyze both compounds in the same sample.

Elution studies of reference n-acetylneuraminic acid revealed that it could be analyzed chromatographically using ammonium acetate--acetic acid buffer at pH 4.4 with detection by the cerate oxidimetric detector system.² Linearity of both peak area and peak height was observed for amounts of sialic acid ranging from 6.5 to 39.1 µg eluted from the anion exchange column (see Fig. 3.2).

Fig. 3.2. Linear response of the cerate oxidimetric system is demonstrated for sialic acid eluted from a chromatographic system.

Stability of sialic acid to hydrolysis conditions. Five samples of n-acetylneuraminic acid were exposed to hydrolysis conditions of 0.1 N sulfuric acid at 80°C for 1 hr. The samples were cooled to room temperature and adjusted to approximately pH 4 with 1 N sodium hydroxide. The samples were analyzed by LC with an average recovery of 97.2% of the sialic acid and a coefficient of variation of 2.2%, indicating these hydrolysis conditions apparently would not affect sialic acid recovery.

Analysis of protein-bound sialic acid in a serum sample. Protein precipitated from a 0.5-ml sample of serum was subjected to the hydrolysis conditions described above. The hydrolyzed sample, after being adjusted to approximately pH 4, was placed on an anion exchange column (0.22 by 100 cm) and eluted with a concentration gradient of acetate buffer. One major chromatographic peak was eluted at about 7 hr; its elution position corresponded with the elution position observed for reference n-acetylneuraminic acid. Four other relatively minor peaks were observed along with the strong peak usually observed at the front of the chromatogram due to sample breakthrough.

Few extraneous peaks were observed in the chromatogram with gradient elution, and thus conditions were sought which would enable faster analysis of sialic acid without the necessity of column regeneration. A number of different concentrations of the acetate buffer were tested to find conditions which would enable separation of the sialic acid peak from the large peak occurring at sample breakthrough. Illustrated in Fig. 3.3 is the chromatogram obtained for a hydrolyzed normal serum sample eluted with 0.2 M ammonium acetate--acetic acid buffer at 60°C. Measurement of the area of this peak and comparison with standards as shown in Fig. 3.2 yielded a sialic acid content, in this serum, of 423 µg/ml. Further studies will be

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Fig. 3.3. Isocratic elution of a normal human serum sample which was hydrolyzed under mild conditions designed to release only sialic acid. Gradient elution, performed previously, had demonstrated that reference n-acetylneuroaminic acid coeluted with the peak designated as sialic acid.

performed with isolated glycoproteins to determine if recovery of sialic acid is linear with the amount of protein.

3.1.2 Experimental results and applications

We have continued analytical separations studies on physiologic fluids to demonstrate useful applications of high-resolution liquid chromatographic systems and detectors that have been developed. Emphasis is continuing on the separation and analysis of protein-bound carbohydrates in sera from breast cancer patients.

Analysis of neutral protein-bound carbohydrates. We have continued the routine analysis of protein-bound mannose, galactose, and fucose in sera from breast cancer patients as described previously.^{1,2} Multiple samples have been analyzed for patients in different stages of the malignancy. Clinical data describing the physical status of the disease and including serum protein analysis were available for some of the patients (see Table 3.1 and Fig. 3.4); additional data of this nature will be available at a later date.

The solid rectangle shown in Fig. 3.4 represents the range of values (fucose/protein, total hexose/protein) found for normal female subjects by LC analysis of hydrolyzed sera.^{1,2} The data shown in this figure for six patients whose clinical status was available are interesting. Three of the patients had their malignancy described as either in full or partial remission at the first sampling and were all within the range of normal values. Two of the three patients were described as having either disease progression or relapse at their next sampling (see patients 1 and 6, Table 3.1) and their analytical data had become abnormal (see Fig. 3.4). The third patient (No. 5, Table 3.1) was described as initially in partial

Table 3.1. Serum protein-bound neutral carbohydrates in patients with breast cancer

Patient number	Sample identification	Mannose (mg/100 ml)	Fucose (mg/100 ml)	Galactose (mg/100 ml)	Protein (mg/ml)	Clinical disease status
1	1523	46.8	5.3	51.6	80	Surgery - complete remission Relapse
	2065	57.2	7.5	58.3	75	
	2408	36.5	4.4	34.8		
2	252	47.2	5.6	43.0		Improving Partial remission No change
	1471	42.8	3.4	39.8	79	
	1604	50.2	3.8	46.1	78	
	2403	48.4	5.2	41.4		
3	1261	42.4	6.7	46.5	83	No change
	1669	49.9	5.9	50.0	75	Partial remission
4	1595	52.3	5.8	59.4	99	Complete remission
	2177	51.8	6.3	47.3	78	Progression
5	686	49.6	6.7	51.2	82	Partial remission
	1366	59.9	7.5	57.5	52	No change
	1854	50.4	8.9	57.3	75	Relapse
	1860	52.1	7.8	50.0		
6	740	46.7	5.4	45.2	86	No change
	1373	43.6	5.9	40.9	95	Partial remission
	1935	48.4	6.0	48.4	61	Relapse
	2466	35.9	8.6	35.8		
	3431	40.0	6.5	38.5		Progression
7	1312	65.9	6.6	62.1	79	No change
	1463	46.9	5.5	51.2	75	No change
	1599	39.3	5.2	37.1	81	No change
	2253	40.4	3.9	36.9		
8	1602	54.2	7.8	59.2		
	1815	46.7	6.6	51.6		
	3183	32.8	5.3	33.1		
9	680	44.6	6.9	46.8		
	1033	45.7	7.4	49.8		
	1229	52.5	10.0	60.0		
	3144	39.5	5.6	43.0		
10	1704	44.2	8.7	44.0		
	3315	43.1	5.8	55.3		

Table 3.1 (continued)

Patient number	Sample identification	Mannose (mg/100 ml)	Fucose (mg/100 ml)	Galactose (mg/100 ml)	Protein (mg/ml)	Clinical disease status
11	699	46.9	8.5	52.8		
	913	44.1	8.4	52.6		
	1295	44.5	8.0	42.7		
	1878	38.8	7.3	34.8		
12	2402	42.4	4.5	40.9		
	2742	30.6	6.1	35.6		
13	391	50.6	5.7	49.5		
	646	42.2	5.9	44.2		
	1015	51.9	6.1	57.9		
	1464	41.7	4.9	42.2		
14	346	51.6	5.9	54.0		
	1064	48.6	6.8	42.3		
	1622	44.7	5.7	42.7		
	2929	44.1	5.9	46.2		
15	268	46.0	7.6	60.9		
	1323	46.6	7.1	47.0		
16	342	71.8	8.2	74.4		
	1397	31.6	4.0	31.5		
17	362	42.5	4.4	50.0		
	887	39.1	4.4	42.3		
	1360	38.3	4.6	38.2		
	1903	46.2	4.0	45.5		
	3543	39.6	4.9	40.0		
18	454	53.9	6.7	62.1		
	885	47.3	8.4	66.8		
19	787	49.6	6.3	51.8		
	1272	40.0	4.6	40.9		
20	1758	55.1	4.5	59.1		
	2133	40.1	4.8	35.7		
21	2364	50.2	7.2	52.8		
	2932	47.6	6.3	48.5		
22	1232	49.4	6.4	56.1		
	1509	53.2	7.5	56.2		
23	392	57.4	5.3	50.8		
	971	51.4	4.9	55.0

Fig. 3.4. The effect of physical status of breast cancer patients on serum protein-bound fucose and mannose plus galactose.

remission, followed by "no change" and then suffered a relapse at the third sampling. Our analysis showed a change had occurred between the first and second samplings, and this change was magnified by a large drop in serum protein. The analytical change had preceded a clinically observable difference in the disease which is necessary for a biochemical marker of malignancy to be useful. However, patient 4 went from full remission to disease progression, while her fucose-to-protein and hexose-to-protein ratios remained within normal bounds. The remaining three patients (2, 3, and 7, Table 3.1) displayed little change in their clinical status and their analyses also remained normal; patient 7 did appear to be improving as indicated by a decline in both fucose-to-protein and hexose-to-protein ratios, although clinically there was no apparent change in her status.

3.2 Blood Sample Preparation System

N. E. Lee and J. E. Mrochek

Genetic mutation is commonly monitored by the determination of variant cellular isoenzymes using electrophoretic separation techniques coupled with staining of specific proteins. Meaningful results necessitate the analysis of large numbers of blood samples which, in turn, require a large amount of technical manpower to prepare cellular hemolysates for analysis. The blood sample preparation system described here is designed to separate whole blood into plasma and cells, to wash the cells, collecting samples of each fraction, and to facilitate the preparation of a lysed cell sample for electrophoretic analysis.^{5,6} Evaluation of early prototypes by personnel at ORNL and the University of Michigan demonstrated the feasibility of adapting all steps in the procedure to a sample preparation system;

however, problem areas were uncovered which required additional development effort.

3.2.1 Experimental system

A major problem uncovered by electrophoretic studies of samples prepared by the early prototypes has been the inability to fully sediment the stroma or cell debris in the lysate at the maximum rotor speed (2300 rpm) available in these early systems. Personnel at the University of Michigan found that centrifuging the system-prepared hemolysates at 18,000 rpm for 20 min did result in suitable samples for electrophoretic analysis, whereas samples without the high-speed spin yielded undesirable smearing of the electrophoretic patterns. Interlaboratory visits and communication between ORNL and University of Michigan personnel suggested that more efficient lysing procedures and more vigorous mixing of the hemolysate and the organic phase (carbon tetrachloride) might enhance the sedimentation of cell debris.

A third-generation prototype system (Figs. 3.5 and 3.6) has been fabricated to provide for more vigorous mixing of the cells during the washing and lysing steps of the procedure. The new prototype employs a rotating seal to enable a partial vacuum to be maintained above the liquids in the sample compartments of the rotor, causing air bubbles to be drawn into the liquids through the exit channels. In addition, a solenoid valve can be activated to cut off the vacuum and introduce bursts of air through the same exit channels to also enhance mixing.

Another problem area uncovered during work with the early prototypes was an undesirable liquid transfer during acceleration. The rotor was redesigned⁷ (Fig. 3.6) with modified syphon channels to eliminate this

Fig. 3.5. Front view of improved blood sample preparation system.

Fig. 3.6. Close-up view of new rotor designed for use in the blood sample preparation system.

problem. However, due to increased efficiency within the rotor, coupled with decreased cross-sectional area of the syphon channels, additional problems have surfaced. The rotor is fabricated of Plexiglas and, due to the inherent nature of its use, must be thoroughly sealed. The seal must withstand vigorous cleaning as well as contact with carbon tetrachloride, which is required during the lysing process to aid in sedimenting the stroma to yield hemolysates that will provide clean electrophoretic band patterns. As a result of this repeated contact of the rotor chambers with small quantities of carbon tetrachloride during lysing, severe crazing has occurred in the extreme outer regions of the chambers and along the cross-sections of the syphoning channels. To overcome these problems, alternative materials (i.e., polycarbonate) are being considered for rotor fabrication.

Another severe limitation encountered with this prototype system has been the elimination of the in situ rotor cleaning step. Operator workload has been increased excessively; however, a separate rotor washing station may overcome this problem.

3.2.2 Studies of system variables

Several lysing procedures were evaluated to optimize hemolysate preparations. The standard procedure used with this system has been to lyse the red blood cells by dynamically introducing 16 cc of distilled water into the rotor containing the cells; accelerating, agitating, and braking several times to facilitate mixing, followed by the dynamic addition of 8 cc of 0.05 M HCl made up in 0.005 M KH_2PO_4 ; repeating the mixing procedure; and adding 4 cc of carbon tetrachloride and again repeating the mixing procedure, accelerating to maximum rpm and centrifuging for 15 to 20 min to remove stroma. The following alternative lysing procedures were tested:

- (1) Doubling the volume of carbon tetrachloride to 8 cc.
- (2) Increasing the volume of water to 20 cc and carbon tetrachloride to 8 cc, while eliminating $\text{HCl/KH}_2\text{PO}_4$.
- (3) Increasing the volume of water to 24 cc and $\text{HCl/KH}_2\text{PO}_4$ to 8 cc, while eliminating carbon tetrachloride.
- (4) Adding 8 cc of 0.05 M KH_2PO_4 (pH 4.0) together with 16 cc of water and 4 cc of carbon tetrachloride.
- (5) Adding 24 cc of 0.005 M KH_2PO_4 (pH 6.0) and 4 cc of carbon tetrachloride.

The results obtained by each of these procedures were equally poor. A commercial hemolysing agent, Saponin, was obtained, and concentrations of 0.01%, 0.1%, 0.25%, 0.5%, 0.75%, and 1.0% by weight in distilled water were prepared. The standard lysing procedure described above was followed except that Saponin-water replaced water. Samples of the hemolysates from each run were then subjected to starch gel electrophoretic analysis which showed promise for concentrations of Saponin equal to or greater than 0.5%.

3.2.3 Electrophoretic results on experimentally prepared hemolysates

The criterion for judging the quality of the hemolysates obtained from this system is electrophoresis and subsequent staining of various isoenzyme systems. And although activity for several isoenzyme systems has been demonstrated, totally acceptable hemolysates have not yet been prepared using prototype systems. This is primarily due to insufficient aggregation of the cell stroma when carbon tetrachloride is added. When the standard lysing procedure, described above, is used with either 0.5%, 0.75%, or 1.0% Saponin solution, and the resulting hemolysate is subjected to an additional

20 min in a Sorvall centrifuge at 18,000 rpm, the heretofore nonsedimented stroma form a nicely packed bead and electrophoretic results are excellent.

The enzymatic activity of lactate dehydrogenase (LDH) as obtained directly from the system and after additional Sorvall centrifugation is evidenced by a comparison of starch gel electrophoresis results. Figure 3.7 (top) clearly indicates a high degree of band smearing for samples from the system, while (bottom) a clean, well-defined band resulted after Sorvall high-speed spin. A further comparison in Fig. 3.8 (top, system; bottom, Sorvall) clearly shows the advantage of the Sorvall centrifugation step in eliminating band smearing when starch gel electrophoresis for malic dehydrogenase (MDH) is performed.

For the initial studies conducted with this prototype system, only about 30% of the total capability for air injection was utilized and samples were centrifuged at 2200 rpm for 20 min after lysing. Later studies have utilized the full capability for air injection, and centrifugation has been extended to 30 min at 2600 rpm. Acrylamide gel electrophoresis and subsequent staining of the LDH isoenzymes revealed improved resolution and decreased band smearing without additional Sorvall high-speed spinning.

3.3 Automated Elution Electrophoresis

R. K. Genung and S. K. Whatley*

Earlier reports⁸⁻¹⁰ of the development of automated elution electrophoresis demonstrated the feasibility of attaining the following advantages over the electrophoresis systems most commonly used in clinical laboratories (i.e., electrophoresis on cellulose acetate strips):

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Fig. 3.7. Lactate dehydrogenase isoenzyme determination by starch gel electrophoresis of cell hemolysates prepared using the blood sample preparation system. Note the improvement in electrophoretic pattern after sedimenting stroma by high-speed centrifugation.

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Fig. 3.8. Malic dehydrogenase isoenzyme determination by starch gel electrophoresis of cell hemolysates prepared using the blood sample preparation system. Note the improvement in electrophoretic pattern after sedimenting stroma by high-speed centrifugation.

- (1) A simplification of the operational sequence required for each analysis¹⁰ to a rapid and easy sample introduction;
- (2) Usage of high voltage gradients (with forced cooling of the column) and forced elution, resulting in a reduction of the time required for analysis to approximately 1 hr (typically 4 to 24 hr);
- (3) An increase in resolving capability; and
- (4) Automation of the results with quantitation of the separated constituents in the eluate by a flow photometer or colorimeter.

While the prototype system successfully demonstrated the operating concept, much work remained to develop an elution electrophoretograph appropriate for use in routine clinical analyses. Also, no attempt was made to optimize the buffer system or other operating parameters.

Recent development efforts have been directed toward defining routine operating procedures which would give reproducible results with acceptable resolution. Some investigation of different column designs, packing materials, and buffer systems has been performed. All work has been done with serum protein samples.

3.3.1 Experimental system parameters

Columns. Both horizontal and vertical column arrangements were investigated. The horizontal system is shown in Fig. 3.9; the same column was used in the vertical system with minor adaptations to allow electrolysis gases to escape. Column diameters of 3.4 mm and 4.2 mm were used, and effective lengths of bed packing varied from 20 to 40 cm (protein migration was always anodic for the buffer systems used, and sample introduction septa were accessible at both the center and cathodic end of the packing).

Fig. 3.9. Schematic diagram of the elution electrophoresis system.

Packing materials. Two packing materials were tried, Bio-Gel P-2 and Ultrogel AcA 54. The addition of 4% agarose to the usual polyacrylamide gel matrix distinguishes the Ultrogel from the Bio-Gel. The exclusion range of the Bio-Gel was 100 to 1800 daltons, while the range for Ultrogel was 6,000 to 70,000 daltons based on measurements with globular proteins. The molecular weight of the smallest serum protein, albumin, is approximately 69,000 daltons. Therefore, porosity of the packing material was of negligible importance.

Buffer systems. Two buffers with varying molarities and ionic strengths were tried. A Tris-barbital--sodium-barbital buffer which has produced high resolution with other electrophoresis systems was tested at pH 8.6 for ionic strengths of 0.01, 0.1, and 0.3. A phosphate buffer was used routinely at pH 8.6 and at 0.125 and 0.25 M concentrations.

The barbital system (see Table 3.2) was unsatisfactory for two reasons. First, the system was stable only in the pH range of 8.2 to 8.9 and formed precipitates outside that range. Thus, the pH changes occurring in the electrode chambers degraded the buffer, and the resulting precipitates were washed through the eluate monitoring system. The second disadvantage of the buffer system was its high uv absorbance which also varied significantly with pH changes in the electrode chambers. Both effects resulted in artifact peaks demonstrable by electrophoresis in the absence of a protein sample.

The sodium phosphate buffer gave a much lower background absorbance with approximately one-half the changes in absorbance observed with the barbital buffer, even though larger pH changes occurred in the electrode chambers. This buffer was acceptable for routine use.

Table 3.2. Variation in buffer absorbance and pH values during elution electrophoresis

	pH	uv absorbance	Percent transmittance
<u>Tris-Barbital--Sodium-Barbital</u>			
Ionic strength, 0.01			
Before electrophoresis	8.6	0.290	51.0
After electrophoresis - upstream	9.2	0.340	45.5
- downstream	8.3	0.340	45.5
Ionic strength, 0.3			
Before electrophoresis	8.6	0.94	11.5
<u>Sodium Phosphate</u>			
0.25 <u>M</u>			
Before electrophoresis	8.5	0.04	91.5
0.125 <u>M</u>			
Before electrophoresis	8.5	0.02	95.5
After electrophoresis - upstream	10.4	0.05	89.5
- downstream	7.5	0.04	91.0

Voltage gradients and elution rates. Electrophoresis was performed with applied voltages ranging from 200 to 1500 V (producing from 5 to 40 mA of current) for time periods of 15 to 90 min. Elution flow rates varied from 0 to 15 ml/hr across the column during electrophoresis. Elution flow rates after hydrostatic electrophoresis were routinely 6 ml/hr. Protective flows away from the anodic electrode chamber were routinely 2 ml/hr.

3.3.2 Visualization studies

A 0.1% solution of bromophenol blue dye was used in a ratio of 1:1 with both 4.3% albumin preparations and whole serum samples. Introduction of the albumin-dye sample into the column clearly demonstrated the need to extract the microsyringe from the septum very slowly. Otherwise, sample rather than packing would fill the void created by forcing the syringe into the column. Samples drawn into the introductory port washed very slowly back into the column during a run and produced broad, trailing peaks.

Initially, sample introduction and elution were performed without electrophoresis. Separation of stained albumin from excess dye was easily achieved in this manner. Three color regions were observed with whole serum samples, probably marking excess dye, albumin, and globulins. It was observed that sample bandwidths depended, as noted, on sample introduction and that the protective flows directed out of the electrode chambers were effective. Samples either introduced or eluted from column areas outside the packing were observed to mix excessively.

In some experiments, elution flows were used to resolve dyed albumin from excess dye, followed by the establishment of hydrostatic conditions in

the column. Electrophoresis at 1000 V using the phosphate buffer produced no visual movement of dyed albumin within 45 min.

3.3.3 Status of development

Reproducible results have been obtained by these authors with this system, but not with resolution that will compete with electrophoresis on cellulose acetate strips as originally demonstrated.⁸⁻¹⁰ No significant differences in resolution were obtained by varying the parameters described within the ranges defined. It is probable that performance-governing parameters for this system have not yet been identified. Presently, efforts are being made to define conditions favorable for electrophoretic mobility. These efforts include studies of the resistances for gradients associated with the dc-field applied across the column as well as the current-carrying properties of different buffer systems. Studies with a Tris-borate buffer are planned since borate may make glycoproteins in serum more negatively charged by complexing carbohydrates in these macromolecules. This higher negativity might then make them more susceptible to electrophoretic migration.

3.4 Centrifugal Chromatography

A new, removable-column rotor has been developed which greatly enhances the experimental flexibility and operation of the prototype system for centrifugal elution chromatography.^{9,11} New inorganic immunochemical sorbents have been prepared which appear to overcome problems (pluggage, etc.) exhibited by gels when used with the centrifugal elution chromatograph system. The new immunosorbents have been used with the removable-column rotor to study affinity separations of IgG from human serum.

Additional studies will emphasize the determination of several serum proteins (e.g., human immunoglobulins) simultaneously from a single sample.

3.4.1 Development of a removable-column rotor

The new, removable-column rotor (Fig. 3.10) accepts up to eight chromatographic columns. The assembly consists of a plastic center insert for partitioning the sample and eluent stream among the separate flow channels. A flow channel consists of a chromatographic column followed by a flow-through cuvet. The final element is a retainer clip with center screw which is used to compress and secure the assembly within the rotor. Center inserts presently available permit operation in either the four-column or eight-column mode. Four-column sets are available with 0.238-, 0.318-, 0.397-, or 0.476-cm ID by 10 cm in length. The assembled rotor, containing four chromatographic columns, is shown as a part of the centrifugal elution chromatograph in Fig. 3.11.

3.4.2 Preparation and testing of inorganic immunochemical sorbents

In a previous report,¹ affinity separations were discussed which utilized a gel (Sephadex G-25) as the support for an immobilized antibody (antihuman IgG). Although affinity separations of human IgG from human serum were achieved, the gel exhibited unsatisfactory characteristics with regard to flow of eluent through the gel bed. Under the centrifugal forces used to produce the desired eluent flow rate, the bed tended to compress and bridge, forming both densely packed and void regions within the flow channel. After searching the literature, it was concluded that inorganic supports such as alumina or titania might be more suitable for use with our centrifugal elution system. Such particles would be more rigid than gels and less expensive than controlled-pore glass.

Fig. 3.1C. Removable-column rotor for centrifugal elution chromatography. Components include center insert, columns, flow-through cuvettes, and retainer clips.

Fig. 3.11. Assembled rotor containing four columns, shown as a part of the centrifugal elution chromatograph.

The first immobilization technique attempted was adsorption of anti-human albumin on 70- to 100-mesh alumina. Although we were able to adsorb approximately 22 O.D. units of antihuman albumin per gram of alumina, the immobilized antibody exhibited insufficient immunological activity. A technique was then developed for covalent attachment of proteins to alumina using cyanogen bromide activation of the support. Alumina (70 to 100 mesh) was washed first with distilled water to remove brown coloration and then with 0.1 M sodium phosphate buffer, pH 7.0. Fifteen grams (dry basis) of alumina and 20 ml of 0.1 M sodium phosphate buffer were placed in a beaker in an ice bath. The solution pH was maintained at 11.0 during addition of CNBr (4.5 g). The reaction mixture was then moved to a coldroom and gently rotated for 3 hr. The alumina was washed with 0.1 M sodium phosphate buffer to remove adsorbed but noncovalently bound protein. Finally, the immunosorbent was reequilibrated with the eluting buffer for the ensuing experiments in centrifugal elution chromatography.

3.4.3 Experimental results on the affinity separation of IgG from human serum

The immunosorbent prepared by covalent attachment of antihuman IgG to alumina was slurry packed into four columns 0.318 cm ID by 10 cm long. The rotor was assembled as previously described and placed in the centrifugal elution chromatograph housing. The rotor was spun at 400 rpm with a steady flow of 0.1 M sodium phosphate buffer (pH 7.0) through the four columns. A 40- μ l volume of human serum, diluted to 1 ml with buffer, was dynamically injected into the splitting vanes of the rotor insert. The nonadsorbed proteins are eluted as a large peak on the chromatogram (Fig. 3.12). A second peak consisting of nonspecifically adsorbed proteins was eluted with

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SAMPLE: 10 μ l / COLUMN OF HUMAN SERUM
 STATIONARY PHASE: ANTI (HUMAN) IgG IMMOBILIZED ON
 70-100 MESH ALUMINA
 ELUENTS: 0.1 M NaH_2PO_4 - Na_2HPO_4 BUFFER, pH 7.0
 1.0 M NaCl IN 0.1 M PHOSPHATE BUFFER, pH 7.0
 3.0 M NaSCN IN 0.1 M PHOSPHATE BUFFER, pH 7.0

BASELINE REFERENCED TO ABSORBANCE
 PROPERTIES OF ELUTING BUFFERS

ROTOR: 4 COLUMN MODE
 COLUMN DIMENSIONS: 0.32 cm I.D. x 10cm
 ROTOR SPEED: 400 RPM

Fig. 3.12. Affinity chromatogram for the separation of human IgG from normal human serum on a centrifugal elution chromatograph. Immunosorbent was antihuman IgG covalently attached to alumina.

1.0 M NaCl in phosphate buffer (pH 7.0). Finally, the peak for the immunosorbed protein of interest, IgG, was eluted with 3.0 M NaSCN. Serum sample sizes studied were 100, 50, 25, and 10 μ l. The 10- μ l sample produced a sizeable IgG peak (third peak, Fig. 3.12), reflecting excellent sensitivity of the detection system.

Additional experiments will be performed to optimize the sample size of human serum. The capability of the system for performing rapid determinations of several different serum proteins (e.g., human immunoglobulins) from a single sample will be demonstrated.

3.5 References for Section 3

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4. BIOENGINEERING RESEARCH AND DEVELOPMENT

Insight into many biological mechanisms and processes has increased significantly during the past 20 years. We are currently investigating techniques that will allow certain biological systems (especially microorganisms or active fractions of microorganisms) to be used in a process mode. This investigation has advanced to the point where some of these systems have been adapted to serve as specific process steps in various areas of technology. This application represents a radical departure from the traditional use of biological processing with activated sludge and trickle-filter units. Important biochemicals used in support of these and other efforts are being produced and isolated. Pertinent enzyme-catalyzed reactions, as well as techniques for stabilization of the enzymes, are being studied.

4.1 Enzyme Catalysis

B. Z. Egan and J. P. Eubanks

Previous reports^{1,2} described the production of hydrogen by the use of hydrogenase and ferredoxin enzymes to catalyze the reduction of water with sodium dithionite. Experiments aimed at increasing the rate of hydrogen production were continued. Studies were also initiated to determine kinetic parameters associated with the hydrolysis of lactose by lactase. These parameters were important in the design and interpretation of engineering experiments described in Sect. 4.5.

4.1.1 Rates of hydrogen production using ferredoxin-hydrogenase

One of the most effective variables for changing the rate of hydrogen production by reduction of water with sodium dithionite is the concentration

of ferredoxin and hydrogenase. A Clostridium pasteurianum cell extract was prepared in such a way that about 10 ml of cell extract containing ferredoxin and hydrogenase was obtained per gram of dry cells. The cell extract was used without further separation and purification of the components. Enzyme concentration was measured as the volume of cell extract added to the dithionite solution. The cell extract was then distilled at a reduced pressure after dialysis in order to obtain higher enzyme concentrations than were present in the cell extracts.

Hydrogen formation was measured by pressure changes using a Warburg manometer apparatus. Measurements of hydrogen production rates using this apparatus allowed us to collect 12 to 15 data points in the first 10 min, compared to 1 to 2 points using gas chromatography. The reaction is frequently complete in 10 min. The Warburg flask and manometer was calibrated using CO_2 produced from the reaction of sodium bicarbonate and hydrochloric acid. A typical reaction mixture contained 4 ml of 1 M HCl and 0.100 ml of 0.100 M NaHCO_3 at 40°C. Figure 4.1 shows a calibration curve obtained for one of the Warburg flasks. A manometric fluid with a specific gravity of 1.000 was used. A second "dummy" flask and manometer was used to correct for any changes in temperature and barometric pressure during a run. It was necessary to maintain a constant temperature within 0.1°C. A typical flask volume was 25.5 ml.

The effect of increasing the enzyme concentration in the reaction mixture on the hydrogen production rate is shown in Fig. 4.2. Measurements were made on small volumes of solution to facilitate easier measurement of pressure changes. Each reaction was run in 0.05 M potassium phosphate buffer (pH 6.6) at 40°C. Flask 1 (curve 1, Fig. 4.2) contained 0.0175 g

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Fig. 4.1. Calibration curve for Warburg flask and manometer.

Fig. 4.2. Hydrogen production rates at high enzyme concentrations.

of $\text{Na}_2\text{S}_2\text{O}_4$ in 5 ml of buffer plus 0.25 ml of cell extract; flask 2 (curve 2, Fig. 4.2) contained 0.0140 g of $\text{Na}_2\text{S}_2\text{O}_4$ in 4 ml of buffer plus 0.5 ml of cell extract; flask 3 (curve 3, Fig. 4.2) contained 0.0100 g of $\text{Na}_2\text{S}_2\text{O}_4$ in 1.0 ml of buffer plus 2 ml of enzyme. In each case, the final $\text{Na}_2\text{S}_2\text{O}_4$ concentration was about 0.003 g/ml. To obtain curve 4 (Fig. 4.2), 6.0 ml of cell extract was dialyzed against 4 liters of 0.005 M potassium phosphate (pH 6.6) saturated with argon. The dialysate was evaporated at a reduced pressure to 0.5 ml and transferred to a Warburg flask with 0.5 ml of water. One milliliter of buffer containing 0.0140 g of $\text{Na}_2\text{S}_2\text{O}_4$ was added, and the hydrogen production was measured. The maximum initial rate obtained in curve 4 (Fig. 4.2) corresponds to a hydrogen production rate of about 6.0 liters (STP) of hydrogen per hour per liter of solution. Further increases in the rate should be obtained by separation and purification of the enzymes.

4.2 Enzyme Stabilization and Immobilization

Certain advantages are to be gained in bioprocess development by attaching the biochemical catalysts (with or without their microorganisms) to a solid support. This approach is being investigated for several enzyme systems.

4.2.1 Hydrogenase-ferredoxin system (B. Z. Egan and J. P. Eubanks)

Several efforts have been made to immobilize the hydrogenase-ferredoxin enzyme system using the Clostridium pasteurianum cell extract. Results with polyacrylamide gels and collagen were reported previously.¹ We have investigated three more possible methods of immobilizing this

enzyme system: (1) hollow fibers, (2) covalent binding through gluteraldehyde to aminoethyl Bio-Gel P-150,* and (3) adsorption on alumina.

A schematic diagram illustrating the hollow fiber method is shown in Fig. 4.3. A hollow fiber cartridge (HLD P-10, Amicon Corporation, Lexington, Massachusetts) was washed and filled with 0.05 M potassium phosphate buffer (pH 6.6). The buffer inside the fibers was displaced with about 5.5 ml of cell extract. A solution of sodium dithionite containing a total of 0.21 g of sodium dithionite in 60 ml of 0.05 M phosphate buffer was circulated through the outer jacket at 3.5 ml/min. The dithionite solution was collected in a closed reservoir and recycled to the hollow fiber cartridge. Gas samples were taken above the dithionite solution in the reservoir at timed intervals and analyzed for hydrogen by gas chromatography. Hydrogen evolution was measured for 2 days with the system at room temperature. A total of about 0.5 ml (23 micromoles) of hydrogen was produced.

In a second method, Bio-Gel P-150 (containing aminoethyl groups) was reacted with gluteraldehyde, and the enzymes were then bound to the aldehyde. One gram of Aminoethyl Bio-Gel P-150 was reacted with 20 ml of a 12.5% glutaric dialdehyde solution (pH 6.8). The resulting gel was reacted with an equal volume of cell extract containing ferredoxin and hydrogenase. The reaction of the enzyme-treated gel with sodium dithionite was measured in a batch reaction at 40°C. Based on the rate of hydrogen production, the activity of the bound enzymes was estimated to be $\leq 0.5\%$ of the activity of the free enzyme. Apparently, the enzymes did not bind completely to the gluteraldehyde-treated gel, since enzyme

* Product of Bio-Rad Laboratories, Richmond, California.

Fig. 4.3. Schematic diagram for enzyme immobilization in hollow fibers.

activity remained in the enzyme solution used to treat the gel. Additionally, the gel did not inactivate the enzyme. It was also determined that dithionite did not react with the gluteraldehyde-treated gel alone.

In another method, the enzyme system was also adsorbed on alumina. Two grams of alumina was washed with buffer solution and then mixed with 4 ml of cell extract. After several hours the enzyme solution was removed and the alumina was washed with buffer solution. The resulting solid was reacted with 10 ml of solution containing 0.070 g of $\text{Na}_2\text{S}_2\text{O}_4$ in 0.05 M phosphate buffer (pH 6.6). The hydrogen production rate is shown as curve 1 in Fig. 4.4. The enzyme activity remaining in the cell extract used to treat the alumina was measured in a similar reaction. In this case, 0.5 ml of this cell extract was added to the dithionite solution. The hydrogen production rate is shown as curve 2 in Fig. 4.4. While the activity of the alumina-adsorbed enzymes was higher than the Aminoethyl Bio-Gel P-150 immobilized enzymes, the adsorbed activity on the alumina was still low--probably less than 1% of the activity of the free enzymes. As shown in Fig. 4.4, enzyme activity remained in the cell extract used to treat the alumina; the supernatant contained about 10% of the activity of the original cell extract. Use of gluteraldehyde in an intermediate step in the adsorption did not significantly affect the activity of the bound enzymes. In this procedure, 2 g of alumina was mixed with 2 ml of cell extract and the recovered solid was treated with 4 ml of a 5% glutaric dialdehyde solution. The resulting product, when reacted with the dithionite solution, gave a hydrogen production rate comparable to the non-gluteraldehyde-treated material (curve 3, Fig. 4.4). The use of the

Fig. 4.4. Activity of ferredoxin hydrogenase on alumina.

alumina-supported enzymes in a small, tapered fluidized bed is described in Sect. 4.3.5.

4.2.2 Immobilization of catechol oxygenases, lactase, and immunoglobulins (D. W. Holladay and Janie Cohill)

Previously,¹ many attempts with various immobilization techniques had yielded unpromising results (either no or inacceptably low activity) in the effort to immobilize catechol oxygenases from Trichosporon cutaneum. In consideration of the success recorded by others^{3,4} in immobilizing enzymes to alumina through either adsorption or covalent linkage, several experiments were initiated with this support. Alumina possesses potential as a relatively desirable bioreactor support. The advantages of alumina include (1) inertness, (2) low flow resistance, and (3) good fluidization properties. The disadvantages are (1) friability, and (2) nonbiospecific adsorptive action.

Crude oxygenase preparations were contacted (for sorption) with washed alumina (pH 7.6) in both batch reactions and as slug feeds to fluidized alumina beds. In those cases, a continually interfering complication to catechol assays was the considerable affinity between catechol and alumina. After this spurious effect was negated, very little enzyme could be detected as being actively adsorbed to the alumina. Alumina was also activated with aminopropyltriethoxysilane (APTS) in acetone, followed by activation of the added amine with glutaraldehyde, so that the crude enzymes could attach to the free aldehyde group. It was determined from a control prepared exactly as the derivative (but without enzyme addition) that most of the catechol that was removed from a batch reaction with the alumina-APTS-GAH-enzyme derivative was due to nonbiospecific adsorption.

The most recent immobilization method attempted with catechol oxygenase was diazotization.⁵ This technique consists of (1) derivatization of a support to yield a terminal amine, (2) reaction with this aminoalkyl derivative to yield a p-nitrobenzamidoethyl derivative, (3) reduction of this latter compound with sodium dithionite, so that the diazotized p-aminobenzamidoethyl derivative will react with tyrosyl residues on the enzymes. However, the complexity of the reactions involved in this procedure increases the possibility of several side reactions that may interfere with the desired sequence. After the crude extract was reacted with the diazo derivative, no enzyme activity could be detected.

Increased attention in the enzyme engineering research community is being focused on the significant problem of enzyme stability (particularly for those with little previous study) since, without prolonged stability, an immobilized enzyme is inferior to the whole bacteria or soluble enzyme. As additional information becomes available, new immobilization techniques and stabilization efforts (pre- and post-treatment) will be applied to the endocellular catechol oxygenases vital to the bacterial degradation of phenols.

There were two other projects in the biotechnology group that were dependent upon a knowledge of protein chemistry and protein-support compatibility. These projects were two successful applications of the support alumina for bioreactor studies: (1) the immobilization by simple adsorption to alumina (with subsequent crosslinking) of the enzyme lactase (for use in baseline enzyme and bioreactor studies), and (2) the immobilization of antibodies (against human IgG) to cyanogen-bromide activated alumina (for use in clinically related serum protein assays). The latter

immobilization technique is very new (based on Weetall's work⁶), and its potential for activation of inorganic supports should generate much future interest because of its possible use in enzyme engineering applications.

4.3 Bioprocess Development

A bioprocess is an entire collection of steps (at least one of which involves a biological entity) where a known feed material is modified or transformed by a chemical or physical process into the desired product. The biological entity may be either a living organism or an active fraction derived from a living organism. Since each of the steps constituting a bioprocess is subject to variations, the development of the bioprocess must determine the effects of changes on the entire process to arrive at the optimum configuration.

The existence of a bioprocess implies a change of incoming material to desirable products. Compared to typical chemical processes, bioprocesses are particularly applicable to systems where (1) reacting species are at low concentrations, (2) specific reactions are required, or (3) desired products can only be obtained by biochemical reaction.

As in any process development, the questions that must be addressed are: What is the appropriate type and size of equipment needed? What is the optimum sequence of unit operations to achieve the desired products? Among other things, the following must be considered: (1) the viability of the organisms used, (2) the reaction rates attainable, and (3) the economics of the overall process compared with various competitive processes. Potential areas where bioprocessing may have an attractive future include: (1) removal of organic contaminants from aqueous streams,

(2) removal of sulfur from finely ground coal before further processing, (3) removal of trace metallic or organometallic constituents from an aquatic environment, and (4) recovery and/or production of products of value from worthless or low-value feed streams.

Biological process steps are being investigated and developed for use in energy production, resource recovery, and the abatement of environmental pollutants. The approach is oriented in the direction of bench-scale engineering studies that will be the first step toward proving process feasibility. Currently, the two specific process areas which are being investigated are: (1) the use of continuous-flow bioreactors for the abatement of organic pollutants in the effluent streams of energy production facilities, especially coal conversion processes; and (2) the use of bioreactor systems for the concentration or removal of dissolved metals (including useful resource materials and radioactive waste materials from process waste streams).

4.3.1 General bioreactor development (D. W. Holladay and Mildred McClure)

For about 1 year, a program has been evolving with the purpose of developing the best plan of treatment for removal of biohazardous chemicals from waste liquors. Waste liquors of particular interest are those that will be by-products of coal gasification/liquefaction processes.

In a previous report,⁷ expressions were derived for the conversion obtained with plug flow of reactants in an immobilized-enzyme packed bed, using as the rate expression (r), either (1) simple Michaelis-Menten (M-M), (2) M-M with product inhibition, or (3) M-M with substrate inhibition, where the integration is made for retention time,

$$\tau = C_i \int_0^x \frac{dx}{r}.$$

The applicability of M-M kinetics usually assumes the most idealistic and compliant of systems, whereas in practicality of operation there may be considerable complications (e.g., external and internal mass transfer diffusional resistances). These models, however, often serve to provide initial insight into the kinetics and rate mechanisms of enzyme systems.

Previously, continuous stirred-tank bioreactors (CSTRs) and packed-bed bioreactors (PBRs) had been operated on a bench scale to study the conversion parameters for the degradation of phenol in a controlled high-nutrient medium by a single pure organism (a yeast). The results that were obtained¹ delineated certain guide-line values for the conversion of phenol that could be expected as a function of reactor volume and flow rate. However, operational difficulties were posed by the bioreactors whose active agents were limited to containment of only one strain. Unless extensive and expensive efforts are made to guarantee aseptic conditions, the bioreactive agent will be invaded by indigenous laboratory populations (almost always resulting in the destruction of the pure strain).

In order to circumvent the complexities of maintaining a single population, we decided to switch to a more natural concept (already the industrial operational procedure) of using mixed symbiotic populations of bacteria as our bioreactive agents. From a search of the literature, it was concluded that one of the best industrial operations utilizing biodegradation similar to our projected needs was the Bethlehem Steel Company's activated sludge unit that has been successfully treating weak ammoniacal liquors (from coking processes) for 14 years. Arrangements were made to obtain a sample from Bethlehem's aeration unit to serve as a seed inoculum for our bioreactors. In exchange for this sample, we aided

Bethlehem in determining the bacterial strains present in their sludge since they had never ascertained the nature of their population. A service microbiological identification laboratory at the Biology Division reported that the Bethlehem population contained between 10 and 15 bacterial species, including the following: Bacillus, Staphylococcus, Pseudomonas, Citrobacter, Proteus, and Escherichia coli. Fortunately, for safety considerations in handling this population, no pathogenic species were observed.

First, we had to determine if this population could be transplanted and established in our process. The Bethlehem population is a mixed, very symbiotic, group of aerobic bacteria. Years of adaptation have made these bacteria finely balanced so that they may tolerate (and in most cases, degrade) many toxic organic and inorganic chemicals. Among the chemicals that the bacteria may tolerate are phenols, cresols, catechols, and thiocyanates. They do not have significant capability to degrade ammonia and cyanide.

Thus, our first goal was to begin operation of a CSTR using the Bethlehem sludge as the bioreactive agent and a scrub liquor from the coal hydrocarbonization process at ORNL as the feed. Our next goal was to obtain operation of this bioreactor comparable in conversion to the Bethlehem process. If this process could be established, then our subsequent goals were to develop three-phase (solid support for active surface, liquid feed, and air to supply oxygen to the aerobic bacteria) packed beds and three-phase fluidized beds using the symbiotic population. When a stable operation was achieved for these bioreactors, the optimum type or combinations could be deduced for the specific conversions required and a

more in-depth study of the mass transfer and hydrodynamic characteristics of the reactors could be pursued; sound, basic correlations would be required for future scale-up. It is of interest that the hydrodynamics of the three-phase fluidized bed have not yet been adequately correlated and no clear correlations exist for mass transfer in three-phase packed fluidized beds.

4.3.2 Continuous stirred-tank reactor (D. W. Holladay)

Some baseline figures for the Bethlehem plant are a presumed capacity of 150,000 gpd of weak ammoniacal liquor with a phenol content of 5000 ppm. The actual operating conditions at the Bethlehem plant for treatment of 110,000 gpd of liquor with a phenol content of 1500 ppm will be used for comparison with the operation of the ORNL scrub liquor CSTR (see Table 4.1). The production or degradation rates have been calculated for phenols only, as complete description awaits development of a full battery of analytical tests for such ions as ammonium, chloride, and sulfate (also our first liquors were low in ammonium since the coal hydrocarbonization process was not in final operational form).

If the Bethlehem facility were operated at its maximum capacity of 4500 ppm of phenol influent, a degradation rate of about 1.35 g/day-liter would be obtained, thus comparing favorably with our degradation rates for comparable retention time. The current rate of bio-oxidation in the ORNL CSTR is being achieved with an air flow rate of ~ 3.5 liters/min as measured through an air flowmeter. It will be of interest to determine if the CSTR can operate effectively at an overall retention time of less than 15 hr. Such a retention time seems unusually low for this type of bioreactor, and already certain serious disadvantages appear to be

Table 4.1. Phenol conversion in continuous stirred-tank reactors

Operating parameters	Bethlehem CSTR	ORNL CSTR
Weak ammoniacal liquor flow (or equivalent liquor)	110,000 gpd	120 ml/hr
Dilution water	300,000 gpd	200 ml/hr
Phenolic flow to bioreactor	410,000 gpd	120 ml/hr
Aerator volume	265,000 gal	2.5 l
Clarifier volume	100,000 gal	1.0 l
Total bio-oxidizer volume	365,000 gal	3.5 l
Sludge recycle	330,000 gpd	
Total flow to aerator and clarifier	740,000 gpd	120 ml/hr
Aerator retention time	8.5 hr	21 hr
Clarifier retention time	3.2 hr	8.3 hr
Overall bio-oxidation retention time	21.2 hr	29.3 hr
Degradation rate based on 1500 ppm phenol	0.62 g phenol/ day-liter aerator	1.75 g phenol/ day-liter aerator
(365 ppm after Bethlehem dilution)	0.44 g phenol/ day-liter bio-oxidizer	1.25 g phenol/ day-liter bio-oxidizer
Effluent phenol level	0.05-0.5 ppm	0.5-1.0 ppm
Effluent thiocyanate level	1-20 ppm	1-5 ppm

associated with low retention times. Although the phenols are reduced to the 1- to 5-ppm level, other inorganic biohazards such as thiocyanate and cyanide may be incompletely oxidized, and thus the effluent may be unacceptably elevated in these limiting reactants.

The operation of the CSTR is elucidated somewhat by consideration of Fig. 4.5, which shows the conversion of several key constituents over an extended operational period (including startup). After a variety of startup and operational problems, present phenol degradation is greater than 99% for inlet concentrations up to 1500 ppm at a 120-ml/hr flow rate (phenol effluent 0.5 to 1.0 ppm). Thiocyanate degradation for these conditions is 90%, and total carbon conversion is 90%. The CSTR was seeded with Bethlehem sludge on days 1, 3, 6, 20, and 28. Since analyses were not made during weekends, some days of the operational period are not shown in Fig. 4.6. The slow buildup of the bacterial species that degrade inorganics such as thiocyanate was attributed primarily to low retention times, insufficient nutrients, and perhaps to an incorrect antifoam agent. To indicate the significance of the latter factor, the cell population growth in the CSTR is shown as a function of operational time (Fig. 4.6). Significant cell population was not detected until the antifoam was changed from Nalco 75 (a carbon-like powder) to Dow Anti-foam B. In recent days, the antifoam used by Bethlehem [71-D5 (Nalco)] has been obtained and is in use. The difficulty in determining the correlation factor (bacterial yield) will be discussed later in the section on proposed models.

The CSTR has been in operation continuously for 3 months and has obtained stability sufficient to produce effluents at the very lower limits of detectability of the phenol assay for levels greater than

Fig. 4.5. Degradation of biohazardous chemicals in a CSTR.

Fig. 4.6. Sludge concentration in the CSTR vs days of operation.

500 ppb (producing consistently phenol effluents of 0.5 to 5.0 ppm). However, even if these values were determined more accurately, it is not likely that our effluents have achieved the low values of 0.02 to 0.5 ppm reported by Bethlehem for their process. However, our best low thiocyanate effluent values have been consistently lower than those reported at the Bethlehem facility.

One disadvantage of the CSTR as a bioreactor is its vulnerability to chemical shocks and feed perturbations. Such perturbations are shown in Fig. 4.5 and are perhaps better explained in Fig. 4.7 (where the oscillations in conversion can be directly related to sudden increases in the inlet phenol concentration). However, with increasing operational time, the CSTR appears to develop some resistance to shocks.

An experiment that remains to be completed (but is now in progress) for the CSTR entails determination of the maximum influent concentration that may be treated at a maximum flow rate to yield acceptably low phenol, thiocyanate, and cyanide effluent levels. Acceptable levels would be phenol, 0.02 to 0.5 ppm, with thiocyanate, cyanide, and ammonium each less than 1 ppm. In conjunction with this determination, the wash-out point ($C_i = C_o$) of the CSTR must be established. Finally, the maximum phenol conversion could be determined, although the other biohazards might still require further processing (e.g., in a series of CSTRs). All of these kinds of data could be presented in the form of Fig. 4.8, showing the conversion of phenol in the CSTR as a function of flow rate (or retention time).

The mixed population bioreactor has one main advantage over the pure single species bioreactor previously studied. The CSTR (with its symbiotic

Fig. 4.7. Phenol conversion as a function of time and initial concentration in the CSTR.

Fig. 4.8. Phenol conversion as a function of flow rate in the CSTR, establishing washcut.

population) operates over a much wider flow rate range; the limit for the pure species bioreactor was ~120 ml/hr.

A mathematical model of a CSTR for biological systems was derived based on the excellent treatise by Atkinson⁸ concerning biochemical reactors. A series of mass balances for a CSTR fermenter gives:

for microorganisms,

$$S_O K_O r M V_r = F M + K M V_r ; \quad (1)$$

for substrate,

$$F C_i = F C + r M V_r ; \quad (2)$$

for product,

$$F P_i + S_P K_P r M V_r = F P ; \quad (3)$$

where

r = rate of microbial reaction,

M = dry weight of organism per unit reactor volume,

V_r = bioreactor volume,

F = volumetric flow rate, and

$S_O K_O$ and $S_P K_P$ are yield coefficients.

With a reasonable assumption of a small floc for the bacterial distribution, the rate expression can be estimated by:

$$r = \frac{k_1}{\rho_O} \frac{C}{(1 + k_3 C)} ; \quad (4)$$

and combination of Eqs. (1) and (4) gives:

$$C = \frac{1 + \kappa \tau}{S_O K_O \tau} \left[\frac{k_1}{\rho_O} - \frac{k_3}{S_O K_O \tau} (1 + \kappa \tau) \right]^{-1} . \quad (5)$$

Equation (5) gives the effluent concentration for a CSTR with a biological floc as the bioreactive agent. Note that according to this model, the substrate concentration C within the fermenter is independent both of C_i and the cell concentration M . The expression in Eq. (5) is of little utility unless the constants k_1 , k_3 , and $S_O K_O$ can be determined. For a bacterial floc in a batch reactor, if we assume a simple growth-associated system, then

$$M - M_i = S_O K_O (C_i - C) . \quad (6)$$

If we assume a high hydrodynamic shear and thus small bacterial floc size, then

$$\frac{1}{M} \frac{dM}{dt} = \frac{S_O K_O}{\rho_O} \frac{k_1 C}{(1 + k_3 C)} . \quad (7)$$

Combination of Eqs. (6) and (7), followed by integration leads to

$$\frac{1}{t} \ln \frac{M}{M_i} = \frac{G_{\max} k_3 \Delta}{S_O K_O + k_3 \Delta} - \frac{S_O K_O}{S_O K_O + k_3 \Delta} \frac{1}{t} \ln \left(\frac{\Delta - M_i}{\Delta - M} \right) , \quad (8)$$

where

$$\Delta = S_O K_O C_i + M_i .$$

The time $t = 0$ can be selected to correspond to any position in the experimental phase of the bacterial growth. Now, Eq. (8) is of the form $y = a - bx$, so knowledge of $M(t)$, M_i , C_i , and $S_O K_O$ allows a linear presentation of the data leading to a and b and thus k_1 and k_3 . Also, $S_O K_O$ is a yield constant described by:

$$\frac{dM}{dt} = S_O K_O rM , \quad (9)$$

$$\frac{dC}{dt} = -rM . \quad (10)$$

Thus, $S_O K_O$ can be obtained from a plot of $M - M_i$ vs $C_i - C$. An initial effort was made to use this model [Eqs. (6)-(10)] in a batch growth of Bethlehem sludge cells to obtain the pivotal constants $S_O K_O$, k_1 , and k_3 . Due to operational difficulties, the first attempt was a failure, but the effort should be repeated in view of improvements in growth conditions that have been made.

4.3.3 Three-phase packed-bed reactor (D. W. Holladay)

The same three-phase PBR as described previously² was seeded with the Bethlehem sludge. It was slightly less than 1 in. ID and 3 ft long, thus enclosing a geometric bed volume of 560 ml (initial void volume was 350 ml, giving a porosity of 0.625). It was randomly packed with 1/4 in. Berl saddles and equipped for both air and liquid influent ascending flow. Seeding required about 1 week, starting with a single injection of 10 ml of Bethlehem sludge (~ 20 g settleable solids) and then additional loading by recycling effluents from the CSTR (spiked with phenol and phosphate) that were known to contain viable (unsettled bacteria ($\sim$ g/liter)). Growth on the packing, as would be expected, became visible first in the bottom section and then expanded to cover the entire column length. Also, as more surface area became covered by bacteria, the column performance improved proportionately. However, after 2 months of operation, the biomass (although at its maximum conversion ability) reached sufficient density to significantly affect liquid and air flow rates. The manner in which the column will be regenerated will be noted later.

The initial goal of the PBR experiment was to establish a degradation rate $(C_i - C)Q_L/V_R$ (units of g/day-liter reactor) that could be compared with

the rate for the CSTR and the FBR. The data obtained for this first operation of the PBR are shown in Fig. 4.9.

It may be concluded from these data that for a three-phase PBR (bioreactor) with a fixed air rate (Q_G), a family of conversion curves (as a function of flow rate) will be obtained dependent on the inlet concentration. It appears at this initial stage that if the air flow rate is increased (maximum rate not determined) or if the bacterial population increases in the bioreactor, the family of curves shifts to higher conversions. The behavior of the family of degradation curves $(C_1 - C)Q_L/V_R$ also seems to follow this trend. Without attempting to analyze the complex mass transfer mechanisms indicated by these relationships, certain operating conditions for a particular PBR can be deduced from Fig. 4.9. The optimum flow rate required for the reduction of an inlet concentration could be estimated from the family of curves. The production rate is not a maximum at this point.

It was previously stated that the primary goal of the initial studies with the CSTR and PBR bioreactors was to establish optimum operating conditions giving maximum degradation rates compatible with an effluent acceptably low in phenols. Present "best" values are given in Table 4.2. Both degradation rates may increase as the process improves, but it seems likely that their rates will remain about the same (i.e., the rate in the PBR is approximately twice that in the CSTR). Caution should be exercised in the extrapolation of these results to predict specific conversion for other reactor sizes since the conversion is a not-understood complex function of reactor dimensions, bacterial rate mechanisms, and three-phase mass transfer characteristics. The PBR conversion rate could safely be

Table 4.2. Comparison of degradation rates of phenols in a CSTR and a three-phase PBR

Bioreactor type	Reactor volume (liters)	Flow rate (ml/hr)	Phenol conc. in effluent (ppm)	Degradation rate $\frac{\text{g phenol}}{\text{day-liter reactor}}$
Continuous stirred tank reactor (CSTR)	3.5	200	0.5-1.0	2.0
Three-phase packed bed reactor (PBR)	0.560	100	1-5	5.0

extrapolated to reactors of the same length but with different cross-sectional areas.

The biggest disadvantage of the PBR is its proclivity for flow blockage induced by cell biomass growth on the support surface. The PBR in question was operated about 35 days before the biomass multiplied to an extent sufficient to seriously affect air-liquor flow rates. This disadvantage can be minimized, however, if a quick, efficient technique can be found for controlling excess cell growth or if a method can be developed for rapid in-situ regeneration of the column. Preliminary tests indicate that the latter provision may be very feasible using eluents of detergent and 0.1 to 0.5 N acids. Studies are under way to determine minimum turnaround times for a PBR.

Much remains to be investigated in the characterization of the PBR. The details of the curves in Fig. 4.9 require further flow rate tests, varying first Q_L with constant Q_G and then the reverse test. Liquid and air flow rate interdependencies need to be documented, particularly as they relate to the pressure drop behavior of three-phase systems. Reynolds

number calculations indicate a laminar flow regime for the liquid flow rates being studied.

It may be recalled⁹ that there is an established sequence for correlation of hydrodynamic data in three-phase flow systems. The computational sequence for hydrodynamic design based on known flow rates (turbulent flow), inlet pressure, inlet temperature, bed length, and bed properties is as follows:

- (1) The average pressure in the packed section will be required for the determination of the single-phase gas friction loss. If the bed is very long, the calculation would have to be made on increments of the bed in view of the assumptions in the derivation of the Ergun relation for gas flow rates.
- (2) The determination of the constants for each single-phase friction loss in the Ergun equation is made, using

$$N_{re} = \frac{D_p G}{\mu(1 - \epsilon)} , \quad (11)$$

$$D_p = \frac{6(1 - \epsilon)}{S} , \quad (12)$$

$$\zeta = \frac{-\Delta P}{\Delta L} + \rho , \quad (12a)$$

for the equation

$$\zeta \left(\frac{g_c \rho D_p^3}{\mu^2} \right) \left(\frac{\epsilon}{1 - \epsilon} \right)^3 = N_{re} (\alpha + \beta N_{re}) . \quad (13)$$

Equation (13) can then be used for prediction of any friction loss variation with flow rate.

- (3) Calculate X and ζ_{Lg} with

$$X = \left(\frac{\zeta_L}{\zeta_g} \right)^{1/2}, \quad (14)$$

and

$$\log_{10} \left(\frac{\zeta_{Lg}}{\zeta_L + \zeta_g} \right) = \frac{0.416}{(\log_{10} X)^2 + 0.666}. \quad (15)$$

- (4) Then the two-phase friction loss is obtained from knowledge of ζ_L and ζ_g .
- (5) Calculate liquid saturation (fraction of void volume occupied by a phase) from:

$$\log_{10} R_L = -0.774 + 0.525 \log_{10} X - 0.109 (\log_{10} X)^2. \quad (16)$$

- (6) Calculate density of the flowing mixture from:

$$\rho_m = \rho_L R_L + \rho_g (1 - R_L). \quad (17)$$

- (7) Finally, the two-phase pressure drop is calculated from:

$$\zeta_{Lg} = - \left(\frac{\Delta P}{\Delta L} \right)_{Lg} + \rho_m. \quad (18)$$

4.3.4 Three-phase fluidized bed reactor (C. W. Hancher and G. B. Dinsmore)

For phenol conversion on a tapered fluidized bed reactor (TFBR), Phenobac (a commercially available dry-bacteria mixture)² was used as our bacterial starting source. The bioreactor system used for these tests consisted of the following: (1) a feed tank and pump, (2) a tapered fluidized bed bioreactor with a recirculation pump and air introduction equipment, and (3) the necessary control equipment [pH controller with a caustic pump and dissolved oxygen sensor (Fig. 4.10 and 4.11)].

Fig. 4.10. Schematic diagram of the tapered fluidized-bed reactor for phenol removal.

Fig. 4.11. Photograph of the tapered fluidized-bed reactor for phenol removal.

A solid packing support, -50 +70 mesh coal, was provided for the adherence of the bacteria. The normal recirculation rate of 400 ml/min resulted in a fully mixed system. The feed rates varied from 9 to 23 ml/min at phenol concentrations ranging from 500 to 2400 mg/liter. (Phenol is toxic to the bacteria when concentrations exceed 750 mg/liter and exposure time exceeds several minutes.)

The first test period, from May 23, 1975 to July 29, 1975, is shown on Table 4.3. This table indicates the weekly average test conditions and phenol reduction rates. Prior to this first period, Phenobac had been added to a dilute phenol feed (0.025 vol %) at a rate of about 50 g/day for the 3-liter bioreactor system volume over a period of about 3 weeks. Starting the week of May 23, feed concentration and flow rates were increased. The phenol removal rate continued to increase until a maximum rate of 1776 g of phenol per day per liter was attained (Table 4.3). About July 30, the rate in the bioreactor decreased rapidly to near zero. Two explanations for this rapid decrease are: (1) disruption of air (these bacteria die very quickly without enough oxygen), and (2) bacterial degradation due to the high phenol level of the feed.

The bioreactor was cleaned, and test 2 was begun. The bacterial population of test 2 was low; the reason for the slower growth rate is unknown.

4.3.5 Fluidized-bed behavior of ferredoxin-hydrogenase adsorbed on alumina (B. Z. Egan and J. P. Eubanks)

Ferredoxin-hydrogenase was adsorbed on alumina by thoroughly mixing 37 g of 70-100 mesh alumina with 25 ml of cell extract. The alumina was then washed with 400 ml of buffer solution to remove nonadsorbed enzymes.

Table 4.3. Weekly average test condition and phenol reduction rate

Date	Flow rate		Retention time (hr)	Feed concentration (mg/liter)	Waste concentration (mg/liter)	Conversion, $X = \frac{C_i - C}{C_i}$	Degradation Rate
	(ml/min)	(liters/day)					(g/day-liter) $Q \frac{(C_i - C)}{V_r}$
TEST 1							
5/23	15	21.6	3.33	580	20	0.965	4.04
5/30	13	18.7	3.85	780	< 1	0.999	4.86
6/5	23	33.2	2.17	750	< 1	0.999	8.30
6/20	24	34.6	2.08	650	< 1	0.999	7.50
7/2	22	31.6	2.27	1600	700	0.562	9.50
7/14	17	24.5	2.94	2200	160	0.927	16.66
7/21	17	24.5	2.94	2400	900	0.675	12.20
7/29	15	21.6	3.33	2400	300	0.875	15.20
TEST 2							
8/15	15	21.6	3.33	700	610	0.128	0.65
8/22	15	21.6	3.33	600	140	0.766	3.32
8/29	15	21.6	3.33	700	250	0.642	3.22
9/5	9	13.0	5.55	710	120	0.830	2.56
9/12	12	17.3	4.18	500	80	0.840	2.42

The alumina was placed in a tapered column and fluidized with a 0.05 M potassium phosphate buffer solution (pH 6.6), containing 3 g of $\text{Na}_2\text{S}_2\text{O}_4$ per liter. The substrate solution was contained in a closed system and recycled to the column. The apparatus and operations were contained in a glove box with an argon atmosphere. A schematic diagram of the system is shown in Fig. 4.12. The total solution volume in the column was 470 ml. The dithionite-buffer solution reservoir contained about 1 liter. Gas samples were removed periodically with a syringe, and the hydrogen was measured on a gas chromatograph. The results are shown in Fig. 4.13. The first run (curve 1) lasted for 27 hr. The substrate solution was then replaced with fresh dithionite-buffer solution, and a second run (curve 2) of 72 hr was made. Again, a fresh dithionite solution was added and a third run (curve 3) was continued for 44 hr. The enzyme activity decreased with each succeeding run, but some enzyme activity was retained for several days.

4.4 Supporting Bioanalytical Studies

C. W. Hancher, D. W. Holladay, G. B. Dinsmore, and M. McClure

We are continuing to develop and adapt analytical procedures for use in bioprocessing. In connection with processes to remove organics from processing streams, we have devised procedures for analyzing feed and effluent streams for phenol, catechol, and thiocyanate. A standard enzymatic assay for glucose (using a commercially available testing kit) was adopted for use in studies related to the hydrolysis of lactose to produce glucose.

Fig. 4.12. Schematic diagram of tapered-bed apparatus using ferredoxin-hydrogenase adsorbed on alumina.

Fig. 4.13. Hydrogen production in a fluidized bed containing ferredoxin-hydrogenase adsorbed on alumina.

4.4.1 Assays of organics degraded in bioreactors

During the monitoring of the various bioreactors presently under study, an effort was made to assay for all of the organic and inorganic constituents of the feed and effluents that posed potential biohazards. However, the complexities of several of the assays coupled with extensive analytical time required have limited routine daily assays to only a few of the most interesting compounds. Comprehensive assays are also made on a periodic basis. Most of the assays in daily use are described below:¹⁰

A. Phenol concentrations greater than 500 ppb:

- (1) Withdraw 10 ml of sample.
- (2) Add 0.2 ml of 5% ammonium chloride solution.
- (3) Add 0.3 ml of 2% aminoantipyrine solution.
- (4) Add 0.3 ml of 8% potassium ferricyanide solution.

All solutions were adjusted to a pH of 10.0 with glycine-NaOH. The phenol concentration was given by the absorbance at 510 nm; the calibration curve shown in Fig. 4.14 was established with pure phenol.

B. Phenol concentrations less than 500 ppb:

- (1) Withdraw 50 ml of sample (The sample was passed through a Millipore filter prior to assay in order to remove interfering organisms).
- (2) Add 1.0 ml of 5% ammonium chloride solution.
- (3) Add 0.3 ml of 2% aminoantipyrine solution.
- (4) Add 0.3 ml of 8% potassium ferricyanide solution.

This mixture was placed in a separatory funnel, and the color was extracted with 3 ml of chloroform. The color was determined by absorbance at 460 nm. The calibration curve shown in Fig. 4.15 was established with pure phenol.

C. Thiocyanate:

- (1) Add 10 ml of sample to 50-ml volumetric flask.
- (2) Add 3 ml of ferric nitrate solution.
- (3) Dilute to 50 ml and measure absorbance at 550 nm in a 1-cm cuvette.

Fig. 4.14. Calibration curve for assay of phenol concentrations greater than 500 ppb in a 10-ml sample.

Fig. 4.15. Calibration curve for assay of phenol concentrations less than 500 ppb in a 50-ml sample.

- (4) Prepare ferric nitrate solution. (Place 50 g of ferric nitrate in 500 ml of distilled water, add 25 ml of concentration nitric acid, and dilute to 1 liter.)

The calibration curve shown in Fig. 4.16 was established with a pure thiocyanate solution.

D. Catechol:

- (1) Withdraw 1.0 ml of sample.
- (2) Add 1.0 ml of 10% sodium molybdate solution.
- (3) Add 0.5 ml of 0.5 N HCl.
- (4) Add 1.0 ml of 0.5% sodium nitrate solution.

The calibration curve for catechol has been reported in a previous semiannual.¹

These assays have been used to monitor the contactor liquor and clarifier effluent each day; also, the total carbon in these solutions has been determined daily with the use of a Beckman total carbon analyzer. Finally, dry cell weights have been obtained daily in order to monitor cell growth in the CSTR bioreactor.

4.4.2 Assay for glucose

Calbiochem* supplies a test kit (Stat-PakTM) for determining glucose concentration by a series of enzymatic reactions:

The amount of NADPH produced (measured by absorbance at 340 nm) is proportional to the amount of glucose in the sample.

* Calbiochem, Los Angeles, Calif. 90063.

Fig. 4.16. Calibration curve for assay of thiocyanate concentrations less than 50 ppm in a 50-ml sample.

4.5 Immobilized Lactase as a Test System to Evaluate Bioreactors

C. W. Hancher, B. Z. Egan, W. Funkhouser, and G. B. Dinsmore

Since Charles³ and co-workers had fully tested the chemical system of lactase enzyme immobilized on alumina particles, it was chosen as a test system to evaluate bioreactors. The lactase enzyme hydrolyzes 1 mole of lactose to 1 mole of glucose and 1 mole of galactose (Fig. 4.17). The resulting glucose can be easily analyzed using a Calbiochem glucose standard reagent assay kit.

The lactase-lactose chemical system was chosen to determine the operating characteristics of the tapered fluidized-bed bioreactor and determine its advantages as compared with a conventional straight column fluidized-bed bioreactor.

4.5.1 Enzyme immobilization and life study

Lactase enzyme obtained from the Wallerstein Laboratories* was immobilized on alumina (-70 +100 mesh) from the Carborundum Co.** by the method of Charles.³ The alumina had a surface area of $4.0 \text{ m}^2/\text{g}$, a porosity of $0.32 \text{ cm}^3/\text{g}$, and a bulk density of 1.2 g/cm^3 . The Charles procedure is as follows:

Lactase-Alumina Catalyst

A slurry containing 250 g of alumina and 500 ml of a solution of lactase in citrate-phosphate buffer in a 1-liter bottle is gently agitated for 30 min at room

* Wallerstein Co. (a Division of Travenol Laboratories, Inc.), Morton Grove, Ill. 60053.

** The Carborundum Co., Niagara Falls, N.Y., 14302.

Fig. 4.17. Hydrolysis of lactose to glucose and galactose.

temperature. The enzyme solution is decanted (stored under refrigeration for subsequent reuse) and 500 ml of a 5% glutaraldehyde solution in citrate-phosphate buffer is added to the bottle containing the solids. The resulting slurry is then agitated for 80 min at room temperature. The glutaraldehyde solution is decanted and the enzyme solution previously stored is reintroduced. This is followed by another 30 min of agitation. The resulting catalyst is washed in buffer and is then ready for use.

The activity of the immobilized lactase was tested using the following procedure:

- (1) Prepare a buffer of 0.2 M $\text{Na}_2\text{H}_2\text{PO}_4$ and 0.1 M citric acid, adjusting the resulting solution to pH 3.5 with 50% NaOH.
- (2) Wash the solids in 10 ml of buffer per gram of solids.
- (3) Make up a lactose solution containing 20 g of lactose per liter of buffer.
- (4) Mix 10 ml of lactose solution and about 0.2 g of immobilized enzyme on alumina for 5 min at 37°C; withdraw a sample of this solution.
- (5) Assay the sample for glucose (assuming no free enzyme is present in solution).

The enzyme that we immobilized had an initial activity of 8×10^{-5} mole of lactase per minute per gram of solid (Fig. 4.18), which decreased to 5.5×10^{-5} mole of lactase per minute per gram of solid

Fig. 4.18. Immobilized lactase activity vs time.

after 3 days of use. The next 50 days of use caused only a very slight drop in activity to 5.0×10^{-5} mole of lactase per minute per gram of solid. The initial activity reported by Charles was 12×10^{-5} mole of lactase per minute per gram of solid.

4.5.2 Test hydraulic data

Alumina particles were used to determine the hydraulic pressure drop of both a tapered bioreactor (48 in. long with a diameter tapering from 3 in. at the bottom) and a straight bioreactor (48 in. long, 2 in. diam). The experimental apparatus is shown in Fig. 4.19. The alumina particles, No. SAEHS-33 from the Carborundum Company¹² were dry screened and the -70 +100 mesh particles used. Fig. 4.20 shows a comparison of the volumes at various heights of the two bioreactors. The pressure drop and bed expansion coefficients (expanded volume/settled volume) obtained at various flow rates and bed loading are shown in Fig. 4.21 and 4.22.

The hydraulic pressure loss in the tapered column tends to decrease as the solid fluidized bed expands; the average column cross-sectional area decreases. Fig. 4.22 graphically indicates that the pressure loss of alumina packing is about the same with or without the immobilized lactase enzyme. The pressure loss is less in the straight bioreactor than in the tapered bioreactor. Since the packing is not expanded the entire tapered length, the average cross-sectional area of the tapered bed is less than that of the 2-in.-diam bioreactor. This results in a higher average linear fluid velocity at equal volumetric flow rates. At 200 ml/min and 500 g of packing, the tapered

Fig. 4.19. Equipment layout for immobilized lactase reactor for conversion of lactose to glucose.

Fig. 4.20. Comparison of volumes at various heights of a 3-to 1-in.-diam x 43-in.-long tapered reactor and a 2-in.-diam x 48-in.-long straight reactor.

Fig. 4.21. Hydraulic pressure loss (g/cm^2) and bed expansion coefficient (expanded volume/settled volume) vs flow rate (ml/min) at various bed loadings for 3- to 1-in.-diam x 43-in.-long tapered columns.

Fig. 4.22. Hydraulic pressure loss (g/cm^2) and expansion coefficient (expanded volume/settled volume) vs flow rate (ml/min) for 500 g of alumina solid for the 2-in.-diam x 48-in.-long straight column.

bioreactor pressure drop was 22.2 g/cm^2 (1.8-in., av. diam) while the 2-in.-diam straight bioreactor was 19.5 g/cm^2 . The maximum flow rate for 500 g of alumina packing for the 2-in.-diam straight bioreactor is 180 to 240 ml/min for packing with out without the immobilized enzyme. The maximum rate for the tapered bioreactor with 500 g of packing with immobilized enzymes is 600 ml/min.

4.5.3 Comparison of the tapered and straight cylindrical bioreactors as systems for converting lactose to glucose

Two bioreactors were tested to determine the difference in their ability to convert lactose to glucose: (1) a tapered bioreactor (48 in. long with a diameter tapering from 3 in. at the top to 1 in. at the bottom); and (2) a cylindrical bioreactor (48 in. long, 2 in. diam).

A solution containing 50 g of lactose per liter of 0.01 M phosphate buffer (pH 3.0 at 41 to 43°C) was contacted with the same immobilized enzyme packing in both types of bioreactors. The comparison was made during the period between the 14th and 20th day of packing life (Fig. 4.18) when the enzyme activity was 5.0×10^{-5} mole of lactase per minute per gram of solids.

The glucose production rate (mole/min) and lactose conversion (percent conversion = moles of glucose produced x 2.0, divided by moles of lactose in feed) are shown in Fig. 4.23 and 4.24. A comparison of production rate and conversion at a flow rate of 200 ml/min is presented in Table 4.4.

Fig. 4.23. Glucose production and percent conversion of lactose vs flow rate at a lactose feed concentration of g/liter for the 3- to 1-in.-diam $\times$ 43-in.-long tapered bioreactor.

Fig. 4.24. Glucose production and percent conversion of lactose vs flow rate at a lactose feed concentration of 50 g/liter for the 2-in.-diam x 48-in.-long straight bioreactor.

Table 4.4. Comparison of the tapered and the straight cylindrical bioreactors as lactose conversion systems

Operating conditions: feed, 50 g of lactose per liter;
pH, 3.0; temperature, $\sim 42^{\circ}\text{C}$;
flow rate, 200 ml/min

Bioreactor	Immobilized packing weight (g)	Glucose production rate (mole/min)	Lactose conversion (%)
Tapered ^a	430	12×10^{-3}	43
	710	16×10^{-3}	58
Cylindrical ^b	430	11×10^{-3}	44
	710	14×10^{-3}	52

^a48 in. long with a diameter tapering from 3 in. at the top to 1 in. at the bottom.

^b48 in. long, 2 in. diam.

Operation with the tapered bioreactor is much easier than with the straight individual bioreactors because the increasing diameter of the tapered model results in a decrease of the liquid velocity, which allows the solid-liquid interface to rise to a height equaling the escape velocity of the solid particle. This stable solid-liquid interface produces a braking effect on the solids with higher velocities at the lower levels of the bioreactor.

These two bioreactors show equivalent performance with regard to the conversion of lactose to glucose, as indicated by the test data (compare Figs. 4.23 and 4.24). The percent of lactose conversion is plotted vs the amount of immobilized enzyme, divided by the flow rate, in Fig. 4.25, and the resulting graph indicates that the two bioreactors give comparable results.

Fig. 4.25. Percent conversion of lactose vs immobilized enzyme/flow rate for two bioreactors at a lactose feed concentration of 50 g/liter.

4.5.4 Immobilized lactase-lactose enzymatic kinetics

In order to apply a kinetic model to the results of chemical testing of the immobilized lactase-lactose system, the following steps must be taken: (1) a kinetic model must be chosen, (2) the kinetic data must be determined, and (3) the results obtained with the model must be compared with actual data.

Many others have reported that immobilized enzyme systems can be modeled using the Michaelis-Menten equation:⁷

$$R = \frac{V_{\max} S}{K_m + S},$$

where

R = reaction rate (moles per minute per gram of catalyst),

S = substrate concentration (moles/liter),

$V_{\max}$ = maximum rate (in terms of R),

K_m = concentration at one-half of $V_{\max}$ (in terms of S).

For the situation of "competitive inhibition," the competitive inhibition concentration, I, and the rate constant, KI, must be included in the rate equation:

$$R = \frac{V_{\max} S}{K_m [1 + (I/KI)] + S}$$

where

I = inhibitor concentration (moles/liter),

KI = inhibitor rate (moles/liter).

The kinetic constants in these equations can be determined from a reciprocal plot of $1/S$ vs $1/R$ (Fig. 4.26). If no inhibition

Fig. 4.26. Graphic procedure for evaluation of Michaelis-type kinetic data.

takes place, then the plot of $1/S$ vs $1/R$ has an intercept of $1/V_{\max}$. If inhibition does occur, then it can be determined to be either competitive or noncompetitive. The competitive plot coincides with the noninhibition plot on the $1/R$ axis. The noncompetitive plot coincides with the noninhibition plot on the $1/S$ axis.

Two series of tests were performed to determine the kinetic constants for the immobilized lactase-lactose systems: (1) test 1 - immobilized lactose and lactase at four different concentration levels; (2) test 2 - conditions same as those in test 1, plus the addition of fixed concentrations of glucose and galactose at each of the few levels. These tests were conducted using the procedure described previously for the immobilized lactase activity tests (Sect. 4.5.1). A 2-min end point was selected to determine the initial rate. The kinetic constants were determined to be: $V_{\max} = 13 \times 10^{-5}$ moles per minute per gram of catalyst, $K_m = 9 \times 10^{-2}$ mole/liter, and $K_I = 4 \times 10^{-3}$ mole/liter. Subsequently, the initial rate was determined using end points earlier than 2 min. These tests indicated that the rate at 20 sec may be approximately 10 to 15 times faster than that at 2 min. Therefore, the kinetic constants are probably low by a factor of 10 or more. The determination of kinetic constants will be redone with a more precise procedure that allows for the determination of rates at very short intervals after time zero.

4.6 Growth of Brevibacterium fuscum

D. W. Holladay and J. Hopkins

Certain bacteria (as differentiated from the yeast Trichosporon cutaneum) contain unstable hydroxylases but have oxygenases more stable than those from yeast. The most stable oxygenase yet reported is that derived from Brevibacterium fuscum;¹¹ after purification, this catechol oxygenase maintained its activity after several weeks' storage at 4°C (previously isolated oxygenase could not). This increased stability led to the conclusion that it could be advantageous to investigate B. fuscum as an alternative source of catechol oxygenase. Initial studies delineating inoculum growth were made by D. R. Stallions.*

A small sample (~ 100 mg) of a seed culture of B. fuscum (obtained from the American Type Culture Collection)** was carefully added to 1 liter of autoclaved, aerated medium of the following composition:

0.5 g of ammonium sulfate
2.0 g of monobasic potassium phosphate
3.0 g of dibasic potassium phosphate
2.5 g of sodium chloride
0.2 g of magnesium chloride
0.5 g of dried yeast extract
1.0 liter of tap water

* Biology Division, ORNL.

** American Type Culture Collection (ATCC), 1231 Parklawn Drive, Rockville, Md., 20852.

The pH was adjusted to 7.2 with KOH. In addition, 50 g of 5% glucose (to provide a carbon source) was autoclaved separately and then added to the medium along with the seed culture.

An accepted method for obtaining large quantities of cells with phenol-degrading capabilities is (1) to grow from seed a large quantity of cells with glucose as the carbon source, (2) to induce these cells to metabolize phenol by removing the glucose and exposing the cells to a low concentration (0.1 to 0.5%) of phenol as the only carbon source.

The batch growth of the cells was monitored by two methods: (1) a dry cell weight was taken by depositing and then drying 1 ml of fermentor solution on a preweighed Millipore filter, and (2) the disappearance of glucose was followed using a TES-TAPE which gave a color test for residual glucose accurately in the range of 0 to 0.5%. The TES-TAPE is based on the enzymatic system glucose oxidase--peroxidase--orthotolidine. After 4 days of growth, the daily increase in cell weight ceased, indicating that the cells had stopped growth and had become stagnant at a concentration of about 7 g/liter. The cessation in growth was not due to a lack of glucose but was traced to a shortage of nitrogen. The cells grown in the 1-liter batch were saved for future seed. A large-scale fermentation was begun in the controlled-fermentation laboratory using the information gained from the small-scale test.

About 10 liters of medium was prepared as described above except that sufficient nitrogen was supplied as 1% urea (autoclaved separately). The medium was seeded with the ATCC culture and was fermented for

3 days. Air was supplied at a rate of one-half the volume of the fermenting medium per minute. The temperature was maintained at 30°C, and the pH was controlled at 7.2. Cell growth was monitored by periodically determining glucose and dry cell weights; the results are shown in Fig. 4.27. The cell growth was interrupted earlier than desired due to time limitations. (One area of interest was the determination of the maximum concentration of cells obtainable under these conditions.)

The cells were stored at 4°C prior to being harvested with our small continuous Sharples centrifuge. About 50 g of cells was recovered and frozen prior to being reseeded in freshly autoclaved media which contained phenol instead of glucose as carbon. The cells were grown in the medium plus phenol for about 28 hr with temperature, pH, and air supply being monitored as described previously. The phenol level was assayed by using aminoantipyrine as the standard (see Sect. 4.4.1); results showed that 3 g was utilized by the cells before harvest.

When the cells were harvested, they were found to have remained at their initial glucose-growth concentration. The mechanism by which the cells metabolized phenol but did not experience a net gain in weight remains unexplained. Since the cells showed unusual resistance to breakage, it was necessary to resort to the acetone-drying technique to achieve any semblance of enzyme recovery.

Because of the difficulty in achieving good cell fracturing, only dilute quantities of crude cellular extract were available for analysis for catechol oxygenase activity in our standard enzyme assay.

Fig. 4.27. *Brevibacterium* growth in a fermentor as monitored by dry cell weight and absorbance at 620 nm.

The degradation of catechol by the crude extract is shown in Fig. 4.28, where the increase in A_{260} is due to the conversion of catechol to muconic acid.

4.7 References for Section 4

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Fig. 4.28. Activity of the crude extract of Brevibacterium fuscum as monitored by conversion of catechol to muconic acid.

5. ENVIRONMENTAL STUDIES

We have continued our efforts relative to the isolation and identification of soluble organic constituents and pollutants, the determination of chlorination effects on cooling waters, and have organized a national conference on the Environmental Impact of Water Chlorination.

5.1 Automated Analysis of Dissolved Organic Compounds in Polluted Waters

R. L. Julley, W. W. Pitt, Jr., G. Jones, and J. E. Thompson

Characterization of the organic constituents and pollutants in process effluents, aquatic ecosystems that receive effluents, surface waters that are sources of drinking water, and potable waters, themselves, is of vital importance for understanding possible environmental health effects. The epidemiological relations of human diseases to trace environmental contaminants in such waters cannot be determined without quantitative measurement and characterization of both individual pollutants and broad chemical groups or families.

It has been the objective of this program, funded by the National Science Foundation (NSF-RANN) to examine natural waters and the effluents from coal-processing plants. Preliminary examinations have also been made on an estuarine water sample, a potable water supply, and water containing an algal bloom. The latter study was conducted in cooperation with Dr. Roger Minear of the Environmental Engineering Department, University of Tennessee-Knoxville. The major objective of that study was to determine the utility of high-resolution liquid chromatography for separating organophosphorus compounds.

5.1.1 Natural waters

The identification and quantitation of trace organics in several natural waters were objectives of the program funded by NSF-RANN. After completion of the first series of examinations of six different natural waters, that phase of the program was deemphasized; the results have been reported previously.¹⁻³ High-resolution anion exchange chromatography was the principal separations method utilized for this study. The separated constituents were examined and identified using a multi-component analytical scheme consisting of uv spectrometry, gas chromatography, and mass spectrometry.⁴ Several conclusions from this study were the following:

(1) Many organic constituents are adequately concentrated and separated from aqueous solutions using vacuum distillation for concentration of the trace pollutants and high-resolution liquid chromatography for separation.

(2) The multicomponent analytical technique is very successful for characterization and identification of low-molecular-weight (<500) compounds that are relatively nonvolatile.

(3) A relatively high portion of the total organic carbon in natural waters consists of apparently high-molecular-weight compounds or polymers such as humic and fulvic acids, which, for adequate characterization, require additional procedures other than those utilized in the routine multicomponent analytical scheme.

5.1.2 Effluents from coal-processing plants

The fossil-fuel coal has a high probability of becoming the major energy source of our nation during the near future with the concomitant

necessity for coal-conversion to gaseous and liquid fuels. Thus the determination of refractory organics in process effluent from coal-gasification and -liquefaction plants is of much importance with respect to assessing the environmental impact of the anticipated large number of process plants. In addition to assisting in the evaluation of environmental effects, including that on mankind, the data obtained on the chemical composition will be useful for providing feedback information for modification of engineering and plant flowcharts. Analytical results for two aqueous process streams from a char-oil process were reported previously.³ During this report period, high-resolution chromatography has been applied to the determination of nonvolatile organic constituents in aqueous effluents from other coal-liquefaction processes. Aqueous stream samples from two coal-liquefaction experiments (Atmospheric Hydrocarbonization and In-Situ Programs) have been analyzed by high-resolution liquid chromatography. All samples were chromatographed without prior concentration (and in some cases diluted), and each chromatogram showed many uv-absorbing and cerate-oxidizable constituents (i.e., Fig. 5.1). The multicomponent analytical identification and quantitation procedure is being used to identify the individual compounds in the samples.

Since the predominating compounds in coal process aqueous wastes are phenolic, the chromatographic conditions are being optimized for separation of phenol-type compounds. A chromatogram of eight phenolic compounds, indicating the capability of the system, is shown in Fig. 5.2. A slightly better and considerably faster separation than that shown was achieved using isocratic elution with 2 M ammonium acetate at 60°C.

Fig. 5.1. High-resolution chromatogram of trace organics in the off-gas scrubbing liquors from an atmospheric hydrocarbonization experiment.

Fig. 5.2. High-resolution chromatogram of phenolic standards
 (28 µg/liter of each compound).

Information concerning phenolic and other compounds in coal-processing waste streams is of great importance in assessing the possible environmental impact of coal-conversion plants. The data on chemical composition of such effluents will also provide informational feedback for modification of engineering and plant flowsheets. For example, the high-resolution liquid chromatography system is being used to analyze the efficacy of the biological treatment of the phenolic wastes from coal-liquefaction experiments. Figure 5.3 shows chromatograms of an aqueous waste stream from an atmospheric hydrocarbonization experiment before and after biological treatment.

5.1.3 Estuarine of marine waters

Estuarine and marine sites are being used for both conventional and nuclear electrical power generating plants. Maintenance of high production efficiencies currently requires the treatment of the cooling systems of such power plants with biocides or antifoulants. The effects of these biocides on the organic constituents in the cooling waters may have considerable environmental significance. Information concerning the dissolved organic species in such waters is necessary for the prediction and understanding of possible chemical reactions and potential formation of environmentally harmful reaction products. Thus a preliminary investigation was made concerning the concentration and chromatographic analysis of an estuarine water sample.

A 40-liter grab sample was obtained from the Cooper River estuary at Charleston, South Carolina, placed in polyethylene containers, and immediately frozen in dry ice at the sampling site. The following analytical results were determined for the sample: pH, 8.0; chloride,

Fig. 5.3. High-resolution chromatograms of trace organics in the off-gas scrubber liquor from an atmospheric hydrocarbonization experiment. Top, before biochemical treatment; below, after biochemical treatment.

10,260 mg/liter; organic carbon, 8.0 mg/liter; inorganic carbon, 6.0 mg/liter; organic nitrogen, 0.03 mg/liter; and ammonia (as N), 0.14 mg/liter.

A preliminary concentration of a 1-liter aliquot was made using a vacuum distillation method. The resulting copious precipitation of solids because of the high salt content of the brackish water caused considerable difficulty in redissolution of the organics in the small volume of eluent buffer solution required for chromatographic analysis.

A second 1-liter aliquot was passed through a weak cation exchanger (Rexyn 102, 16-50 mesh, Fisher Scientific Company) to exchange the Na^+ for H^+ , thus reducing the salt content. Concentration of this sample still resulted in a large amount of precipitated solids. Consequently, the large volume of eluent buffer required to satisfactorily dissolve the organics resulted in a concentration factor too low for the detection of any constituents during chromatography.

Subsequently, a 5-liter aliquot was passed through the weak cation column and reduced to 500 ml in a rotary evaporator. The concentrate was frozen and lyophilized. The resulting freeze-dried residue was dissolved in 50 ml of distilled water, centrifuged to remove the solids, and again freeze-dried. This residue was dissolved in 5 ml of eluent buffer and the solids removed by centrifugation. Fourteen uv-absorbing and/or cerate-oxidizable peaks were separated and isolated from preparative-scale chromatography of the concentrate. The eluate fractions containing these chromatographic peaks are being processed through our routine multicomponent identification procedure.

5.1.4 Organophosphorus compounds

Organophosphorus compounds occur in natural and process waters.⁵ Identification of these compounds is of both practical and academic interest. This is particularly true, since eutrophication of reservoirs and natural lakes is often attributed to increased phosphate concentration. Phosphorus is considered to be the major limiting factor in waters with respect to formation of biomass, and an increase in phosphorus concentration permits an increase in plant and animal growth rates, thus an increase in total biomass.⁶ Therefore, an understanding of the behavior of organophosphates on our high-resolution liquid chromatographs is necessary to facilitate their identification and quantitation in natural and polluted waters.

A preliminary investigation of the separation of organophosphorus compounds using the high-resolution UV Analyzer was made in cooperation with Dr. Roger Minear of the Environmental Engineering Department, University of Tennessee-Knoxville. A culture of Chlamydomonas reinhardtii, an algae associated with eutrophic waters, was inoculated with ^{32}P as orthophosphate (1.0 $\mu\text{Ci}/200$ ml culture). The water medium was separated from the algal mass by centrifugation and 1.0 ml was chromatographed on the dual-column UV Analyzer. Radioactivity in the chromatographic eluate fractions was determined by liquid scintillation counting techniques. Analysis of the radioactivity data indicates that over 60 organophosphorus compounds were separated during the chromatographic run of 120 hr. Of the separated peaks, 20 were separated during the first 30 hr and over 30 were separated during the last 50 hr. The analytical results are being corrected for radioactive decay and plotted. A first conclusion from

this scouting test is that high-resolution liquid chromatography may be a very useful adjunct to the isolation and identification of organophosphorus compounds.

5.1.5 Potable waters

Approximately 100 liters of potable water from a metropolitan water supply was obtained at the water treatment plant and returned to Oak Ridge National Laboratory for concentration and analysis by high-resolution liquid chromatography.³ The water sample was obtained after sand filtration and contained a 2.1 mg/liter chlorine residual (amperometric method⁷) and 1.6 mg/liter organic carbon. Previously, in the water treatment, the water had been chlorinated (chlorine concentration of 12 mg/liter) at the water intake and passed through an open settling basin (3-day residence time). The grab sample was stored at ice-water temperatures for the several days required for transportation to ORNL and thawed prior to low-temperature (<35°C) vacuum concentration. The chlorine residual was destroyed by the addition of a stoichiometric amount of thiosulfate solution, and the sample was concentrated 3600-fold by vacuum distillation in preparation for subsequent preparative-scale anion exchange chromatography, carbohydrate analysis, and cerate-oxidimetric analysis.

Only eight major uv-absorbing peaks and/or cerate-oxidizable chromatographic peaks were detected during the preparative-scale chromatographic separation. Eluate fractions containing the chromatographic peaks were collected and processed through the routine multicomponent identification procedure. The following four organic constituents were identified and quantified in the eluate fractions: inositol, 0.5 ppb;

O-methylinositol, 11 ppb; mannitol, 0.7 ppb; and succinic acid, 8 ppb. Six unknowns were characterized. The gas chromatographic and mass spectral characteristics of the trimethylsilyl derivatives⁴ of these unknowns are given in Table 5.1. In addition, a methylene chloride extraction of the solids precipitated during the concentration procedure (principally inorganic phosphate and sulfate salts) contained dioctylphthalate, dibutylphthalate, glycol-1-palmitate, and one unknown (Table 5.1).

Thirty chromatographic peaks were separated from the drinking water concentrate using the analytical carbohydrate analyzer.⁸ Analysis of this same concentrate using the analytical-scale UV-Analyzer indicated 21 uv-absorbing peaks and 23 cerate-oxidizable peaks.⁹ In the last analysis, the uv-absorbing and cerate-oxidizable peaks were not correlated; thus some may correspond to the same organic constituents.

5.1.6 Continuous monitoring systems for dissolved pollutants

The objective of this program is the development of automated analytical systems for isolation and identification of specific pollutants and monitors that can detect properties of specific pollutants on a continual basis for application in environmental monitoring. The initial emphasis is being placed on two types of monitors: (1) a continuous chemical oxygen demand monitor (CCOD) and (2) a surface-fluorometer.

A CCOD monitor that measures fluorescent Ce(III) resulting from the reduction of Ce(IV) by any oxidizable compound in the stream being monitored is being assembled and is about 75% complete. The monitor is based on an earlier version¹⁰ that gave results comparable to those obtained by the standard COD tests in the low ppm range.

Table 5.1. Gas chromatographic and mass spectral characteristics of unknown organic constituents in a drinking water sample

Anion exchange eluate fraction	Gas chromatographic retention position ^a		Mass spectral properties ^b				
	OV-1	OV-17	MW	(1)	(2)	(3)	(4)
20-33	17.00	17.55	346	253	331	346	-
72-75	~10	-	262	170	120	143	186
72-75	11.40	12.00	369	219	177	281	369
72-75	15.26	15.84	320	233	189	231	320
83-91	10.62	10.84	381	117	191	190	219
185-192	10.00	10.60	244	93 ^c	95 ^c	157	185
d	16.56	16.97	292	277	292	105	242

^aTMS derivatives.

^bMolecular ion and principal peaks are listed. Silicone-related peaks are not included.

^cIndicative of possible chloro-organic.

^dMethylene chloride extract of inorganic salts precipitated during concentration procedure.

A bifurcated, fiber optic fluorometer (Fig. 5.4) for surface fluorometric measurements to determine dissolved fluorescing pollutants has been constructed. The use of bifurcated fiber optics allows the measurement of fluorescence at a zero angle (i.e., excitation and emission paths are the same). This minimizes the depth of fluid required for measurement. By using filters of the same wavelength (or removing the filters altogether), the fluorometer can be used as a reflecting-light turbidimeter. It is now being evaluated for the determination of trace polynuclear aromatic hydrocarbons in coal-liquefaction/gasification liquors.

5.1.7 New and modified instrumentation

Because of cross-contamination incidents between water samples with relatively low organic concentrations and those with considerably higher organic concentrations, the high-vacuum distillation system⁴ has been modified. The intricate heated and baffled system has been removed, and round-bottom glass vessels with external heaters have been installed. This new system should reduce cross-contamination between consecutive concentrations of water samples to a minimum.

The Hall Electrolytic Conductivity Detector* designed for detection of organic compounds containing halogens, sulfur, or nitrogen has been installed on the MicroTek MT-222 gas chromatograph.¹¹ This new detection capability should be of considerable benefit in the examination of chloro-organic compounds.

The component parts of the plasma emission spectrometer detector for gas chromatography have been received and the mounting table constructed and positioned on the MicroTek MT-222 gas chromatograph. The parts are scheduled for assembly and check-out in November 1975.

*Tracor Instruments, 6500 Tracor Lane, Austin, Texas 78721

Fig. 5.4. Bifurcated, fiber-optic fluorometer.

5.2 Environmental Effects of Antifoulants

R. L. Jolley and J. E. Thompson

In contemporary technology, chlorine is the principal biocide for removal of biological surface films in the cooling systems of electrical power producing plants.¹² This is required to maintain high production efficiencies. Chlorination of waters containing low concentrations of organics with ppm chlorine concentrations results in the formation of chloro-organics.¹³ Consequently, the practice of chlorinating cooling waters is being evaluated with respect to the formation of chlorinated organics. As previously reported, the methodology developed with process effluents for identification of chlorinated organic compounds and for determination of the biotoxicity of the chlorinated compounds is being applied in this investigation.^{3,13-15}

5.2.1 Chlorine-containing organics in chlorinated cooling waters

The chlorination yield of chlorinated organics (as Cl associated with chloro-organics) in a Mississippi River water sample was found to be 1 to 3% of the chlorine dosage. This sample had been treated in the laboratory using reaction conditions similar to those employed at the Allen Steam Plant.

In this experiment, the water sample was chlorinated with 3.4 mg/liter chlorine to a 1.2 mg/liter chlorine residual using the coupled ³⁶Cl tracer--high-resolution chromatographic procedure.¹⁴ The lesser chlorination yield was computed assuming no isotopic dilution of the ³⁶Cl in the chlorinating agent with the inert chloride of the water sample; the higher yield was computed assuming complete isotopic dilution occurs within a fraction of a second.

The cooling water sample for this experiment was a grab sample from the cooling water inlet (prior to chlorination) for the Allen Steam Plant, which is operated by the Tennessee Valley Authority and located on the Mississippi River near Memphis, Tennessee. The sample was stored in a polyethylene container at -60°C and thawed just prior to use. The following analytical results were determined for the sample: pH, 7.3; chloride, 7.7 mg/liter; organic carbon, 9.0 mg/liter; inorganic carbon, 16.3 mg/liter; organic nitrogen, <0.05 mg/liter; and ammonia (as N), 0.15 mg/liter.

The chlorine requirement (demand) of the cooling water sample was determined using standard methods⁷ with a chlorine reaction time of 5 min. At the Allen Steam Plant, the cooling waters are chlorinated daily for 30 min with an average chlorine dosage of 1 to 2 mg/liter at ambient to ambient-plus- 11°C temperatures and a reaction time of 2 min to achieve a chlorine residual of 0.5 mg/liter or less. After discharge from the cooling tubes, the chlorinated cooling water may be diluted 2- to 3-fold, depending upon the number of generating units in operation at that time.^{16,17} The reaction time in the discharge conduit varies from 14 min at maximum flow of the cooling waters to 28 min at minimum flow. The flow rate is dependent upon the number of condenser units operating (three units at Allen Steam Plant) and the ambient temperature of the cooling water, which determines the flow rate necessary to achieve the desired temperature drop in the condenser units. The discharge conduit empties into an open ditch which returns the cooling waters to the Mississippi River. The residence time of the chlorinated cooling waters in the open ditch ranges from about 17 to 34 min before entering the Mississippi River.¹⁸

The chlorine requirement (demand) of the Mississippi River water sample was determined using standard methods⁷ with a chlorine reaction time of 5 min. The linear equation expressing the relationship between the chlorine dose and chlorine residual in the chlorinated cooling water is the following:

$$Y = 0.38X - 0.09,$$

in which Y is the chlorine residual in mg/liter and X is the chlorine dose in mg/liter.

The 1-liter aliquot of cooling water was chlorinated with 3.4 mg of chlorine gas containing 0.033 mCi of ³⁶Cl. After a chlorination contact time of 15 min at a chlorine dosage of 3.4 mg/liter, the chlorine residual of about 1 mg/liter was destroyed with a slight stoichiometric excess of thiosulfate solution. The chlorinated sample was then concentrated 1470-fold in preparation for subsequent chromatography. The ³⁶Cl material balances for the chlorination and concentration steps are given in Table 5.2. The poor material balances suggest the need for further investigation, but are believed, at this time, to be due to two main problems: (1) the difficulty in manipulating small volumes of radioactive materials, and (2) the difficulty of redissolving coprecipitated organics in small volumes after the concentration step. The possible formation of volatile compounds (e.g., chloroform) may contribute to the poor material balances and should be evaluated.¹⁸⁻²¹

Over 50 chlorine-containing organics were chromatographically separated from the 1470-fold concentrate of the ³⁶Cl-tagged chlorinated Mississippi River water sample. As in the chlorination of sewage effluents^{14,15,21} and another cooling water sample,²² more than 99% of the ³⁶Cl activity

Table 5.2. Radioactivity (^{36}Cl) distribution and material balances for chlorine generation and concentration in radioactive tracer experiment with cooling water

Stage and experimental component	Activity in experimental component (10^7 counts/min)	Percent of activity entering stage	Percent of initial activity ^a
Chlorine generation stage:			
Chlorine generator	0.44	4.52	4.52
Potassium iodide solution	0.01	0.08	0.08
Chlorinated effluent	7.01	72.04	--
Stage material balance	7.46	76.64	--
Concentration stage:			
First condensate	0.06	0.80	0.58
First centrifuged sediment	0.10	1.37	0.99
Second condensate	0.01	0.07	0.05
Second centrifuged sediment	0.43	6.19	4.46
Concentrate (for chromatography)	5.31	75.75	54.57
Stage material balance	5.90	84.18	--
Overall material balance	--	--	65.35

^aThe initial activity used in this experiment was 9.73×10^7 counts/min at 96% counting efficiency.

was associated with the chloride ion peak. About 1.3% of the radioactivity was associated with the other chromatographic peaks and resin (see Table 5.3). This activity represents stable ^{36}Cl -tagged chloro-organics. This fact is concluded by analogy with previous studies.^{3,13,15,21}

Results of this current study indicate that with reaction conditions approximating those at the Allen Steam Plant the total reaction yield of chlorine as chloro-organics was 3.1% of the initial 3.4 mg/liter chlorine dosage. This yield is calculated based upon the reasonable assumption that isotopic dilution of the ^{36}Cl -tagged chlorinating agent occurs rapidly and essentially completely with the inert chloride in the water sample.^{15,23} However, if no isotopic dilution occurred, the reaction yield would be 1.0%.

Table 5.3. Radioactivity (^{36}Cl) distribution and material balance for chromatographic separation of constituents from a 1470-fold concentrate of chlorinated cooling water^a

Component	Activity in chromatographic peaks and resin (10^7 counts/min)	Distribution (%)	Percent of total activity chromatographed
Chloride peak	2.03	98.66	92.69
Other peaks	0.03	1.25	1.18
Resin ^b	0.02	0.09	0.08
Material balance	2.06	99.99	93.95

^aThe ^{36}Cl -tagged 280- μl sample of effluent concentrate contained a total activity of 2.19×10^7 counts/min and a specific activity, after isotopic dilution, of 6.44×10^6 counts min^{-1} (mg Cl)⁻¹.

^bActivity was removed from the resin by ethanol extraction.

5.3 Conference On The Environmental Impact of Water Chlorination

R. L. Jolley

International concern about the effects of chlorination during water treatment has prompted increased attention from the scientific community. The Conference on the Environmental Impact of Water Chlorination has been organized and will be held at the Oak Ridge National Laboratory October 22-24, 1975. At this conference, scientists in biological, chemical, engineering, environmental, and other disciplines will present papers and discuss major facets of chlorine-related problems. It will stress the need for free interchange of information (on both a formal and an informal basis) to permit the development of new ideas and concepts in all chlorine research. Several structured objectives of the conference are (1) to make an assessment of the available knowledge of the aqueous chemistry of chlorine, (2) to examine effects of chlorine and chlorinated organic products on aquatic ecosystems and man, (3) to assess the biological and ecological implications of chlorine usage for treatment of natural and process waters (e.g., cooling waters and sewage effluents), and (4) to publish the proceedings of this conference in order to provide a permanent record and reference document containing the most recent relevant scientific data on water chlorination. Principal results anticipated from the conference are an increased understanding of chlorination and a better definition of possible environmental problems.

The Conference on the Environmental Impact of Water Chlorination will be sponsored by the Energy Research and Development Administration, the U.S. Environmental Protection Agency, and the Oak Ridge National Laboratory. The conference has been organized by R. L. Jolley, Conference Chairman, of the ORNL Chemical Technology Division. Other members of the planning

committee are C. W. Gehrs, Environmental Sciences Division, ORNL; W. W. Pitt, Chemical Technology Division, ORNL; R. B. Cumming, Biology Division, ORNL; W. A. Brungs, EPA National Water Quality Laboratory, Duluth, Minnesota; and D. H. Hamilton, ERDA Division of Biomedical and Environmental Research, Washington.

The program will consist of invited papers from major investigators in the field. The Proceedings of the conference will be published probably as an ERDA Conference Document.

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6. CHEMICAL ENGINEERING RESEARCH

The Chemical Engineering Research Program includes a number of separate fundamental studies that often benefit the mission-directed development programs of the Chemical Technology Division. The investigations are of broad interest to chemical engineering and important to one or more potential useful applications. The studies may fit into any of the broad divisions of chemical engineering such as reacting systems, separation processes, data correlation, or transport phenomena. Potential applications of these studies are usually related to future energy programs.

Efforts during this report period have included studies of three-phase fluidized beds; oxidation of tritium on permeable surfaces; evaluation of a unique solid-gas separation device; mechanically agitated, nondispersing liquid-liquid contactors (Lewis cells); preparation of uniform microspheres for separation processes; adiabatic combustion of graphite; and development of continuous chromatography. These studies are of interest to various fission and fusion reactor concepts, separation processes, and off-gas processing.

6.1 Three-Phase Fluidized Bed Reacting System

J. M. Begovich

A study of three-phase fluidized bed reacting systems was initiated during this report period. The system of interest consists of solid particles fluidized by the upward cocurrent flow of gas and liquid. The liquid forms the continuous phase, and the gas and solids are discontinuous phases. This type of system is of particular relevance to coal conversion

processes and some bioreactors (see Sect. 4.5), but neither the hydrodynamics nor the various mass transfer processes in these systems are well understood. The available literature information is sparse and often conflicting. The objectives of this study are to provide fundamental data for the actual transport processes that occur in these systems and to correlate the results with reliable literature data into useful forms.

6.1.1 Background

Several factors must be considered to design and accurately predict the operation of a three-phase fluidized bed. These include the various phase holdups, the minimum fluidizing velocities, the mixing characteristics of the solids and the liquid, and the overall volumetric mass transfer coefficients.

The holdup of one phase in a multiphase system is defined as the fraction of the system volume occupied by that phase. Thus, a three-phase fluidized bed has three such volume fractions related by the following equation:¹

$$\epsilon_G + \epsilon_L + \epsilon_S = 1, \quad (1)$$

where

ϵ_G = gas holdup,

ϵ_L = liquid holdup,

ϵ_S = solids holdup.

If wall effects are neglected, the volume fractions are also related to the pressure drop over the bed by Eq. (2):

$$\Delta P = H(\epsilon_L \rho_L + \epsilon_G \rho_G + \epsilon_S \rho_S) g/g_c, \quad (2)$$

where

ΔP = pressure drop across the bed,

H = expanded bed height,

ρ_i = density of the i^{th} phase,

g = acceleration due to gravity,

g_c = conversion constant.

The solids volume fraction can be calculated from the expanded bed height using Eq. (3):

$$\epsilon_S = \frac{M_S}{\rho_S A H}, \quad (3)$$

where

M_S = total mass of solids,

A = cross-sectional area of the bed.

If pressure drop and expanded bed height measurements are accurate, Eqs. (1-3) are sufficient to solve for the three volume fractions. Gas holdup can also be determined by simultaneously stopping all flows and measuring the settled liquid height, H_0 . The gas volume fraction is then calculated from the following:

$$\epsilon_G = \frac{H_L - H_0}{H_L}, \quad (4)$$

where

H_L = dynamic height of the liquid in the column.

Several mass transfer processes can take place in three-phase fluidized beds. Our initial mass transfer studies have looked at gas-liquid mass transfer controlled by resistance in the liquid film. The

overall volumetric mass transfer coefficient, $K_L a$, can be calculated from one of the following equations depending on the extent of liquid dispersion.^{2,3} The gas phase is considered to be in plug flow in all three cases.

Case 1: Liquid phase in plug flow ($Pe_L = \infty$):

$$K_L a \Big|_{pt} = \frac{U_L}{H(F+1)} \cdot \ln \frac{1}{XF + X-F}, \quad (5)$$

where

$$X = \text{approach to equilibrium, } \frac{C_{Leq} - C_{Lout}}{C_{Leq} - C_{Lin}},$$

$$F = \text{extraction ratio, } mU_L/U_G,$$

U_L, U_G = superficial velocity of the liquid and gas phases,

$$m = \text{dimensionless Henry's law constant, } C_{Leq}/C_G,$$

$$Pe_L = \text{Peclet number, } H U_L/E_L,$$

E_L = axial dispersion coefficient in the liquid phase.

Case 2: Liquid phase is completely mixed (CSTR) ($Pe_L = 0$):

$$K_L a \Big|_{CSTR} = \frac{U_L}{H \cdot F} \cdot \ln \frac{X}{XF + X-F}, \quad (6)$$

Case 3: Liquid phase has finite dispersion ($Pe_L = \text{finite}$):

$$X = 1 - \frac{1}{F+1} + \frac{A}{F+1} \quad (7)$$

where

$$A = \left[\begin{array}{c} \frac{\exp(\mu_2 Z)}{\mu_2 \exp(\mu_2)} - \frac{\exp(\mu_1 Z)}{\mu_1 \exp(\mu_1)} \\ \frac{1 - \frac{E_L \mu_2}{U_L H}}{\mu_2 \exp(\mu_2)} - \frac{1 - \frac{E_L \mu_1}{U_L H}}{\mu_1 \exp(\mu_1)} \end{array} \right]$$

Z = dimensionless height up the bed (at top of bed, $Z = 1$);

$$\mu_{1,2} = \frac{- \left[\frac{E_L \cdot NTU}{U_L H} - \frac{1}{F} \right] \pm \sqrt{\left(\frac{E_L \cdot NTU}{U_L H} - \frac{1}{F} \right)^2 + \frac{4E_L}{FU_L H} \left(NTU + \frac{1}{F} + 1 \right)}}{2E_L / FU_L H}$$

NTU = number of transfer units, $K_L a \cdot H / U_L$.

Thus, Eq. (7) is implicit in NTU (or $K_L a$) and must be solved by a numerical technique. When X is known from measurements of inlet and outlet concentrations, NTU is varied until the value calculated by Eq. (7) equals the known value. At this point, NTU is known, and $K_L a$ can be determined.

6.1.2 Experimental apparatus

The experimental apparatus is shown in Fig. 6.1 and consists of a 7.62-cm-ID Plexiglas column with the associated gas and liquid piping. The auxiliary equipment is described in a previous report.⁴ The center section of the column is replaceable so that total column height of 53, 84, 114, 145, 175, and 206 cm can be studied. Gas and liquid enter at the bottom of the bed; the gas enters through a distributor with thirty 1.2-mm-diam holes, and the liquid enters through a 50 x 50 mesh screen. The screen also supports the solid phase. The gas is vented to the

Fig. 6.1. Three-phase fluidization apparatus.

atmosphere while the liquid exits through a glass tee near the top of the column and is sent either to a floor drain or back to the 55-gal feed tank. Solids are prevented from flowing out of the column by a 16-mesh screen across the tee.

Four types of solids have been used which differ in size, density, or shape: -4 +8 mesh alumina beads with an average particle diameter of 6.4 μ m and a density of 1.93 g/cc; -8 +12 mesh alumina beads with an average particle diameter of 2.1 mm and a density of 1.63 g/cc; 2.38-mm Plexiglas cubes; and 6.35-mm Plexiglas spheres with a density of 1.17 g/cc.

The three-phase holdups were determined using Eqs. (1), (3), and (4). Thus, ϵ_G and ϵ_S were determined directly, and ϵ_L was calculated as the residual of Eq. (1). The determination of ϵ_G and ϵ_S involved inherent errors. As can be seen in Fig. 6.2, the fluidized bed does not occupy the entire column. The solids concentration is approximately constant in the lower section of the column (to just above sample port 3). A more dilute region exists between sample ports 3 and 5. Above port 5 the column is essentially a bubble column with no solids present. Figure 6.2 is representative of all the packings used. Because the top of the expanded bed is indistinct and the height is difficult to determine, this leads to uncertainties in the calculation of ϵ_S .

The use of simultaneous shut-off of all flows to determine ϵ_G yields an average gas volume fraction over the entire column. To separate the gas holdup in the fluidized bed region from that in the bubble column region, two different initial heights were used at each of the desired flow rates. By assuming that the gas volume fractions are independent of bed height, two simultaneous equations result which can be solved for the two volume fractions.

Fig. 6.2. Fluidization of 8 x 12 mesh alumina with air and water.

The minimum fluid velocities required to achieve fluidization were determined by measuring the pressure drop across the bed. After the desired gas flow rate was set, the liquid flow rate was increased incrementally. The bed is considered fluidized when the pressure drop no longer changes with an increase in liquid velocity. The total column pressure drop was determined by measuring the difference between the liquid heights in the column and sight tube. The pressure drop due to the bubble column, determined earlier at the same fluid flow rate and appropriate height, was subtracted from the total to give the pressure drop across the bed.

Overall volumetric mass transfer coefficients for the absorption of carbon dioxide into water were determined for a bubble column and for the 6.35-mm Plexiglas spheres and -4 +8 mesh alumina beads in the fluidized column. The sampling and titration techniques used were discussed in the previous progress report.⁵ Samples were taken from each sample port in the column so that a concentration profile could be obtained in the bed. Taking the average concentration of a number of samples from the top of the bed and an assumed value for the dispersion coefficient, $K_L a$ was calculated using Eq. (7). The concentration profile down the bed was calculated using this $K_L a$ and the same E_L , again using Eq. (7). This calculated profile was then compared to the experimental profile. If the agreement was not adequate, a new E_L was assumed, and the iteration was repeated.

6.1.3 Hydrodynamic results

Effect of flow rate on ΔP . The effect of gas and liquid flow rate on the pressure drop across the bed is shown in Fig. 6.3. These data,

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SOLID PHASE: 6.35-mm ALUMINA SPHERES
 STATIC BED HEIGHT = 15.1 cm
 ○ $U_G = 10.16$ cm/sec
 ● $U_G = 0.0$ (TWO-PHASE FLUIDIZATION)

Fig. 6.3. Effect of flow rates on overall pressure drop.

typical of each packing used, were taken with 6.4-mm alumina beads using two gas velocities and a variety of liquid velocities.³ A sharp break point exists in the curve for zero gas velocity, which corresponds to a minimum liquid fluidization velocity, U_{Lmf} , of 4.5 cm/sec; this is in excellent agreement with a correlation developed by Wen and Yu.⁶

The addition of a constant gas flow rate caused the break in the curve to shift so that a considerably lower liquid velocity was necessary to achieve fluidization. The minimum liquid fluidization velocity was approximately 2.6 cm/sec at a gas velocity of 10.2 cm/sec, but the break point was not clearly defined.

The liquid velocity at incipient fluidization plotted against gas velocity for three packings is shown in Fig. 6.4.⁷ Two initial bed heights were used at each of five gas velocities. Contrary to expectations, U_{Lmf} decreased with increasing initial bed height, even though the bed height should not affect the fluidization characteristics. These differences could result from end effects, especially since the gas and liquid distributors are separated by a distance of 3.8 cm. At zero velocity, the differences in U_{Lmf} for the two initial bed heights are not significant.

Minimum fluidization results from three packing materials are shown in Fig. 6.4. Tests with the Plexiglas cubes were stopped after a few initial runs. Due to their irregular shape, they grouped together, trapped air bubbles, and rose to the surface. This actually caused the concentration of the Plexiglas cubes to increase up the column, leaving a dilute phase at the bottom.

Fig. 6.4. Minimum fluidization velocities as a function of packing and initial bed heights.

Effect of flow rate on ϵ_G . Gas holdup was not correlated with the liquid and gas flow rates. When measurements of ϵ_G at constant U_G were plotted against U_L , the resulting data were scattered. Any of the following sources of error could account for the scatter:

- (1) It was difficult to measure a definite bed height for the low-density packings or at high fluid flow rates. This height was needed to measure ϵ_S and to solve the simultaneous equations mentioned previously. The extent of this difficulty is illustrated in Fig. 6.2.
- (2) Simultaneous shut-off of the entering and exiting fluid streams was difficult. The shut-off of the liquid overflow was accomplished by squeezing shut the 2.5-cm-ID Tygon tubing. This was difficult to coordinate and was prone to error, because a second person was needed to close the gas valve, and a third person was needed to close the liquid inlet valve.
- (3) Some additional gas emerged from the bed shortly after all fluid streams were stopped. This suggested that gas was trapped in the bed. If more gas remained in the bed, an incorrect indication of the gas holdup would result.

Effect of flow rate on ϵ_S . The solid volume fraction is plotted in Fig. 6.5 against U_L , with initial bed height and U_G as parameters for the -4 + 8 mesh alumina.⁷ The packed bed and fluidized bed regions are distinct, with ϵ_S remaining constant in the first region and decreasing with increasing U_L in the second region. The solid volume fraction

Fig. 6.5. Effect of fluid velocities and initial bed height on solids holdup.

appears to be essentially independent of superficial gas velocity and initial bed height over the range of flow rates used.

Correlation of ϵ_S with U_L and $U_{L_{mf}}$ for the -4 +8 mesh alumina -8 +12 mesh alumina, and 6.35-mm Plexiglas resulted in the following equation:

$$\epsilon_S = 0.509 \left(U_L / U_{L_{mf}} \right)^{-0.076 \pm 0.020} \quad (8)$$

Effect of packing characteristics on volume fractions. Although a large degree of scatter existed in the ϵ_G data (and hence ϵ_L), two qualitative results were observed:

- (1) As particle diameter increased, and other parameters (ρ_S , U_G , U_L , and H) remained constant, ϵ_L and ϵ_G increased while ϵ_S decreased.
- (2) As particle density increased, and other parameters remained constant, ϵ_G and ϵ_S increased while ϵ_L decreased.

6.1.4 Mass transfer results

Two solid materials, the -4 +8 mesh alumina and 6.35-mm Plexiglas, were used in mass transfer experiments. A typical concentration profile up the column is shown in Fig. 6.6 for the alumina.³ Depending on the model chosen, the profile is similar to one of the three shown. The top curve is obtained if it is assumed that the liquid phase is in plug flow. If it is assumed that the liquid is completely mixed, the horizontal line results (i.e., the concentration throughout the column is the same as the concentration in the effluent). If the liquid is characterized by a finite dispersion coefficient, the curve falls somewhere between the

$$U_G = 10.4 \text{ cm/sec}$$

$$U_L = 5.5 \text{ cm/sec}$$

$$H = 42.5 \text{ cm}$$

$$X = (C_{L_{eq}} - C_L) / (C_{L_{eq}} - C_{L_{in}})$$

Fig. 5.6. Theoretical and experimental concentration profiles in a three-phase fluidized bed.

two extremes. For this case, the experimental data indicate that plug flow was the correct model. Data from other runs at different conditions indicated that dispersion was important.

Table 6.1 shows the results obtained for the limited number of operating parameters studied. Two packings were used, but the column was also operated with no packing (i.e., a bubble column).^{3,7} The dispersion and mass transfer coefficients were obtained by mathematically matching the experimental concentration profile, as described previously. This is not a sensitive method for obtaining E_L , but it does give a qualitative indication of the intensity of mixing present in the bed.

Results of runs 5 and 6 show that, compared to a bubble column at similar conditions, the dispersion coefficient was lower, and the mass transfer coefficient was higher for the bed of -4 +8 mesh alumina particles. Conversely, a comparison of runs 2 and 3 shows that the dispersion coefficient was higher and the mass transfer coefficient was lower for the bed of 6.35-mm Plexiglas spheres. This is in qualitative agreement with literature results.^{8,9} The low value of E_L for run 1 is explained by noting (see Fig. 6.4) that the column was operating as a packed bed because the bed was not fluidized at the flow rates used.

6.1.5 Future work

It is obvious that knowledge of both the hydrodynamic and mass transfer characteristics in three-phase fluidized beds is still incomplete. In the next few months we plan to concentrate our efforts on solving the hydrodynamic problems mentioned and on obtaining correlations for the minimum fluidization velocities and for various volume fractions.

Table 6.1. Summary of dispersion and overall mass transfer coefficients

Run	Packing	U_L (cm/sec)	U_G (cm/sec)	E_L (cm ² /sec)	$K_L a$ (sec ⁻¹)
1	6.35-mm Plexiglas	0.88	1.67	4	0.0124
2	6.35-mm Plexiglas	2.14	4.05	125	0.0342
3	Bubble column	2.14	4.05	35	0.0736
4	6.35-mm Plexiglas	3.39	6.45	115	0.0384
5	-4 +8 mesh alumina	3.39	6.45	0	0.1090
6	Bubble column	3.02	6.54	70	0.0498
7	-4 +8 mesh alumina	4.63	8.80	30	0.0890
8	-4 +8 mesh alumina	3.12	12.10	110	0.0595
9	-4 +8 mesh alumina	5.48	11.70	27	0.0773
10	-4 +8 mesh alumina	5.48	11.60	20	0.0993

A 15.24-cm-ID column has been constructed to test the effects of diameter on the operating characteristics of the fluidized beds. Sample ports along the two columns have been connected to a manometer board so that pressure profiles can be obtained across the entire column. This will allow us to use Eq. (2) and thereby solve for the holdups more accurately. Ball valves have been placed on the exiting liquid streams so that gas holdup can also be determined with more accuracy.

Various tracer techniques are being considered to measure liquid holdup. With accurate values for the dispersion coefficient, direct calculation of mass transfer coefficients could be made without the difficulty of matching experimental concentration profiles. Also, a gas chromatograph has been obtained which will permit gas phase analyses, and thus more accurate overall mass balances and mass transfer coefficients.

6.2 Hydrogen Permeation-Oxidation Studies

S. D. Clinton and J. S. Watson

Due to the large permeation rates of tritium through containment barriers at even moderate temperatures (300 to 500°C), the handling of tritium (specific activity of 10^4 Ci/g) in fusion reactors can create hazardous situations. Oxidation of tritium to a nonpermeating and recoverable form could make effective secondary containment possible. In addition, an oxidation technique may be useful for recovering the bulk of the tritium produced in a breeding blanket.

The technique under investigation in this study is oxidation of tritium to water as it permeates through the first containment or heat

exchange surface. An important consideration is the chemical form of the tritium as it leaves the permeated surface. Low concentrations of oxygen are likely to be present in either a helium coolant or reactor containment atmosphere, and higher concentrations of oxygen could be added. Atomic tritium, permeating a metal barrier, should react with oxygen at the metal surface to form the oxide; however, the catalytic effectiveness of certain metals being considered for fusion reactor piping and heat exchangers is unknown. To date, only preliminary data have been obtained with deuterium (stand-in for tritium) permeating through 304 stainless steel and palladium tubes.

6.2.1 Experimental equipment

A schematic equipment diagram for the hydrogen isotope permeation-oxidation studies is shown in Fig. 6.7. The basic system consists of a permeation tube surrounded by a 1 1/2-in.-diam tubular vacuum chamber, a furnace, a VacSorb roughing pump, a 20 liter/sec noble ion pump, and a variable leak valve for introducing oxygen. Two nude ion gauges (millitorr and ultrahigh vacuum) measure the system pressure, and the gas composition within the vacuum chamber is monitored by a mass spectrometer (UTI Model 100C). Since the maximum operating pressure for the spectrometer is 10^{-3} torr, the vacuum chamber contains a 2-mil-diam orifice to permit higher oxygen partial pressures at the permeation tube surface. The primary goal of the experiment is to determine the conversion of a permeating hydrogen isotope (deuterium) to water at conditions which can be expected to prevail within fusion power reactor systems. Deuterium is being used as a stand-in for tritium and, to date,

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Fig. 6.7. Schematic equipment diagram for permeation-oxidation studies.

two permeation metal surfaces (304 stainless steel and palladium) have been tested briefly. Figure 6.8 is a photograph of the permeation-oxidation system and the associated instrumentation.

6.2.2 Results and discussion

The initial permeation-oxidation results have been obtained with deuterium and two metal permeation tubes: 304 stainless steel and palladium. Stainless steel is an obvious choice as a test specimen material; however, palladium was chosen as a control or reference material. At room temperature, the permeation rate of hydrogen through palladium is controlled by the exit surface reaction.¹⁰ Although palladium can adsorb large quantities of hydrogen and the diffusion rate is high, hydrogen does not leave the metal surface at a significant rate for temperatures below 100°C. However, a palladium surface will lose hydrogen rapidly at room temperature in an atmosphere containing oxygen. The release is apparently caused by reaction of the hydrogen atoms with oxygen on the metal surface.

All of the deuterium-stainless steel data were taken without an orifice between the permeation tube and spectrometer (see Fig. 6.7). This limited the preliminary data to a very low total pressure. At 300 to 500°C, and with an oxygen partial pressure of 10^{-6} torr, the conversion of permeating deuterium to water was 40 to 50%. With a palladium tube at 25°C and an oxygen partial pressure at the metal surface of 10^{-3} torr, the conversion of deuterium to water was approximately 80%. It is necessary to obtain more data with a variety of materials at higher temperature and higher oxygen pressures to determine conditions in which permeating hydrogen is effectively converted to water.

PHOTO 2352-75

Fig. 6.8. Photograph of the permeation-oxidation system and the associated instrumentation.

6.2.3 Program status

A 2-mil-diam orifice has been installed in the vacuum system between the permeation tube and gas analyzer. With a pumping speed of 20 liters/sec, the pressure ratio across the flow resistance is between 10^4 and 10^5 (i.e., gas analyzer at 10^{-6} torr and permeation tube at 10^{-2} to 10^{-1} torr). A palladium permeation tube (0.150-in. diam, 0.015-in. wall, and 1-in. long) has been installed, and the system is now undergoing a bakeout period. Testing with palladium should be completed by the end of October, and the next series of experiments will be made with deuterium and nickel.

6.3 Experiments with a Mechanically Agitated, Nondispersing Contactor Using Water and Mercury

C. H. Brown

We have continued development of a mechanically agitated, two-phase contactor that uses an aqueous electrolyte and mercury to simulate systems in which it is desirable to contact two phases without dispersing one phase into the other (e.g., the extraction of rare earths from fluoride fuel carrier salt to an intermediate bismuth stream in MSBR processing).

As reported previously,¹¹ we have investigated the feasibility of using a polarographic technique for measuring electrolyte film mass transfer coefficients in this type of contactor. During this report period, we have (1) tested three different anode materials; (2) produced cathodic polarization waves which correspond to the reduction at the mercury surface of Fe^{3+} complexed with excess oxalate ions; (3) obtained and calibrated a slow-scan controlled-potential cyclic voltameter; and (4) examined the quinone-hydroquinone redox couple as a possible alternate to the Fe^{3+} - Fe^{2+} couple now being used.

6.3.1 Modifications to experimental equipment

With the exception of the tests made with the quinone-hydroquinone redox couple, all tests made during this period were performed with the equipment described previously.¹²

Prior to studies made on the quinone-hydroquinone system, a slow-scan controlled-potential cyclic voltameter (potentiostat) was obtained from the Analytical Chemistry Division to replace the Hewlett Packard dc-power supply used previously. This is a three-electrode instrument that controls the potential between the mercury surface and a standard calomel reference electrode (SCE) while passing a current between the auxiliary electrode and the mercury surface. Voltages can be scanned between +2 V vs SCE and -2 V vs SCE, at a scan rate up to 1 V/min. The potentiostat can carry a current of up to 2.5 A between the auxiliary and mercury electrodes.

6.3.2 Experiments with the Fe^{3+} - Fe^{2+} system

The electrolyte used for all experiments performed during this report period was nominally 0.001 M Fe^{2+} obtained from ferrous sulfate, 0.00025 M Fe^{3+} obtained from ferric sulfate, and 0.8 M potassium oxalate. The oxalate ions form a stable complex with both the Fe^{3+} and Fe^{2+} that facilitates direct measurement of the Fe^{3+} reduction wave.

Successful polarization waves were produced with the three anode materials that were tested: copper, iron, and gold; however, the copper and iron reacted with the electrolyte solution. This side reaction introduced scatter in the data and possibly could alter the properties of the solution. To avoid this problem, an anode was fabricated by

plating gold on a 0.0625-in.-thick sheet of nickel, which was formed to fit the inner perimeter of the electrochemical cell. The gold anode was inert to the solution and introduced no stray reactions to the polarogram.

Figure 6.9 shows a polarogram, measured with the electrolyte described above, in the 5- by 7-in. Plexiglas contactor using the gold anode with no agitation, and phase volumes of about 1.8 liters each. The current through the cell is plotted as a function of the mercury surface potential relative to the standard calomel electrode (SCE). The current increases from zero at zero applied potential to a relatively constant value at an applied potential of about -0.35 V vs SCE (standard calomel electrode). In this region, continuous electrolysis is taking place in the cell, causing reduction of $\text{Fe}(\text{C}_2\text{O}_4)_3^{3-}$ at the mercury cathode. In the region of applied potential from -0.35 V vs SCE to -0.80 V vs SCE, the current through the cell increases slightly. In this region, the current is limited by diffusion of the $\text{Fe}(\text{C}_2\text{O}_4)_3^{3-}$ to the mercury surface where it is reduced. The diffusion current can be related to the mass transfer coefficient through the electrolyte film, as discussed previously.¹¹

The half-wave potential is defined as the potential at which the current is equal to one-half the limiting value. The measured half-wave potential for the ferric oxalate complex is shown in Fig. 6.9. The half-wave potential of -0.245 V measured in the contactor agrees well with a value reported in the literature of -0.24 V vs SCE for the reduction of ferric oxalate.¹³

Under ideal conditions, the diffusion current is directly proportional to the polarized electrode surface area and the bulk concentration of the limiting ion. Two tests were performed to determine that we were

Fig. 6.9. Cathodic polarization wave for $\text{Fe}(\text{C}_2\text{O}_4)_3^{3-}$ measured in the 5- by 7-in. rectangular contactor with no agitation.

actually polarizing the mercury surface. In the first test, the anode surface area was decreased by about 48%. This had no effect on the magnitude of the diffusion current, indicating that the mercury surface (cathode) was polarized rather than the anode. In the second test, the concentration of the ferric ion was doubled, but no concomitant increase in diffusion current was seen. Since the diffusion current is directly proportional to the concentration of the limiting ion (Fe^{3+}), one would expect the current to double. The only explanation for this behavior is that most of the Fe^{3+} had been reduced by some contaminant in the system, possibly present in the mercury. This would cause the ferric ion concentration to be present at only a very low level during cell operation due to electrolytic oxidation of the ferrous ion.

To eliminate the possibility of reductant being present in the mercury, a fresh supply of purified mercury was obtained from the Analytical Chemistry Division. A test was performed using the purified mercury and an electrolyte with the same nominal Fe^{3+} and Fe^{2+} concentrations given above. Preparation of the electrolyte in the absence of oxygen precluded any possible oxidation of Fe^{2+} to Fe^{3+} . Again, the anode surface area was decreased with no discernible decrease in the diffusion current, indicating that the mercury surface was polarized. An increase of the Fe^{3+} concentration from ~ 0.25 millimolar to ~ 0.5 millimolar resulted in increasing the diffusion current by a factor of 2, indicating that the wave being measured is the ferric ion reduction wave. The half-wave potential, however, was -0.75 V vs SCE, which is about three times the reported value.

5

In order to calculate the aqueous film mass transfer coefficient from the polarographic data, it is necessary to know the bulk concentration of the oxidized species accurately. The method presently being used for this measurement is a polarographic technique using a dropping mercury electrode. Samples of the electrolyte used in the second of the two tests mentioned previously were analyzed for Fe^{3+} and Fe^{2+} by this method. Results indicated that the Fe^{3+} and Fe^{2+} concentrations were 1.7 millimolar and 0.28 millimolar, respectively, which is in poor agreement with the expected values of 0.50 millimolar Fe^{3+} and 1.0 millimolar Fe^{2+} . One possible cause for the poor agreement is that the Fe^{2+} oxidized to Fe^{3+} while in the sample bottles. This was not expected, however, since the electrolyte had been sparged with argon to remove dissolved oxygen, and the sample bottles were purged with argon to remove air.

To determine if the analytical results reported above were in error because of analytical technique or preparation errors, two standard solutions were prepared and samples of these solutions were analyzed. One solution was prepared with 56 $\mu\text{g}/\text{ml}$ Fe^{3+} , and the other solution was prepared with 56 $\mu\text{g}/\text{ml}$ Fe^{2+} ; both solutions were 1 M in $\text{K}_2\text{C}_2\text{O}_4 \cdot \text{H}_2\text{O}$. Analytical results indicate that both solutions contained both Fe^{3+} and Fe^{2+} and had the same composition, 50 $\mu\text{g}/\text{ml}$ Fe^{3+} and 27 $\mu\text{g}/\text{ml}$ Fe^{2+} . Further investigation will be necessary to determine the correct method for preparing and/or analyzing iron oxalate solutions of a desired concentration.

6.3.3 Experiments with the quinone-hydroquinone system

A possible alternate to the Fe^{3+} - Fe^{2+} system for measuring electrolyte-phase mass transfer coefficients is the reversible reduction of quinone to hydroquinone at the mercury cathode.

The reaction under consideration is:

Since hydrogen ion is a reacting material, as is quinone, a strong buffer must be present as a supporting electrolyte. The buffer causes the H^+ concentration to be essentially constant across the interfacial electrolyte film, because the rate of establishing the buffer equilibrium is relatively rapid compared to the quinone diffusion rate.¹⁴

A qualitative test was made with the quinone system to determine if good polarization waves could be measured and if the quinone electrolyte is inert to mercury. The electrolyte was 0.01 M hydroquinone and 0.005 M quinone with a 0.05 M phosphate buffer at a pH of 7.0. Satisfactory polarization waves were obtained in a small cell with a large copper anode and a mercury pool cathode. The electrolyte was chemically inert to mercury during the tests. The quinone electrolyte color was observed to change from a light yellow to deep brown within several hours. This phenomenon is due to the decomposition of quinone by uv light. Further studies will be performed in the 5- by 7-in. contactor to determine if this system is suitable for mass transfer measurements.

6.4 Preparation of Small Particle Sorbents

W. W. Pitt and Guy Jones

Numerous chemical processes involve interaction and mass transfer between solid particles in fixed beds and gas or liquid streams. This is particularly true in several energy-related processes. Generally, the limiting factor on the efficiency or effectiveness of the process is the resistance to mass transfer, both between the stationary and mobile phases and within the stationary or solid phase. These resistances can be reduced by decreasing the size of the particles in the fixed beds. However, smaller particles require higher pump pressure to force the fluid flow through the bed. The pressure drop across a packed bed of a given size particle can be minimized by using uniformly sized spheres as the bed material.

Sol-gel processes for preparing uniformly sized metal oxide spheres in the range of several hundred microns in diameter have been developed and successfully demonstrated at the Oak Ridge National Laboratory.¹⁵ The goal of this research is to extend the capability of these processes down to particle sizes less than 10 μ .

As reported previously,⁵ 2- to 20- μ spheres of erbium oxide gel could be formed by using a turbulent, two-fluid nozzle with a right-angle entry. During this report period, efforts have been directed towards improving the uniformity of size by imposing a vibration on the drive fluid. During the course of the investigation, it became apparent that the use of isoamyl alcohol as the drive fluid was unsatisfactory. The high drying rate of the isoamyl alcohol often caused the sol feed nozzle to plug with gelled material. Consequently, we returned to 2-ethyl

hexanol as the drive fluid. Also to minimize plugging, a larger diameter (0.4 mm) sol feed nozzle was used. To reduce the entrainment of small gel particles in the recirculating alcohol, the tapered column was replaced by the apparatus shown in Fig. 6.10.

With these modifications, an attempt was made to produce gram quantities of $\sim 10\text{-}\mu$ erbium oxide spheres using the following operating parameters:

Sol flow rate	- 0.1 ml/min
Drive fluid flow rate	- 380 ml/min
Vibrator frequency	- 400 Hz

Approximately 2 g of spheres were produced and subsequently fired to 1400°C . The 100X magnification of some of the product spheres shown in Fig. 6.11a indicates that spheres in the range 5 to $50\text{ }\mu$ were produced. The 1000X scanning electron micrograph in Fig. 6.11b shows that the particles are very porous and also very firable. Firing to a higher temperature would probably have reduced both of these properties.

Although the product spheres were disappointingly nonuniform, they were used to pack a 1.5- by 500-mm column, and their properties for sorption chromatography are under investigation.

6.5 Adiabatic Combustion Graphite

R. M. Canon

Adiabatic graphite oxidation studies are being conducted in a continuing program that was first described in a previous report in the series.⁵ These experiments involve the operation of an adiabatic burner to oxidize graphite rods. Burner operation utilizing pure oxygen feed

Fig. 6.10. Schematic diagram of apparatus for producing sol-gel microspheres using a turbulent, two-fluid nozzle.

(a)

(b)

Fig. 6.11. Erbium oxide spheres fired to 1400°C.

(a) Optical microscope with side lighting.
100X magnification

(b) Scanning electron microscope.
1000X magnification

and product gas recycle have been effective in controlling burn rates and graphite temperatures.

The reactions occurring at the interface are:

Reaction (1) is thermodynamically favored over reaction (2) at all operating temperatures in the presence of excess carbon. Reaction (3) is slow at all common burner temperatures. In addition, a gas phase reaction occurs:

This reaction is sufficiently rapid to convert most of the carbon monoxide in the boundary layer. The major reactions are exothermic; therefore, once the reaction is initiated, the oxidation is self-sustaining. Essentially all of the heat is removed by the product gases.

6.5.1 Experimental procedure

Graphite rods, 2.6-cm diam by 56-cm long, are placed vertically into a 4.75-cm-diam furnace. The solid graphite rods are type H-327, with five 0.18-cm holes drilled axially to various depths to allow insertion of thermocouples. Burning occurs on the outside of the graphite rods which are preheated to approximately 1000°C under an argon atmosphere before oxygen is admitted. A portion of the product gases are recycled during operation.

Several graphite rods have been burned in an attempt to determine the important variables. Previous studies of carbon rod combustion suggested that the oxidation is mass transfer controlled at velocities as high as 165 m/sec;¹⁶ this has been confirmed by our investigations in which graphite rods have been used. Gas velocities up to 12 m/sec have been studied, and it was found that reaction rates increase with gas flow rate. No dependence of reaction rate on graphite temperature was observed in the temperature range studied (1000°C to 1200°C).

Typically, a "spike" in carbon monoxide concentration of a few percent is observed in the product gas immediately after startup. The carbon monoxide concentration then decreases to 1 or 2% and remains at a low value throughout the burn. This behavior is shown in Fig. 6.12. Also shown are typical carbon dioxide concentrations over a 30 min run. The carbon dioxide concentration rises quickly, then gradually declines as less area is available for burning. Exit oxygen concentrations significantly below 30% have not been obtained with the 56-cm rods. Longer rods would lower the O₂ exit concentration and therefore increase O₂ utilization.

Diffusion theory suggests that the flux of oxygen to the graphite surface, and therefore the burn rate, is dependent on O₂ concentration:

$$\text{Burn rate} \propto \ln \left[1 + X_{O_2} \left(\frac{O_2 + CO_{2\text{flux}}}{O_{2\text{flux}}} \right) \right] \quad (5)$$

where X_{O_2} is the mole fraction O₂ in the bulk gas stream.

Fig. 6.12. Typical gas composition at burner exit (remainder O₂).

Since CO_2 flux caused by reaction (3) is small compared to the O_2 flux for the burn rates and temperatures of this study, a satisfactory approximation is

$$\text{Burn rate} \propto \ln(1 + X_{\text{O}_2}). \quad (6)$$

Using reaction (6), it is possible to adjust the experimental burn rates to the value that would be obtained if the feed were pure oxygen (no recycle) and if all other conditions remained the same. This is the "Concentration Adjusted Burn Rate" (CABR), and is defined as

$$\text{CABR} = (\text{Burn rate}) \left[\frac{\ln(2)}{\ln(1 + X_{\text{O}_2}^{\circ})} \right] \quad (7)$$

where $X_{\text{O}_2}^{\circ}$ is the inlet O_2 mole fraction.

The dependence of burning rate on gas flow can then be estimated, and the results are demonstrated in Fig. 6.13. Average burn rates for the particular gas flows are shown.

A temperature control loop was installed to establish the feasibility of graphite temperature control by variation of recycle rate. The exit gas temperature was sensed, and the recycle gas flow rate was varied to hold the temperature at the desired value. Figure 6.14 shows the effectiveness of the control system. Graphite temperatures were in excess of 1200°C with exit gas temperature at 600°C ; therefore, the set point was reduced to 540°C . The graphite temperatures could not be shown because four of the five thermocouples failed during the temperature excursion. The remaining thermocouple indicated temperatures less than 1200°C just prior to run termination.

Fig. 6.13. Burning rates for 2.6-cm-diam, 56-cm-long graphite rods.

Fig. 6.14. Exit gas temperature (recycle control in operation).

The burning rate is not constant along the axis of the rod. In general, the highest local burn rate occurs at some distance down the rod, not at either end of the rod. This produces an hourglass shape in the unburned portion. Except at very high burn rates, there is also a tendency for the leading end of the rod to cool below combustion temperature. It is anticipated that higher inlet concentrations of oxygen and/or higher recycle gas temperatures will overcome this tendency. A recycle heater has recently been installed to allow control of the inlet gas temperature.

No quantitative study has been made on fines production from this burner. However, it can be stated qualitatively that quantities of fines are very low, probably less than 2% of carbon burned and certainly less than 5%.

A theoretical model has been developed that is capable of predicting the carbon burn rates to within 15%. It predicts the hourglass shape of the remaining rod, but not the severity in which it occurs. The area of locally high burn rate may cause flow disturbances which further increase mass transfer in the neck region. This effect is not included in the model. Graphite and gas temperatures are also predicted to within approximately 15%; however, gas compositions are not as accurate. Work continues to refine the model.

Further experiments are under way to confirm the results shown in Fig. 6.14 and to establish the slope of the line more accurately. In addition, limited studies will be made to fully demonstrate the effectiveness of the control system. No extensive work is planned in this area since the stability of the system is obvious from its response to

manual operation. The mathematical model predicts that the hourglass burning shape can be avoided by proper selection of oxygen rate and recycle gas temperatures. Experiments are planned to determine if this is the case.

6.6 Perfusion-Impaction Particulate Separator

M. Manning*

Based on a recent patent disclosure,¹⁷ investigation of a novel device for removal of particulate matter from gas streams was initiated. The innovation in collector design is the incorporation of a wetted porous surface in an impaction particulate separator, as shown in Fig. 6.15. In this device, a gas stream is accelerated through a nozzle and directed towards a collection surface. In the vicinity of the surface, the gas stream is deflected parallel to the surface, while entrained particulate material impacts on the surface. Conventional impaction separators must be shut down periodically for cleaning. The porous surface allows perfusion of a liquid to wash the surface continuously, thereby eliminating periodic shut down.

6.6.1 Theory

The collection of particulate material from gas streams by impaction separators has been described by W. E. Ranz.¹⁸ From consideration of the equations of motion for both the gas and particulate material, it was shown that the collection efficiency, η , of an impaction separator of characteristic length, D_c , depended on three dimensionless groups:

*Currently with MIT Practice School.

Fig. 6.15. Perfusion impactor.

$$N_D = \sqrt{\frac{\rho_P V_O D_P^2}{18 \mu_G D_c}}$$

$$N_{\rho_G} = 9 \rho_G^2 V_O D_c / \rho_P \mu_G$$

$$\text{and } N_s = \frac{L}{D_c},$$

where

ρ_P = particle density, g/cm³

V_O = average jet velocity, cm/sec

D_P = particle diameter, cm

μ_G = gas viscosity, g/cm-sec

D_c = nozzle diameter, cm

ρ_G = gas density, g/cm³

L = nozzle to plate spacing.

The first dimensionless group is the square root of the ratio of the distance required to stop a particle of initial velocity, V_O (assuming the drag force follows Stokes' law), to the distance characteristic of the collection system. The second dimensionless group is a parameter accounting for gas inertia effects when Stokes' law does not apply to particle motion. The third dimensionless group, the ratio of the nozzle-to-collector spacing to the nozzle diameter, was found to be important whenever the ratio was smaller than 1. Ranz presents graphical results of calculated efficiency in terms of these three parameters for a variety of impaction geometrics.

K. R. May¹⁹ also determined by dimensional analysis that jets issuing from slots and impacting on a planar surface should satisfy the conditions of dynamic similarity if

$$\frac{\rho_G D_P^2 V_o}{\mu_G D_c} = \text{constant.}$$

This is consistent with the analysis by Ranz.

6.6.2 Experimental apparatus

The apparatus shown in Fig. 6.16 was constructed to measure the collection efficiency of a perfusion-impaction particulate separator. Air from the supply regulator is fed through a filter dryer and Millipore filter to a set of rotameters. The rotameters control the gas flow rates through separate sections of the Sinclair-LaMer aerosol generator.²⁰ Liquid dioctyl-phthalate (DOP) vaporizes into the gas stream in the boiler and is superheated with salt nuclei in the reheater. During passage through a vertical double-walled tower, the DOP condenses on the salt nuclei forming a nearly monodisperse aerosol. The aerosol may be isokinetically sampled from the stagnation tanks located at the inlet and exhaust of the particulate separator. Particle sizing and counting is performed by a Royco particle analyzer. A syringe pump perfuses water through the collector surface at a constant rate.

The particle collection efficiency can be simultaneously determined for several particle sizes with this experimental apparatus. Variation of gas flow rates through the jet can then be employed to check the theoretical model previously developed by Ranz. Preliminary experiments are planned for the next reporting period.

Fig. 6.16. Perfusion impactor test apparatus.

6.7 Continuous Chromatography

F. H. Wilson, C. D. Scott, and R. D. Spence

The development of a continuous annular chromatograph was described in the previous semiannual progress report⁵ and a prototype has been built. Results of separation studies of a Blue Dextran--CoCl₂ feed in a Sephadex G-10 resin are presented in this report. Studies made with other resins and feeds are underway to evaluate the device.

6.7.1 Theory

A mathematical model for the annulus chromatograph gives a simple expression for the angular position ($\bar{\theta}$) where the maximum concentration occurs at the bottom (exit) of the chromatographic bed.⁵

$$\bar{\theta} = \frac{\omega L}{U} [\epsilon + (1-\epsilon) k] \quad (9)$$

where

$\bar{\theta}$ = angular position, rad

ω = angular velocity, rad/sec

L = bed height

U = superficial velocity, cm/sec

ϵ = bed void fraction

k = distribution coefficient, $\frac{\text{g-moles solute/cm}^3 \text{ stationary phase}}{\text{g-moles solute/cm}^3 \text{ mobile phase}}$

If no adsorption occurs, as with Blue Dextran on Sephadex resins, the equation becomes:

$$\bar{\theta} = \frac{\omega L \epsilon}{U} \quad (10)$$

In experiments with Blue Dextran on Sephadex 25, $\bar{\theta}$ was indeed proportional to L as predicted by Eq. (10). Assuming no sorption of Blue Dextran, the void fraction was found to vary from 0.22 to 0.29.

6.7.2 Experimental procedure

The hardware used in the work reported here has been described in the previous progress report.⁵ A change was instigated during this report period; better separation was obtained by submerging the feed nozzle in the resin bed. The submerged nozzle dug a trench in the bed as the bed rotated, but the trench was smoothed by a blade following shortly behind the feed nozzle.

The angular position of the bands at any point below the feed can be measured visually. However, band widths were measured using a light source and light detector mounted into a holding device built especially for this purpose (see Fig. 6.17). Light from the source is focused with a lens onto the resin. Part of the reflected light is focused onto a photovoltaic silicon detector. The lenses and a filter selective for the Blue Dextran color (approximately 620 nm) are built into the body of the detector. The device also responds to the red CoCl_2 band, but with a lower sensitivity. The signal from the silicon detector is amplified and then measured on a recorder. The recorder signal is proportional to the solute concentration. This detector proved to be sensitive to imperfections in the Plexiglas walls; therefore, accurate calibration of the device for solute concentration was not possible. The detector was also sensitive to stray light, necessitating isolation of the apparatus from outside light. The device worked well at a fixed point

ORNL DWG. 75-15123RI

Fig. 6.17. Schematic diagram of band detecting device.

on the annulus for determining relative concentrations. It permitted measurement of vertical band widths, but it was not accurate for horizontal band widths.

The separation of Blue Dextran and CoCl_2 using Sephadex G-10 in the prototype system has been investigated with initial efforts directed toward achieving smooth concentrated bands of well-separated solutes. In the first experiment, the Blue Dextran band contained "fingers" (small extensions of dye above the main band). This problem was reduced when the concentration of Blue Dextran was lowered from 8.62 to 1.41 g/liter. In at least one case these "fingers" occurred under the spacers at the top of the column. Since Blue Dextran does not adsorb on the resin, the "fingers" may have resulted from interaction between the Blue Dextran and the Plexiglas spacers. Several methods for introducing the feed were investigated; these are summarized in Table 6.2.

The separation results are presented in Table 6.3. Runs 1 to 8 were made with feed condition No. 6, described in Table 6.2, using a Sephadex G-25 coarse resin layer over the Sephadex G-10 resin bed. Runs 9-13 were made with feed condition No. 7. For each run, the angular position at the band exit was noted, and the band widths and band separation were measured at several bed heights after steady state had been attained. Figure 6.18 is a photograph showing the appearance of the column operating at steady state in which two separated bands are clearly visible. The lower band is Blue Dextran, and the upper band is CoCl_2 .

6.7.3 Results

The band exit position relative to the feed point for each run is shown in Table 6.3 along with the annulus rotation rate and the eluent

Table 6.2. Operation of continuous annular chromatograph for different feed systems

Feed condition No.	Feed point	Resin level	Water level	Comments
1	Above resin	Midway spacers	Above feed point	Good separation, stairstep band
2	Above resin	Midway spacers	Above feed point	Good separation, smaller stairsteps ^a
3	Above resin	Below spacers	Above feed point	Bad separation, no stairsteps; feed tended to disperse over top of resin
4	Above resin	3/4 from top spacers	Regulated; resin surface dry at feed point, but wet before and after feed point	Conditions could not be maintained long; band disrupted soon after feed point due to rewetting phenomenon
5	Below resin	Above spacers	Above feed point	Good separation, smooth bands; dispersion along groove left by feed line
6	Below resin	Coarse resin above spacers over fine resin	Above feed point	Good separation, smooth bands; irregular interface can form between resins causing irregularities in bands
7	Below resin	Above spacers with blade for smoothing	Above feed point	Good separation, smooth bands; blade stopped dispersion along groove

^aTwo Teflon baffles were placed between each spacer.

Table 6.3. Band positions and widths for separation of Blue Dextran and CoCl_2 in a continuous annular chromatograph using Sephadex G-1C resin (bed height, L , = 50 cm)

Run No.	$\omega \times 10^2$ (rad/sec)	$U \times 10^2$ (cm/sec)	θ (rad)		$\frac{\omega_2 - \omega_1}{25 \omega_1}$, cm^{-1}		ϵ from Eq. (10) and Blue Dextran	k_{CoCl_2} from Eq. (9)
			Blue	CoCl_2	Blue	CoCl_2		
			Dextran		Dextran	$\times 10^2$		
1	1.05	1.28	1.33	2.14	2.17	2.67	C.32	0.30
2	1.05	1.33	1.33	2.12	1.54	2.3	C.34	0.30
3	1.05	1.37	1.38	2.21	1.7	3.4	C.36	0.34
4	1.01	1.95	0.94	1.53	--	--	C.36	0.39
5	1.02	1.93	0.97	1.60	0.22	1.3	C.37	0.37
6	1.03	2.41	0.69	1.16	1.1	1.7	C.32	0.33
7	1.03	1.92	0.93	1.53	0.54	1.2	C.35	0.37
8	2.60	1.89	2.41	3.98	3.7	2.3	C.35	0.35
9	1.11	2.10	1.01	1.64	-0.48	0.91	C.38	0.39
10	1.05	1.63	1.11	1.77	0.49	2.38	C.34	0.32
11	1.05	2.15	0.93	1.54	0	1.37	C.38	0.40
12	1.05	2.68	0.74	1.19	0	1.40	C.38	0.37
13	1.05	3.40	0.58	0.92	--	--	C.38	0.35
						Av.	0.36	0.35

Fig. 6.18. Photograph of continuous annular chromatograph in operation (upper band - CoCl_2 ; lower band - Blue Dextran).

superficial velocity. Also reported in Table 6.3 is the fractional increase in band width per unit height.

$$\frac{\omega_2 - \omega_1}{(x_2 - x_1) \omega_1},$$

where

$\omega_{1,2}$ = band widths at positions x_1 and x_2 ,

$x_{1,2}$ = positions in the column below the feed point.

In this study, x_1 was 15 cm below the feed point and x_2 was 40 cm below the feed point. The greater the difference in $\bar{\theta}$ and the lower the values of $\frac{\omega_2 - \omega_1}{(x_2 - x_1) \omega_1}$ for Blue Dextran and CoCl_2 , the more effective the separation became.

The results reported by Scott and Moon⁵ demonstrate that Blue Dextran is not sorbed, and Eq. (10) can be used to determine the void fraction, ϵ . The void fraction for each run was calculated from Eq. (10) by using the angular position, $\bar{\theta}$, at the exit for Blue Dextran, and the results are listed in Table 6.3. Note that ϵ was essentially constant for all runs and was independent of flow rate. Knowing ϵ , the distribution coefficient for CoCl_2 , k_{CoCl_2} was calculated for each run by using Eq. (9). These values ranged from 0.30 to 0.40, but did not appear to be a function of flow rate.

An overall value for ϵ and CoCl_2 distribution coefficient were also obtained as follows. Figure 6.19 shows a plot of the band angular position at exit, $\bar{\theta}$, vs the inverse superficial velocity, u^{-1} . According to Eqs. (9) and (10), these plots should be linear. A least squares analysis of the data gave a slope of 0.0182 for Blue Dextran and 0.0295 for CoCl_2 .

Fig. 6.19. Band exit position vs inverse superficial velocity for Blue Dextran- CoCl_2 , Sephadex G-10; $L = 50$ cm, $\omega = 1.01$ to 1.05 rads/sec.

(The regression lines are shown in Fig. 6.19.) The Blue Dextran slope corresponds to a void fraction of 0.35. Using this ϵ and this CoCl_2 slope, the distribution coefficient for CoCl_2 was calculated to be $0.33 \frac{\text{g-moles/cc resin}}{\text{g-moles/cc water}}$. This overall or average value compares favorably with the individual values calculated from each run and shown in Table 6.3.

The band widths were measured at one-half the peak height and were used to generate the fractional increase per unit of height reported in Table 6.2. The peak width increased less for the Blue Dextran band than for the CoCl_2 band; this would be expected if the Blue Dextran does not adsorb on the resin. The rate of Blue Dextran band width increase gives a measure of the dispersion characteristics of the bed itself. Some runs reported in Table 6.3 gave negligible increase in the Blue Dextran band width and some gave a significant increase. Extrapolation of band width data for both Blue Dextran and CoCl_2 shows significant initial band widths. This initial dispersion is probably caused by the injection system and the spacers at the top of the column. Investigation of dispersion in the column will continue. Further development of the model is planned to permit more quantitative analysis of band width data.

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7. CONTROLLED THERMONUCLEAR PROCESSING

Chemical process problems for controlled thermonuclear reactors (CTRs) are largely centered around purifying and recycling tritium containing fuel from the plasma exhaust and from a tritium-breeding blanket. Extremely high degrees of tritium containment are required in each of these operations. Efforts during this report period included scoping studies of tritium-handling systems for an Experimental (Fusion) Power Reactor (EPR), evaluation of methods for recovering tritium from blanket materials, studies of tritium recovery from vacuum pumps, measurements of tritium sorption rates from liquid metals, and initiation of an experimental study of tritium release from solid blanket materials.

7.1 Scoping Studies of a Tritium-Handling System for an Experimental Fusion Power Reactor

J. S. Watson, S. D. Clinton, P. W. Fisher, and R. Cherdack*

The experimental power reactor (EPR) operating at 150 MW(t) from fusion reactions would consume 200,000 Ci, or 20 g, of tritium per day. If the fraction burned is 1%, this would correspond to a tritium process rate of 20 MCi, or 2 kg, per day. This will be, by far, the largest tritium-handling system built for a fusion experiment by the mid-1980s. The tritium-handling system will include equipment to evacuate the reactor vessel, purify and recycle fuel to the reactor, separate deuterium and tritium for injection, extract tritium from the breeding blanket, and control tritium releases by containment and atmosphere cleanup. In size and complexity, the EPR system will represent significant extrapolations

*Burns and Roe Company.

from the Tokamak Fusion Test Reactor (TFTR) tritium system, including larger quantities of tritium, more complete and more reliable containment, and larger equipment, as well as additional process steps. The EPR may provide the first realistic demonstration of all major components of a fusion power reactor tritium-handling system. It will be the first operating fusion device to provide on-site purification and recycle of unburned fuel. (TFTR plans currently call for collection of unburned fuel on solid getters. The fuel is, perhaps, recovered and purified off-site.) The EPR will include the first on-site isotope separation equipment and, probably, the first on-line experiments for recovering tritium bred in a CTR blanket.

The EPR will be a major experiment in many areas of plasma physics and fusion reactor technology. Tritium-handling operations and experiments will constitute only a portion of the overall program; therefore, the tritium-handling equipment (like most other parts of the system) must have a relatively high degree of reliability. The first goal of the tritium-handling system will be to ensure that the probability and consequence of environmental releases will be acceptable. The second goal will be to ensure, with relatively high probability, that tritium releases or equipment malfunction will not hinder other portions of the experiment. The third goal will be to obtain data and experience in designing tritium-handling equipment for later fusion devices, and for demonstration and commercial reactors.

During FY 1975, tritium-handling experiments were limited to scoping studies (i.e., definition of problems and listing process options which could be used); no attempt was made to develop a specific design. Com-

parisons were made of the merits of various process options and the conditions under which they appeared to be practical; however, in most cases, no firm choice was made. During FY 1976, many options in all portions of the EPR will be eliminated; specific (preliminary) designs will then become useful.

Perhaps the most useful results of the scoping studies of the EPR tritium-handling system are the establishment of estimates of the magnitude of the tritium-handling problems and a better understanding of the developmental work needed to evaluate some proposed techniques and to provide the process systems required for an EPR. Although ORNL tritium experimental programs have been conceived and planned specifically to meet the anticipated needs of the CTR development, the scoping studies have shown a need for additional or expanded efforts in two areas:

1) recovery of tritium from lithium metal blankets, and 2) handling of tritium in solid feed systems. Methods for recovering tritium from lithium blankets are currently being studied at ORNL (and elsewhere), but a significant expansion of this effort is needed. Specific process techniques needing evaluation are discussed. Far less effort has been placed upon the feed system, and all of the process and containment aspects of solid pellet feed should be evaluated. Additional work is needed to properly couple the injector gas recovery stream to the main plasma exhaust system.

Further advances in the tritium-handling system design will eventually require additional information from other portions of the EPR studies. In the next few years, the EPR program must attempt to define the fueling system, divertor system (if needed), and blanket cooling

and breeding systems. In addition, an effort is needed to estimate purity requirements for all EPR feed streams and operating cycles for all systems.

7.1.1 Design criteria

The following criteria were formulated to provide guidance for the design of the tritium-handling systems:

- (1) All systems containing tritium in either gaseous or potentially gaseous form in significant quantities shall be enclosed. The atmosphere of these enclosures shall be maintained at a slight negative pressure and processed through a cleanup system designed to remove tritium.

This criterion was established to limit normal leaks to the reactor building as well as to reduce the probability of large leaks due to accidents. It is consistent with the present practice for all new systems being constructed to handle large quantities of tritium.

- (2) Tritium-bearing systems shall not release their inventories as a result of design-basis earthquakes, tornadoes, or the missiles generated by them. Protection must be provided for support systems, such as cryogenic refrigerators or electric power supplies, which could affect tritium containment. This criterion was established to reduce the likelihood of releases resulting from natural disasters.

- (3) If possible, all tritium-handling systems shall be located in the reactor building. In any case, such systems shall be enclosed within containment structures that are reasonably leak-tight (comparable to BWR secondary enclosures), maintained at a slight negative pressure, are missile protected, and are of suitable seismic design. All containment structures shall have elevated release points. This criterion, which is aimed particularly at mitigating the effects of the maximum credible accidents, also leads to the control of releases due to normal leaks and smaller accidents.
- (4) All containment buildings shall be equipped with atmosphere cleanup systems which remove tritium at a rate and an efficiency necessary to meet the site boundary dose criterion (1 rem) for a maximum credible accident and the air concentration criterion for normal maintenance conditions (5×10^{-7} $\mu\text{Ci/cc}$) established in Section 3.1 of ORNL/TM-5080.
- (5) Where possible, tritium-handling systems shall operate at temperatures and pressures below those at which significant diffusion of tritium through metal walls occurs.
- (6) Monitors shall be provided throughout containment structures, particularly at possible tritium leakage points. All release points from the containment structures shall be monitored. Monitors will be needed to

protect personnel. Local monitors at potential leak points will provide fast response, aid in the protection of personnel, and help in locating failures. Monitors at release points will aid in evaluating the performance of the containment and cleanup system and in determining the environmental impact of any release.

- (7) Those systems containing significant quantities of highly flammable materials shall have inert gas as a blanket in their enclosures.

7.1.2 Selection of techniques

An attempt was made to evaluate the best ways to meet the performance criteria that have already been outlined in Sect. 7.1.1. Some of these evaluations resulted in confirmation of the design criteria contained in Sect. 7.1.1 as well as in the conclusions discussed below.

An examination of the size, operating conditions, diversity, and complexity of the various systems that will pump, process, or otherwise handle large quantities of tritium indicates that some leakage will be inevitable. Therefore, the use of double enclosures at slight negative pressures, with cleanup systems to remove tritium from their atmospheres, is considered to be the only way to ensure minimum leakage to the reactor building. Brief analyses (see Section 4.4.2 of ORNL/TM-5080) indicate the extremely small amount of leakage that is tolerable to ensure compliance with even less stringent criteria than 5×10^{-7} $\mu\text{Ci/cc}$. Also, the use of double-enclosure and cleanup systems is accepted practice

today, and certain portions of the tritium-handling systems (e.g., uranium beds or gas storage cylinders) may represent fire or explosion hazards unless contained within inert-atmosphere enclosures. Many systems for removing tritium from enclosure or reactor building atmospheres are commercially available.

A containment building with a slight negative pressure and a controlled, elevated exhaust was judged to be more effective in reducing doses from a maximum credible tritium accident and probably more economical than a PWR-type containment building. However, future studies of the effect of the normal release of air activation products and the costs of PWR- and BWR-type containments might alter this conclusion.

The exact nature of double enclosures of the tritium-containing system will depend on detailed system designs, access requirements, and physical arrangements. As discussed above, it may be necessary to maintain inert atmospheres in some enclosures, depending on the quantities of hydrogen isotopes or other materials contained by the enclosed system. All enclosure atmospheres will be maintained at a slight negative pressure and will be processed continuously through cleanup systems to remove tritium. Process designs for these cleanup systems will be similar to those for the reactor building.

The atmosphere cleanup system for the reactor building will contain heaters and catalytic or, perhaps, CuO beds to oxidize tritium to tritiated water. The tritiated water will then be collected on dryer beds probably consisting of refrigerated molecular sieves which are regenerable. The exact system flow rate, although not yet developed, will probably be on the order of one-fourth to one-half building volume per hour for rapid

tritium removal. A tritium decontamination factor (DF) of 100 is considered both achievable and desirable. Addition of hydrogen or steam may be necessary for isotopic dilution in order to achieve this DF for low concentrations of tritium. Analyses of the performance requirements of this system are discussed in the following subsections.

The volume of the reactor building will be on the order of 4,000,000 ft³. Based on data from buildings of a similar nature, it should be possible to maintain a pressure of -0.25 in. H₂O with an inleakage rate of less than one-half building volume (2,000,000 ft³) per day. An amount similar to the inleakage would be exhausted from an elevated release point about 100 m high. This exhaust stream would be taken from the outlet side of the reactor building's atmosphere cleanup system.

Tritiated liquids resulting from the regeneration of dryer beds of cleanup systems will be pumped to a small facility for solidification, drumming, and shipment off-site.

7.1.3 Program requirements

Definitive designs for the double enclosures cannot begin until the designs and layouts for major tritium-bearing systems for EPR are available.

Additional information on the behavior of tritium in the environment and the modes and consequences of tritium releases from fusion facilities would be useful in establishing final criteria and designs for tritium containment and atmosphere cleanup systems. Work in these areas is being performed at ORNL and Pacific Northwest Laboratory (PNL).

Work to be done in the future on containment and cleanup systems for the EPR includes design of secondary enclosures, further detailed

examination of tritium-related accidents, selection of optimum cleanup systems, design of a tritiated liquid waste disposal system, and tritium containment in blanket modules.

7.1.4 Main vacuum systems

The main vacuum system consists of the pumps, piping, and valving that serve to lower the pressure appropriately prior to initiation of a discharge and maintain the proper conditions for operation throughout the discharge. This system must remove impurities that are dislodged or outgassed from the walls, fusion ash (helium), and deuterium and tritium which have left the plasma. The system may pump from a divertor exhaust chamber similar to the MATT-1050 design or directly on the main vacuum chamber, perhaps in conjunction with a gas blanket. Because the main vacuum system pumps large quantities of tritium (probably hundreds of grams per day), it is considered a tritium-handling system.

Preliminary information was used to determine the operational requirements for the main vacuum system. Data on divertors, design and operation, and plasma confinement were used to determine the performance requirements for the vacuum system. Some safety criteria were established (Sect. 7.1.1). Various pumping techniques were examined in light of the system requirements. The feasibility of using cryosorption or mercury diffusion pumps was established, along with the preliminary sizing of vacuum ducts.

Requirements. The system will have to meet the following requirements:

- (1) Must be available in appropriately large speeds and capacities, either now or in the future, without major developments or breakthroughs.

- (2) Must provide complete tritium recovery in a reasonable manner (tritium may cost as much as \$7000/g, and several grams will be pumped per day).
- (3) Must pump helium.
- (4) Must achieve low base pressures.
- (5) Must perform in radiation field.
- (6) Must have relevance to future (i.e., beyond EPR) reactors.
- (7) Must demonstrate reliability and maintainability.
- (8) Must show reasonable economics.
- (9) Must be compatible with the general requirements and physical configuration of the EPR.

Selection process. The initial step in the selection process in this study was to evaluate the capability of eight types of vacuum pumps to meet the requirements listed above.

Cryosorption pumps were found to meet all nine requirements to a reasonable degree. These pumps, which consist of an array of molecular sieves cooled by a liquid helium reservoir surrounded by liquid-nitrogen-cooled chevrons, operate by adsorbing volatile gases such as hydrogen and helium on the helium-cooled array and less volatile gases on the outer chevrons. Special-order modules with speeds on the order of 25,000 liters/sec have been manufactured. Because the pumps function by adsorption, they must be regenerated periodically by removing the liquid helium and heating. A regeneration period of several hours at 300°C is required to drive off water vapor, but rapid regeneration at cryogenic temperatures may be possible if only helium and hydrogen isotopes have to be removed.

Mercury diffusion pumps were also found to be capable of meeting the nine requirements. These pumps use directed streams of mercury vapor to sweep the gas being pumped from the inlet side toward the exhaust. The mercury is then condensed and recycled to the boilers and is subsequently transferred in the form of vapor to the spray jets. Liquid-nitrogen-cooled baffles are required to reduce the backstreaming of mercury into the vacuum chamber. These baffled traps reduce the pumping speed by almost 50%, but the resulting speed is still acceptable, as will be discussed later. Only minor developmental work is required to produce pumps of the scale needed for an EPR. These pumps are now widely used in CTR applications. Diffusion pumps, however, also require forepumps, and the two-part pump system should be considered as a unit.

Oil diffusion pumps are essentially the same as mercury pumps, except that oil vapor is used as the pumping medium. Because of the oil's greater volatility, concern over backstreaming and contamination of the vacuum chamber is intensified. The effects of gamma, beta, and neutron radiation on the oil and its contamination by tritium were other concerns which downgraded oil pumps with respect to the mercury pumps. Forepumps are also required for oil diffusion pumps.

Titanium gettering was found to be unacceptable, principally because of difficulties with tritium recovery and helium pumping. Titanium gettering involves evaporation of the metal, which condenses as a very thin film on a surface. This film then adsorbs active gases as oxides, nitrides, hydrides, etc. Once the film is exhausted, a subsequent layer must be deposited. Because of the chemical nature of the process, helium cannot be pumped. The stability of the hydride and the many layers that would

be required in a short time would make tritium recovery extremely difficult. Because of these failings and concerns over evaporation rates, required surface areas, and possible peeling problems, titanium gettering has been ruled out for an EPR and judged to have no relevance to future reactors.

Turbomolecular pumps are basically similar to rotary compressors operating at 20,000 to 30,000 rpm. The largest pumps commercially available have a speed of less than 2000 liters/sec for air. Turbomolecular pumps have difficulty in achieving low base pressures for hydrogen. It was judged highly unlikely that turbomolecular pumps could be designed to achieve the required speeds and base pressures for hydrogen isotopes with reasonable outlet pressures in an economically feasible manner.

Zirconium gettering pumps use an 85% zirconium--15% aluminum metal mixture, arranged for maximum surface area, to pump gases by forming zirconium oxides, hydrides, etc. These pumps differ from titanium gettering pumps in that the getter material is in a permanently fixed configuration and is regenerable. Questions exist concerning the economic feasibility of scaling the presently available pumps to the sizes needed for an EPR. In addition, they require regeneration at 750°C; the number of cycles before deterioration has not been established. As with all getter techniques, the zirconium pumps cannot pump helium.

A cryosorption or cryogenic panel within the vessel, or the divertor chamber itself, was ruled out because of the anticipated effects of bombardment by charged and energetic neutral particles. Also, regeneration would involve complex extraction equipment in a highly activated area, or it could use a multistep process to raise the pressure of the regeneration

gas to a suitable level for the subsequent step in the fuel cycle. The latter would interfere with continuous operation of an EPR.

Ion vacuum pumps use currents of accelerated ions to propel the gas being pumped onto a titanium gettering surface. This technique yields some effective helium pumping, but at tremendous economic costs as well as increased complexity. Complete tritium recovery from these pumps would probably not be possible. It is difficult to visualize how an ion pump could be scaled up to the size required for an EPR. Difficulties are involved with their performance in a magnetic field; in addition, the lack of a reasonable tritium recovery technique remains.

The preceding evaluations indicate that cryosorption and mercury diffusion pumps are the two types that are reasonably capable of meeting the requirements for an EPR. Each type would be used in a system consisting of several pumps attached to the ends of many large ducts connected either to the main vessel or to the divertor exhaust chambers. The pumps would be located outside the shield, and the ducts penetrating the shield would be bent to allow shielding against neutron and gamma streaming.

Based on the estimates of plasma confinement time and the vacuum requirements stated in the Princeton Reference Design,¹ a pumping speed of 3.1×10^5 liters/sec for DT is required. This could be obtained from ten ducts, each 1 m in diameter by 5 m long with a 1-m-diam cryosorption pump attached. A larger number of smaller ducts and pumps could be used if physical arrangements do not allow the use of 1-m-diam ducts. The length of the pump would be about 3 m if proportions were the same as for smaller existing pumps. However, simple extrapolation from existing designs indicates that a 1-m-diam by 3-m-long pump would have a capacity

on the order of 300 liters (STP). This represents only 8 hr of running time between regenerations, and changes in internal arrangement and an increase in overall size will be required to raise this to a 24-hr operating cycle, which will probably be more reasonable. The proper or allowable operating cycle time can only be estimated until more is known of CTR regeneration techniques.

The major advantages of cryosorption pumps are their simplicity of design and operation, their flexibility of orientation, and their cleanliness. Disadvantages include the need for frequent regeneration, the requirements for liquid helium and nitrogen, and the large quantities of tritium stored in what may be considered to be the vulnerable form of a condensed gas. Of course, the inventory will be less if rapid, frequent regeneration is possible.

Mercury diffusion pumps with appropriate liquid-nitrogen-cooled baffles perform nearly as well as cryosorption pumps with similar flange diameters. Since they are throughput rather than absorption-type devices, there are no concerns over regeneration cycles or the buildup of very large tritium inventories. However, they do require forepumps for efficient operation. The forepumps can be similar to the mercury ejectors proposed for the Princeton Reference Design,¹ which will increase the outlet pressure to about 100 torr. This pressure should be suitable for further processing steps.

The major advantages of mercury diffusion pumps are their lack of large tritium inventory and a fairly long history of successful operation. Disadvantages include the need for forepumps, the requirement for cold baffles, the fixed orientation, and the possibility of backstreaming of mercury into the plasma vessel, which causes subsequent contamination.

The analysis is sensitive to the vacuum requirements and ion confinement time. Drops in confinement time or allowable pressures much greater than a factor of 2 will make design of the system considerably more difficult.

Because the vacuum pumps may have to function in gamma and neutron radiation fields, the changes in the properties and performance of all materials due to such exposure used in candidate pumps should be determined. Possible changes in pump design or materials of construction for lower activation and greater endurance may be suggested. The first step in the program would consist of detailed calculations of the radiation flux at the pumps, based on a reasonable physical layout.

Data required from plasma physics concern the vacuum requirements for divertors and/or gas blankets and the particle confinement time. Such data should be forthcoming from future EPR studies using input on scaling laws from plasma experiments.

Information is needed on the long-term reliability, maintainability, and performance of large cryosorption pumps and mercury diffusion pumps. Rapid regeneration capabilities and storage capacities of cryosorption pumps should be determined.

Studies on rapid regeneration of cryosorption pumps are being made at ORNL. The commercial development of large, high-speed cryosorption and mercury diffusion pumps should be encouraged. Reliability and remote maintenance possibilities should be studied.

Work to be done in future EPR studies directly concerning the main vacuum system includes development of roughing systems and preparation of optimum physical arrangements. Work with those involved with blanket

and shield design and neutronics calculations, and with manufacturers, will further clarify research and development needs.

7.1.5 Injector vacuum system

The injector vacuum system consists of the piping, valves, and pumps that are necessary to maintain the appropriate vacuum in the neutral beam injector drift tubes and gas cells. Because a substantial amount of tritium (perhaps tens of thousands of curies per day) will enter the system from the main plasma vessel via the drift tubes and because the deuterium feed to the injectors may become contaminated with tritium, this system is considered a tritium-handling system. The quantity of tritium involved necessitates a reasonably high recovery of tritium from the injector vacuum systems.

Requirements. Based on preliminary data on injectors, a pumping speed and an ultimate pressure requirement were developed. Thus, to satisfy the above criteria, it was determined that the injector vacuum system should have the following characteristics:

- (1) ultimate pressure below 10^{-4} torr,
- (2) capability for pumping small amounts of helium,
- (3) high pumping speed in a system of reasonable size,
- (4) continuous operation or short, infrequent regeneration periods,
- (5) high degree of tritium recovery,
- (6) compatibility with the physical nature of injectors,
- (7) a design based on commercial or potentially commercial technology,
- (8) favorable economics,
- (9) high reliability,

- (10) easy maintainability,
- (11) any required regeneration should produce a reasonable outlet pressure and cause no interference with injector operation,
- (12) low contamination residue, and
- (13) low backstreaming.

The injector vacuum system consists of valves, pumps, and piping. The major selection process involved determining the most desirable type of vacuum pumps. Again eight types of pumps were evaluated based on their capability for satisfying the 13 requirements listed above.

Both mercury diffusion pumps and cryosorption pumps will probably satisfy all the requirements. The vacuum pumps would be attached to the chambers enclosing the gas cells and drift tube on each injector. Valves between the pumps and chamber would provide isolation during regeneration of the cryopumps, roughing down of the injector, or pump maintenance. Alternatively additional injectors could be provided so entire injectors could be valved-off for pump regeneration.

Based on the preliminary data provided, a pumping speed of 70,000 to 100,000 liters/sec is required for a 20-A, 150-keV injector. This can be attained using four or five cryosorption pumps so that one can be regenerated without having a significant effect on the pumping speed. Each pump must be able to store approximately 200 std liters for 24-hr cycling. Two or three large mercury diffusion pumps of a size and a design similar to those of currently available commercial diffusion pumps would also provide the required pumping speed. The advantages of cryosorption and diffusion pumps for this application are similar to those discussed previously for the main vacuum system.

Requirements. The actual beam requirements, both voltage and total power, can be determined only by utilizing plasma physics data. Data are also needed on the long-term reliability and performance of large cryosorption and mercury diffusion pumps. Rapid regeneration techniques and large-storage-capacity designs for cryosorption pumps should be formulated. Work on rapid regeneration is in progress at ORNL. The commercial development of high-capacity cryosorption pumps and low-backstreaming mercury diffusion pumps should be encouraged.

Information needed relative to beam technology involves the efficiency, pressure requirements, and geometry of the injectors. The beam technology program is expected to generate such information.

Future work on the injector vacuum system will include an evaluation of roughing systems and the development of vacuum systems for a more complete range of possible injector designs.

7.1.6 Tritium processing systems

Tritium processing systems include equipment for removing DT mixtures from the main vacuum systems, purifying the DT mixture for recycle to feed systems, separating isotopic impurities (H) from DT mixtures, recovering tritium from blanket modules, preparing tritium-containing wastes for disposal, transferring feed, providing storage for tritium, and vent or atmosphere cleanup equipment directly associated with the process systems. Although the major vacuum pumps are considered with the main vacuum system, they are connected directly to the process equipment and, therefore, must also be considered in process studies.

Range of objectives. The performance objectives of an EPR have not been defined completely; thus we are forced to look at systems with a

variety of capabilities. The final selection of EPR performance criteria will involve considerations of the reliability of desired information, technological extrapolation (scale-up), and costs. These considerations must be made for individual components of the EPR as well as for the overall EPR program. The range of objectives for the EPR tritium-handling system can be divided into three levels: (1) minimum acceptable, (2) intermediate, and (3) maximum reasonable. Obviously, several levels of performance are possible, and these three levels only represent relative ranges of effort. A minimum objective for the tritium system is more likely (but not necessarily) to be adopted if minimum performance is chosen for several other components of the EPR. This would correspond to a "relatively simple" EPR with minimal performance.

Minimum acceptable performance. The tritium systems with a performance level rated as "minimum acceptable" would ensure safe containment of tritium and provide only basic services for plasma experiments. No additional effort would be made to advance tritium technology for CTRs. Containment of tritium is the first objective of any tritium processing system, and much of the atmosphere cleanup equipment, secondary containment methods, and emergency stack gas process equipment will be similar for any performance level chosen. Since this will be a significant portion of tritium process costs, differences in the total cost for the various performance levels may not be as large as initially expected; the savings would occur only in the main process equipment. The process steps would use current or near-term technology; no special effort would be made to use "more difficult" techniques simply because they could be used in future reactors. No attempt would be made to provide a tritium-breeding blanket or the necessary tritium-recovery equipment.

In summary, the minimum acceptable system would only provide support for other portions of the EPR development program; it would make no extra effort to advance tritium technology. Thus additional and separate work would be required to generate tritium technology for future reactors. This approach could speed the implementation of an EPR; however, it would reduce the benefits obtainable from the EPR. The path would require additional tritium-handling development and demonstration systems prior to future reactors.

Intermediate system. Perhaps a more reasonable approach would involve the selection of process steps that could be applied to future reactors. However, this approach still would not provide all of the tritium technology for future reactors because an intermediate EPR is not likely to include all components of later versions. Little or no breeding blanket experiments would be attempted, and the feed and removal systems might be significantly simplified. Although all process steps should be potentially useful for future reactors, an attempt would be made to ensure conservative selections and to avoid more advanced concepts even if they might eventually prove more desirable for such reactors. Thus all EPR process techniques may not be selected for use in future reactors.

Provisions for tritium containment will not be significantly different for the intermediate and the minimum acceptable system.

Maximum reasonable system. A tritium-handling system with a performance level rated as "maximum reasonable" would include an evaluation of techniques for handling all process steps required for future reactors, including a blanket recovery system. The EPR would provide data for extrapolation of techniques to future reactor conditions. The techniques

tested would be those expected to be most appropriate for the later versions; they could, of course, prove less desirable than expected. The EPR would then be the principal tritium experiment in preparation for future reactors.

As now envisioned, the EPR tritium processing system should be designed to:

- (1) limit tritium release rates to acceptably low levels and minimize the probability and consequence of accidental releases,
- (2) contain acceptable inventories of tritium,
- (3) involve reasonable (hopefully, small) costs,
- (4) be compatible with all other portions of the EPR, and
- (5) use current technology or technology which can be expected to be developed early enough for use in EPR.

The first goals of the tritium-handling system will be to provide acceptable containment and to minimize the hazard of personnel exposure and/or machine downtime due to tritium releases. Excessive release of tritium is not only a hazard to personnel, but can also contaminate expensive equipment and jeopardize the mission of the EPR. The desired degree of containment is discussed in Sects. 7.1.1 and 7.1.2. Secondary containment of all process equipment is anticipated, and emergency tritium removal equipment will be installed in the reactor containment building.

Tritium inventories must be limited because (1) they represent a significant cost to the system, (2) high inventories probably imply a higher maximum environmental risk in the event that a major release occurs, and (3) tritium supplies available to EPR will be limited. A

150-MW(t) EPR would burn 20 g of tritium per day (200,000 Ci/day). If the fraction burned is 1%, this suggests a tritium process rate of 2 kg (20 MCi) per day. If a 2-day inventory is maintained throughout the process and storage systems, this would correspond to an inventory of 4 kg (40 MCi). At this point, one cannot make an accurate estimate of the tritium inventory required for an EPR. Different process techniques will require different inventories; also, most of the process steps have not been chosen. The cost of tritium to the EPR is uncertain. Present ERDA policy would make tritium available to ERDA programs such as the EPR at no significant cost, provided the tritium could be supplied by existing facilities without hindering other programs. The quantities of tritium required for the EPR are expected to be available under these terms. If it became necessary to charge the EPR program for the tritium supply, the cost would be significant. If additional production capacities were required for the EPR, the costs would become considerable.

Although the potential hazard is often considered to increase as tritium inventories increase, one should be careful about drawing such simple conclusions. Inventories can vary, depending on the process techniques used, and the difference in the probability of a release may be more important than differences in the inventories.

Fusion power in general, and the EPR in particular, will require considerable advances in several areas of technology. It is necessary to choose present or near-term techniques when they are adequate to increase the probability of overall EPR success to an acceptable level. This situation applies to all portions of the EPR system. The economics or costs of tritium-handling systems do not impose clearly defined limits,

but no process system that significantly increases the overall EPR cost should be selected.

The principal tritium process steps required for EPR (or any fusion reactors) are shown in Fig. 7.1. Vacuum pumps play an obvious and important part in the removal of tritium, deuterium, and impurities from both the plasma chamber (or divertor) and the injectors. Although vacuum pumps must be considered as part of the tritium-processing system, they are more conveniently treated as part of the vacuum systems. The size and pumping characteristics of the vacuum pumps are discussed as part of the vacuum system in Sect. 7.1.4. Recovery of T-D from the pumps is discussed as part of the tritium-processing system. Cryosorption pumps permit complete regeneration of tritium and have no shaft seals to permit leakage to or from the system. They also introduce no impurities, such as lubricants or working fluids, to the system. Thus cryosorption pumps must be considered for the EPR. Mercury diffusion pumps appear to be the most likely competitor to cryosorption pumps; however, mechanical pumps and getter pumps were also investigated.

Two disadvantages of cryosorption pumps are their cyclic operation and their limited capacity for holding D-T. The cyclic nature of these pumps increases the potential tritium inventory; the pumps would have to be regenerated frequently, probably daily. The disadvantage of this type of pump system depends on the other process steps. If some of these steps are cyclic with the same period, the steps may mesh very well. The most likely purification technique, sorption on uranium beds, is a cyclic process and coordinates satisfactorily with cryosorption pumping. The principal disadvantage of mercury diffusion pumps is the possibility of

Fig. 7.1. Schematic of reference EPR tritium system.

introducing mercury into the plasma system or downstream process equipment. Purification systems would probably be damaged by mercury accumulations. Cold traps would drastically reduce the chance of steady mercury contamination of other equipment, but one must still consider the probability of a slow accumulation of mercury and the possibility of severe contamination in the event of equipment malfunction.

Some form of protection may be needed to protect cryosorption pumps from sputtered materials. This protection may be provided by the vacuum duct walls. If necessary, cold baffles could be inserted in the duct for further protection. If a divertor is used, the divertor channels themselves may serve this purpose. Small quantities of material reaching a cryosorption or mercury diffusion pump would probably have little effect on the pump. The sputtered material would coat the outer chevrons which are cooled to liquid nitrogen temperatures. Very little material would be expected to reach the molecular sieve or mercury. Small quantities of material sticking to the outer chevrons would be expected to have little effect. Larger quantities could damage the chevrons or even significantly affect the liquid nitrogen heat load. The need for additional protection for the pump system can be estimated more accurately when more reliable data on EPR particle fluxes at the wall and sputtering yields become available.

Once tritium is recovered from the vacuum pump, it must be purified to remove chemical impurities such as helium, oxygen, and nitrogen. Helium, which is formed from the D-T reaction in the plasma, may be of the order of 1% of the removed gases. Other impurities may result from leakage or outgassing of the equipment. The purification technique most

likely to be employed for the EPR uses uranium beds. These beds will sorb essentially all likely impurities except the noble gases.^{2,3} When heated, they release the hydrogen isotopes but retain the impurities. Uranium beds obviously have limited lifetimes determined by the impurity content of the process streams. The life of uranium in the EPR, however, is expected to be long (years). Noble gases swept into the bed can be pumped off; and if necessary, additional purification can be achieved by routing the uranium bed outlet stream through a palladium membrane. Uranium beds, which are frequently used for tritium storage and purification, are considered current technology. Although other purification methods also look promising, the existing experience with uranium beds gives them an edge. An alternative method will have to promise significant advantages in order to be preferred for the EPR. The cyclic nature of uranium beds couples well with cryosorption pumping.

Other hydride-forming metals besides uranium will need to be considered. Since different metals have different affinities for hydrogen (isotopes) as well as oxygen and nitrogen, a better combination of properties may be found. However, the existence of a more suitable material is not now apparent. Uranium beds are also proposed for storing tritium. Although it is possible that other hydride-forming metals or alloys may be selected for the storage function, uranium will probably be used for purification.

Palladium membranes have frequently been used for purifying hydrogen. (The application of palladium for final purification of tritium from uranium beds has already been mentioned.) The membranes could be selected as the only purification method, but the principal disadvantage of this

approach is that tritium compressors would be required. Mechanical compressors are likely to require significant maintenance and perhaps allow small leaks to or from the system (to or from secondary containment atmosphere). Although palladium membranes alone can readily produce a high-purity hydrogen isotope stream, the production of a reject stream containing little or no hydrogen isotopes without large surfaces and/or high total pressures is difficult.

It is possible to combine the purification operation with the isotope separation steps. The advantages of this operation obviously depend upon which isotope separation technique is chosen. Cryogenic distillation, one of the most promising isotope separation techniques, could separate helium as well as other impurities likely to be present. The merits of this approach, however, also depend on the throughput capacity required for the isotope separation system. All material recovered from the plasma will have to be purified before it is returned to the plasma. However, all material will not have to undergo isotope separation. The material used to fill the torus before each run and the material feed in the form of gas or solid pellets (probably) will not require isotope separation. Only D (and possibly T) supplied through injectors need undergo isotope separation, and a separate purification step is likely to be chosen.

Isotope separation will probably be required at the EPR site to prepare "tritium-free" deuterium for the injectors and to remove the trace of hydrogen produced by D-D reactions and outgassing from the vacuum vessels and equipment. Although these operations could be carried out in a single column or cascade, separate systems are likely to be selected

because the required throughputs will be different. It is even possible that different separation techniques will be chosen. The first system (DT separation) will be the larger. A specific design for the EPR cannot be prepared at this time because the purity requirements for the injectors and the plasma feed have not been estimated. Hydrogen isotope separations have been performed in equipment of reasonably large size, and the data and experience gained from these separations can be expected to be used in the EPR. However, impurity requirements should be specified as soon as possible. Then specific designs can be made to determine whether, as expected, the isotope requirements of EPR can be met at modest cost with current technology. Although we expect no unusually large expenditures for isotope separation equipment, one could specify plasma and feed concentrations which would be difficult to meet.

Satisfactory storage of tritium either as a gas or as a solid tritide (usually uranium tritide) has been demonstrated in other ERDA programs. This is current technology, and either form should be considered "safe." Because solid storage is often considered less hazardous, it will probably be used for most large-scale and long-term storage. Small-scale and especially short-term (surge) storage will be as low-pressure gas.

The blanket recovery system is one of the components least well understood in the EPR tritium-handling system. Detailed evaluations of all of the blanket recovery systems considered for the EPR or later fusion power reactors will not be presented here since a separate report is being prepared on the subject.⁴ Considerable work will be required to select and develop a practical and reliable recovery system. Lithium is currently considered the most likely blanket material; therefore, most of

the major efforts should involve the study of recovery of tritium from lithium. A small side stream of lithium will have to be removed for processing. We are currently considering three processes for recovering tritium from lithium: extraction, probably with a molten salt; permeation; and sorption on hydride-forming metals. The present experimental effort is deficient in all three areas. A significant study of extraction at ORNL is proposed. The work will focus on the recovery of tritium from the salt (after extraction) because this appears to be the most critical step of the process. Alternative techniques have also been proposed. A smaller effort on recovery by permeation is needed, while current studies on sorption processes should be continued. Expanded studies of sorption processes will be recommended when (or if) sorbers with more practical sorption rates are found.

The feed system has not been extensively evaluated in the EPR tritium study because the plasma physics aspects of the feed system have not been clearly defined. If subsequent developments indicate that solid pellet feed should be used in the EPR, relatively more complex tritium-handling equipment would be involved. This is another area where expanded tritium studies will be recommended.

7.1.7 Information required for evaluating tritium processing systems for the EPR

Plasma physics area. Many aspects of the tritium process system will have to be reevaluated as requirements and performance of the plasma system become better understood. Purity requirements must be known for all feed streams: fill, supplementary feed (solid?), and injector feed. How much H_2 , He, and higher-Z impurities can be tolerated in these streams?

How accurately must the DT ratio be maintained? What is the maximum permissible T concentration in the injector feed? To a lesser extent, the impurity concentrations in all exit streams will be important. We will be particularly interested to find out whether massive quantities of high-Z sputter products affect pump performance.

Several options in the EPR design could affect tritium-handling equipment. The probability of adopting these techniques and their performance characteristics should be evaluated. The selection of feed systems is obviously important to the tritium-handling program. Solid-feed equipment will involve different techniques and containment systems. Although no divertor is currently proposed for the EPR, some potential divertor designs would affect tritium removal techniques. Diagnostic devices can also affect tritium containment. Any opening to the plasma chamber must be considered in tritium containment studies. A graphite liner facing the plasma could lead to tritium retention and result in different sputter products and outgassing rates (and composition).

Fundamental data required. The fundamental chemical data required are, for the most part, associated with blanket recovery processes. Sputter products and tritium retention on graphite liner (if adopted) could become more important as studies proceed.

Tritium technology data required. Considerable data are needed on the recovery of tritium from vacuum pumping systems. Cryosorption pumps or mercury diffusion pumps are currently believed to be the most suitable choice for the EPR, but alternative systems should also be considered. Cryosorption pumping is currently under study at ORNL. Particular interest is centered on regeneration at low temperatures and pump sorption

capacity for hydrogen isotopes and helium at various pressures and pumping rates. If mercury diffusion continues to be a promising alternative system, study of these pumps should be initiated.

The recovery of tritium from lithium blankets is the area of most immediate interest. This is perhaps the most difficult developmental effort required for tritium-handling in the EPR. However, as mentioned earlier, a minimal EPR device would not include provisions for blanket experiments. It would also be possible to use a less-efficient recovery system for the EPR. This would allow breeding and material studies in some blanket modules, but it would be less effective in advancing tritium-handling technology. Recovery processes for 500°C lithium blankets are limited by inventory considerations; that is, the maximum allowable tritium concentration is restricted by the allowable tritium inventory in the blanket. If only a small portion of the EPR blanket region is devoted to breeding experiments, much higher concentrations could be permitted before the inventory becomes excessive. Since heat from this module does not necessarily need to be discharged directly to a stream, tritium release rates may also be controlled even when the tritium concentrations are relatively high. Thus it may be possible to operate the recovery system at concentrations significantly higher than those required for future reactors. However, one would prefer to simulate future reactors as effectively as possible and operate the EPR blanket at comparable concentrations.

Isotope separation. Existing engineering data should be more fully evaluated before new experimental work in this area is initiated. As soon as information on allowable DT rates, permissible H concentrations,

and injector feed rates are estimated, one or more conceptual designs of an isotope separation system should be prepared. This will clarify the magnitude of the isotope separation problem. Can the proposed separations be made at minor or acceptable cost? Should the purity requirements be reexamined? Are the estimating data and experience on isotope separation adequate for EPR needs?

Existing related programs. Most of the experimental development required for tritium handling in EPR is also needed for demonstration reactors, other CTR devices, and future power reactors. These efforts need not be funded solely or even principally as EPR programs. The general-purpose goals can be achieved by continuing and expanding existing tritium-handling studies. Specific design and eventual demonstrations, however, should be considered a part of the EPR program. Development of tritium technology for the EPR, demonstration reactors, and other CTR devices has been the goal of Tritium-Handling Studies in the ORNL Chemical Technology Division. This program, which is funded by the ERDA Division of Controlled Thermonuclear Research (DCTR), is directed at solving some of the problems just discussed. It is, however, only a modest-size effort which will need to be expanded during the coming years to meet all the probable needs of the EPR. This program is supported by fundamental studies now in progress in the ORNL Chemical Technology and Chemistry Divisions which are being funded by the ERDA Division of Physical Research. All of these programs benefit from association and consultation with related studies in the ORNL Thermonuclear and Metals and Ceramics Divisions.

Recommendations. All of the tritium studies in progress at ORNL have been outlined and directed in accordance with our best judgments of the needs of the EPR and future reactors; thus far, no significant redirection of these programs has been indicated. Significant expansion, however, will be required during the next few years to meet the needs of the EPR. This expansion will need to occur largely in the area of DCTR development effort. Fundamental efforts funded by the ERDA Division of Physical Research need to be continued; in addition, some expansion (beyond inflation) would be desirable.

Specific expanded efforts for FY 1976 and 1977 include:

- (1) An increased effort to experimentally evaluate the alternative methods for recovering tritium from lithium. (Studies of tritium recovery from other proposed blanket materials should be continued, but no major expansion is recommended unless the most probable blanket choice for EPR is changed.)
- (2) Studies of tritium recovery from vacuum pumps should be expanded. Additional pumping systems (especially mercury diffusion) should be considered.
- (3) ORNL personnel should become involved in the design of the tritium-handling system for the TFTR. Although the tritium systems for the TFTR and the EPR will probably be quite different, useful interchange of information and experience can significantly aid both programs. Such involvement could include direct participation in most of the design efforts or only

consultation and review. More benefit, however, will accrue from active participation.

Specific efforts during FY 1976 relative to the preliminary conceptual design of the EPR should include:

- (1) Preparation of an integrated layout of the tritium system. This layout will have to be modified several times in the future as information becomes available from elsewhere in the EPR studies. However, such a layout is necessary to ensure that several minor items are not ignored in more-detailed studies made subsequently.
- (2) Investigation of the needs of the isotope separation system. The size and cost of isotope separation will be estimated using the best current estimates of feed rates and allowable isotope concentration ranges in all feed streams.
- (3) Consideration of alternative methods for recovering tritium from lithium blankets. These efforts, along with existing experimental programs, will attempt to narrow the choice of alternative recovery techniques.
- (4) Initiation of studies of tritium handling in solid DT feed systems for the EPR.

7.2 Evaluation of Liquid and Solid Tritium-Breeding Blankets

J. S. Watson, P. W. Fisher, S. D. Clinton, and J. B. Talbot

During the past year, a new review and evaluation of proposed methods for recovering tritium from the blanket of fusion reactors has been completed.⁴ A continuous assessment of recovery processes has been made at ORNL for several years, but recent reference designs for fusion reactors have included tritium-recovery systems which needed more thorough and independent evaluation.

Recovery processes are considered for blankets of liquid lithium, molten fluoride salt, and solid lithium compounds. Principal attention is given to liquid lithium because at the present time this appears to be the most likely blanket material for experimental power reactors (EPR) and demonstration reactors, as well as eventual commercial reactors. In addition, the process options for lithium blankets are believed to be less understood than those for molten-salt blankets. However, one must also investigate other materials since blanket designs are still in planning stages.

7.2.1 Lithium blankets

Although reference designs⁵⁻⁸ currently include more lithium metal blankets than any other material, there are still several differences between these designs. Perhaps the most significant difference is the blanket temperature. High temperatures⁵ are desired for thermal efficiency; temperatures up to 1000°C have been proposed. These require that the blanket be constructed of refractory metals such as niobium or vanadium. Other designs⁶⁻⁸ have stayed with more common structural materials

such as stainless steels or other iron based alloys. This, however, restricts the maximum allowable lithium temperature to approximately 500°C.

The maximum lithium temperature greatly affects the tritium process requirements and options.⁹ Maximum allowable tritium concentrations or pressures in high-temperature systems (800 to 1000°C) are restricted by tritium release rates via permeation of the steam generator. This restricts the tritium concentrations to very low values (less than 1 ppm) and the tritium pressures to values probably between 10^{-4} and 10^{-8} torr, depending upon the allowed release rate and the permeability of the steam generator. Maximum allowable tritium concentrations in low-temperature systems (500°C) are likely to be limited by inventory considerations, but there is at this time no way to know this limit. The limit will depend upon the volume of the lithium blanket, the cost of tritium (an unknown value for the quantities and time when tritium will be required for CTRs), and possibly the availability of tritium. Availability and initial costs will depend upon government policy and actions. The cost of tritium must decrease for later models; availability will increase as large fusion reactors go into operation, and thus inventory restrictions will be relaxed.

Metal windows. One of the simplest tritium-recovery techniques proposed uses a large permeable surface through which tritium can move into a recovery system, perhaps to a vacuum pump. Alternative chemical means have been considered for trapping tritium on the low pressure side of the window, but compatibility problems have usually dictated against this technique. Metal windows, as usually conceived, are constructed like a

shell-and-tube bundle heat exchanger. Lithium flows through the tubes, and the required vacuum is pumped from the shell region. Such a system will obviously remove tritium from lithium, but the principal question is whether the window area and costs are tolerable. As the required tritium concentration decreases, the tritium pressure also decreases, and larger windows with more surface area are needed to maintain the required concentrations. Thus metal window costs are closely coupled to allowable tritium inventories or perhaps to tritium costs.

Metal windows could prove uneconomical because of other technical problems. The most likely problem is a low effective permeability of niobium. Although it has been extensively studied, the permeability of niobium under the conditions required for metal windows is still uncertain. Several studies made with thin niobium foils¹⁰ or with nonsteady-state techniques¹¹ suggest a low "effective" permeability and surface effects. The surface effects could result from the presence of impurities such as oxygen on or near the surface. A steady-state study using relatively thick niobium specimens¹² indicated higher "effective" permeability, and the pressure dependence of permeation rate suggests the absence of surface effects. No data are available for the temperature, pressure, and wall thickness required for a metal window recovery system. If the system was ideal (i.e., obeyed Sieverts' law and displayed a constant activation energy), extrapolations to conditions of interest would be simple and reliable. Furthermore, the results from all investigators would agree; however, extrapolations of various results do not agree. Since practical metal window structures are likely to be approximately

1 mm thick, which is about the same as the thickness used by Rudd,¹² his data are believed to be the most useful for our purposes. However, Rudd's data cover a narrow temperature range and higher pressures than those of interest. Since the permeation rate varies as the square root of pressure (Sieverts' law), at least some extrapolation in pressure is justified. Deviations from the square-root dependence could occur during extrapolation over the several orders of magnitude required.

Even if the permeability of a new niobium window is known, the window surface could change in use. Lithium will not transfer oxygen to the niobium; it is more likely to remove oxygen from the original surface. Other materials, however, may be transferred to the niobium surface. Lithium entering the low-temperature (500°C) metal window system will be in equilibrium with the stainless steel surfaces used in the blanket. In addition, nitrogen entering the lithium could transfer to the niobium window. Carbon and nitrogen have been suggested¹³ as potentially troublesome materials. If accumulation of impurities on the niobium surface impairs permeation, the rate of accumulation of impurities will be important.

Although the uncertainties just described indicate that metal windows are not simple well-understood devices which can be applied immediately to lithium blankets, they are considered among the more promising recovery systems for lithium blankets, especially for high-temperature systems. Rough estimates of the cost of metal

window systems are presented in Fig. 7.2. The estimates include the costs for the niobium taken at \$22 per pound, for the shell at \$38,500 per 6000 ft², and for vacuum pumps at \$0.65 per liter/sec pumping speed (based upon present commercial cryosorption pumps). Again, there is uncertainty about the major costs of the assembled shell-and-tube structure. The values are believed to be realistic within a factor of 2 and thus accurate enough to reach meaningful preliminary evaluations. The current ERDA selling price for small quantities of tritium (relative to CTR needs) is \$1/Ci. Actual production costs of tritium are not public information, but they are certainly less than the selling price. The capital costs of vacuum pumps were based on current cryosorption pump models, and economies are expected to occur if the market expands and demand brings larger pumps into production.

The projected costs of a metal window system (excluding building, services, etc.) are shown in Fig. 7.2 as a function of the tritium concentration in a 1000-MW(t) reactor whose blanket contains 4.6×10^5 kg of lithium. At first, the costs decrease with increasing tritium concentration (on the left side of the figure). In this region, the costs of niobium windows and vacuum pumps are the important parameters. Costs pass through a minimum and then increase again (on the right side of the figure) as tritium inventory costs become important and eventually dominate the overall cost. Clearly, under these assumptions,

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Fig. 7.2. Comparison of total cost for tritium windows and cold traps.

there is an optimum tritium concentration to be maintained in the blanket.

The analysis ignores three factors which could prevent one from operating at the optimum concentration: (1) availability of tritium, (2) acceptable permeation losses to the steam system, and (3) hazards associated with a large tritium inventory. The first factor was discussed earlier. Although one must be aware of potential availability problems, the advantages of having large inventories of tritium on hand for the first reactors should be clearly documented to aid in government (ERDA) decisions which will affect availability. The second factor, tritium-release rates by permeation, will limit allowable tritium concentrations in high-temperature systems¹⁴ (about 1000°C) to values below the economic optimum. This, however, is not likely to be a limitation in stainless steel (about 500°C) systems. The permeation into a helium coolant which does occur can be oxidized to a nonpermeable form before it reaches the steam generator.¹⁴⁻¹⁶ The effects of the third factor (hazards from tritium inventory) can be minimized by a careful system design. Figure 7.2 is presented only to demonstrate that metal windows are economically feasible but are likely to be expensive. This is not an accurate cost estimate.

Solid sorbents. Thermodynamic considerations suggest that tritium can be removed from lithium by sorption into yttrium.^{6,14,17,18} To be practical, however, the sorption process must proceed at an acceptable rate. It has been recognized that solid-phase diffusion rates could

be too slow, especially if impurities such as oxygen in the metal surface impede diffusion.^{14,16} Tritium sorption into a particular yttrium specimen has been shown to follow a first-order process such as one might expect from diffusion through a resistance at the yttrium surface. The rate becomes greater with increasing temperature (the highest temperature tested was 400°C). Present data indicate that metal sorbents are likely to be even more expensive than metal windows.

Extraction processes. Tritium recovery from lithium by extraction into a molten salt was first proposed by Maroni.^{19,20} The proposed flowsheet is shown in Fig. 7.3. The process involves two principal steps. First, the tritium is extracted into a molten salt; several salts have been studied -- a LiCl-KCl eutectic appears attractive. Moderately favorable distribution coefficients have been measured, and a mechanically agitated contactor previously studied for pyrometallurgical processing of fission reactor fuels was suggested. There is, of course, little merit in extracting tritium from liquid lithium into a molten salt unless the tritium can be more easily removed from the salt; that is the second step of the process. Maroni suggests an electrolytic cell for recovering tritium from the salt. The electrolytic cell is similar to fuel cells under development at ANL, but the current flow is in the opposite direction, producing rather than consuming hydrogen (tritium).

Many of the potential problems of the system are discussed by Maroni, but some of the major questions cannot be answered with current information. Perhaps the most serious question is the

Fig. 7.3. Schematic of extraction process for recovering tritium from lithium.

effect of the recovery process on the rest of the blanket system. Lithium metal leaving the extraction will contain potassium and chlorine (in equilibrium with the salt). The concentrations of both components will be low, and should have little effect on blanket neutronics. Corrosion or compatibility effects, however, are more difficult to estimate. Although neither Maroni^{19,20} nor DeVan¹³ expects the trace of chlorine and potassium to significantly affect corrosion rates in the blanket, stainless steel corrosion by lithium is complex, and "in-loop" tests, perhaps with magnetic fields, are needed to demonstrate that an extraction process will not damage the blanket.

Contacting equipment for liquid-metal--molten-salt extraction processes has been studied both at ORNL²¹ (for molten-salt reactor processing) and at ANL²² (for pyrometallurgical processing). The ORNL work is continuing and includes development of both mixer-settler and column devices. The study is concentrating on near-zero entrainment devices which are required for the molten-salt reactor systems and which are likely to be desired for CTR systems.

The weakest part of the original flowsheet is believed to be the electrolytic cell. There are important differences between the hydrogen fuel cell and the proposed electrolytic cell which must be considered. First, the electrolytic cell operates at much lower tritium concentrations. This not only affects polarization of the electrodes (current densities are controlled by diffusion of reacting ions to the electrode surface), but it also affects diffusion through the electrode itself. At the pressures of interest, permeation through

solid electrodes is undesirably slow. Two porous electrodes have been suggested. One would use controlled pore sizes to permit only partial penetration of the nonwetting salt at a carefully controlled pressure. The second concept would use a composite electrode with a wettable porous structure facing the salt; the salt would then only penetrate to the composite interface. This arrangement could further hinder diffusion to the active electrode surface and reduce current densities.

All aspects of electrode design are complicated by the presence of relatively large concentrations of free lithium (plus potassium) in the salt, estimated to be several times the concentration of tritium.²³ This results from equilibration with metallic lithium. High free-lithium concentrations enhance the chance of tritium recombination at or near electrode surfaces and probably require tritium removal with low pressures at the electrode surface.

In summary, an extraction process does appear to be promising for recovery of tritium from lithium blankets, but a replacement for the electrolytic cell is needed to recover tritium from the salt. Several alternative approaches have been suggested, and ORNL plans to evaluate modified versions²³ of the extraction process experimentally.

7.2.2 Molten-salt blankets

Molten salts containing lithium and beryllium have long been considered for fusion reactors. The Princeton Reference Design¹⁵ includes a molten-fluoride-salt-blanket and proposes a recovery system. Molten salts have lower breeding ratios than lithium metal,²⁴ but the

Princeton design indicates that adequate breeding ratios are possible.¹⁵ Although the thermal conductivity of the molten fluoride salt is low, it is less affected by magnetic fields, it can be pumped more freely, and turbulence is not suppressed.²⁵

Princeton Reference Design.* In the Princeton Reference Design fusion reactor,¹⁵ tritium is generated in a molten-fluoride-salt (Li_2BeF_4) blanket. The practicality of the blanket processing scheme proposed by Johnson¹⁵ was reviewed by studying each of the process steps and estimating the feasibility and equipment size involved.²⁶

Description of the process. Figure 7.4 shows the proposed tritium-recovery system for the molten-salt loop. The flow rates and concentrations are listed in Table 7.1.

The breeder salt circulates constantly through eight disengagers arranged in parallel about the periphery of the reactor. The salt is sprayed into the disengagers as small droplets to provide an extended area for the dissolved helium and tritium fluoride (TF) to diffuse from the liquid salt. Salt collects in the bottom of the disengager and flows back to the reactor blankets. An unspecified doctor system removes impurities from the Li_2BeF_4 and adds makeup agents to maintain the desired salt composition to a small drag stream of salt.

First, the gas effluent is drawn through a water-cooled trap and then through liquid nitrogen-cooled cyclic condensers. The helium is discharged to a storage system for final control. Solid TF is melted out of the "off-stream" cyclic condenser and collected in a small

* This study was conducted in cooperation with the Oak Ridge Associated Universities program for Laboratory Graduate Participation.

Fig. 7.4. Princeton Reference Design salt blanket processing system.

Table 7.1. Flow rates and concentrations for tritium recovery system for the Princeton Reference Reactor

Li_2BeF_4 salt flow through disengagers	$4.0 \times 10^6 \text{ kg/hr}$
TF concentration in salt:	
Entering disengagers	2.09×10^{-7} mole fraction
Exiting disengagers	1.29×10^{-8} mole fraction
Exhaust gas composition:	
TF	1.0×10^{-4} atm
Helium	1.53×10^{-4} atm
Salt	1.3×10^{-4} atm
Tritium recovery	548 g/day
Helium ash discharge	1120 g/day

receiver. The salt flows intermittently through a purification system and into an electrolysis cell, where a solution of KF and TF is electrolyzed to F_2 and T_2 . The gases emerging from the electrodes are saturated with TF and must pass through the liquid nitrogen-cooled traps to remove the TF. The pure tritium gas is recycled to the primary fuel loop.

To avoid wall embrittlement, the maximum tolerable tritium pressure in the salt was estimated¹⁵ to be 10^{-2} torr or 1.32×10^{-5} atm, but the validity of this limit is questionable.²⁷ The corresponding TF concentration in the entering salt was calculated to be 2.09×10^{-7} mole fraction. The exit concentration corresponding to a TF pressure of 10^{-4} atm is 1.29×10^{-8} mole fraction.

Atomization of a fluid usually involves the application of an external force, such as a gas resistance. However, disintegration will occur in a vacuum if the liquid jet is turbulent throughout and if its surface tension is overcome.²⁸ The jet velocity needed to achieve atomization must be greater than 488 fps to obtain a droplet size of 150 μ . If the diffusion coefficient of TF in molten salt is 10^{-5} cm^2/sec as suggested by Cantor,²⁹ the residence time for mass transfer for a 150- μ drop is 1.3 sec. Therefore, a column height over 637 ft is necessary. The column results because droplets injected into a vacuum at high velocities (488 fps) do not slow down, but accelerate slightly. Adding packing surface to the column would slow the salt particles and increase residence time, but it would also cause coalescence and conductance (pumping) problems.

A preliminary study of a dropwise liquid-gas contacting system was made by Gabor et al.³⁰ Fused NaF-ZrF_4 containing dissolved uranium tetrafluoride was sprayed into a fluorine atmosphere to recover uranium hexafluoride. Experimental equipment was developed to test heat and mass transfer rates. A Spraying Systems Company full-cone spray nozzle (1/8 G3001.4) constructed of Monel was used for the heat transfer tests. This nozzle had a 0.026-in. orifice. The salt was heated to 650°C and sprayed into air at 20°C. The average diameter for a droplet was determined to be 164 μ .

Several problems were encountered in spraying the fluoride salt. Plugging of the spray nozzle was a difficulty, but this was solved by heating the nozzle for fully controlled operation. In other fused-salt process studies, autoresistive heated salt transfer lines have proved successful. The nozzles used were removed and inspected after 20 batch sprayings. The orifice had enlarged from 26 to 32 mils diam, increasing the area by approximately 50%. This experiment suggests that frequent nozzle replacement would be necessary.

The prospect for a spray process for the recovery of tritium from molten fluoride salt appears discouraging. The velocity of the jet must be large to overcome the surface tension of the salt for atomization to occur in a vacuum of 10^{-4} torr. The droplet diameter and residence time in the disengager needed for mass transfer, coupled with the drops accelerating as they fall, lead to a tower of considerable height. If the minimum number of nozzles is 110, the column diameter must also be large to decrease coalescence of the

droplets. Corrosion within the spray nozzles would make frequent replacement necessary for full operation.

The cyclic condensers seem feasible as an integral part of the tritium recovery system. Helium will not increase the surface area required to freeze out the TF greatly,³¹ and refrigeration loads from the condensers will be reasonable.

Other design considerations. The salt traps above each spray tower and the vacuum pump system may be areas of additional design problems. The molten salt entering the spray disengagers at 660°C may be boiling; the uncertainty in the reported values of vapor pressures for molten Li_2BeF_4 is a factor of 10.³²

Tritium loss from the vacuum pump system will be inherent; therefore, a trapping system would be a requirement to prevent tritium escape. The Princeton design specifies that 2% of the tritium will be T_2 which will not be caught in the cold traps. In choosing the vacuum pumps, the TF compatibility with the pump components must be reviewed since traces of TF will inevitably reach the pumps.

Alternative flowsheet. Although the flowsheet just described has some difficulties, slight modifications can be made which are expected to remove these difficulties. It will be necessary to go to much higher tritium pressures to simplify the process. The Princeton design selected the allowable tritium pressure as 10^{-2} torr due to embrittlement problems, but materials are available which are believed to be able to sustain considerably higher tritium pressures at approximately 500°C. High tritium pressures over the molten salt

do not result in significant tritium inventory;³³ this is unlike lithium blankets. If the pressure is increased closer to atmospheric pressure, a very small disengager would be required. If the salt circulation pump is a long vertical shaft unit like those used in molten-salt (fission) reactor tests, the pump bowl itself may be adequate for tritium removal. High tritium pressures will result in higher permeation rates to the helium coolant and through all other exposed piping. Tritium escaping in this matter must be contained, but secondary containment techniques are available^{14,15} or under development¹⁹ which should be able to handle this problem.

Thus although we question the size of the disengagers in the proposed Princeton design, only slight modifications would be required to make the system look more attractive. However, the ability of the proposed structural materials to avoid embrittlement at the required temperatures and pressures may be questioned. If this is satisfactory, the recovery of tritium from molten salts does appear to be easier than recovery from lithium.

7.2.3 Solid breeding materials

Solid breeding materials were first suggested for CTR blankets because they promised low activation blankets [with low-temperature aluminum or "sintered aluminum products" (SAP) structure] and/or low tritium inventories.³⁴ Some have also seen ease of tritium removal or simpler blanket designs with helium-cooled solid blankets, but these advantages are nonexistent in many cases and highly questionable in others. There are many proposed solid blanket

materials and many designs (sometimes even from the same laboratory), and it is difficult to make simple checks to verify or devise the feasibility of all of these systems. As of this writing, no solid blanket design has been prepared which is capable of meeting all of the requirements of the CTR blanket; however, certain designs are able to satisfy some of the specifications.

Without neutron multiplication, no proposed solid "breeding blanket" except Li_2O can reach tritium-breeding ratios greater than unity. For other solid blankets, the availability and performance of a neutron multiplier (beryllium) are important. The availability of beryllium (world supply) has been estimated to be insufficient to support a substantial fusion power economy.³⁵ Solid beryllium used as a neutron multiplier also would undergo considerable swelling and other forms of radiation damage.³⁶

Solid blankets with helium coolants present heat removal difficulties. Several designs have been prepared which apparently can handle the heat load, but possible "hot spots" from local plasma discharges onto the wall have not been fully analyzed.³⁷ Liquid blankets place more heat capacity in contact with the wall. Although solid breeding modules have been considered for three experimental fusion power reactor scoping studies,³⁸⁻⁴¹ none chose solid materials for their reference designs.

Recently, data have been reported which permit some evaluations of tritium recovery from solid blankets. Tritium diffusion rates in such materials are generally low, and very small blanket particles are required if low inventories are to be achieved. Recent data from

LiSiO_2 and LiAlO_2 ⁴² indicate that with 2- μ particles, tritium removal rates are adequate. The data do not cover a range of particle size sufficient to confirm the proposed simple solid diffusion-controlled process, but release-vs-time curves suggest the model. Available data on Li-Al, however, present an unclear picture. Release rates do not suggest the solid diffusion-controlled model,⁴² and recent sorption studies²⁶ suggest surface resistance to diffusion and/or surface sorption.

The fine solid blanket powders must be enclosed, perhaps in a tubular structure, and flushed with helium to remove the released tritium. Pressure drops through the powder can be large if substantial flow rates are required. Both sintering or further fragmenting the bed into even finer particles must be avoided. Fine powders sinter far below their melting point, and sintering temperatures may limit the operating temperatures of some solid blankets, especially Li-Al. On the other hand, under radiation, blanket powders may fragment even further if helium formed by lithium transmutation (or the T_2O product formed in some materials) cannot diffuse out of the particles.

In summary, solid lithium-containing materials do show promise for CTR blankets, although problems still remain which could limit or prevent their use. Breeding ratio is perhaps the most serious problem, but neutron multiplication (another questioned technique) could solve this problem. Heat transfer and tritium recovery may be possible; however, these are not simple processes which can be designed with current information.

7.3 Tritium Sorption Studies

S. D. Clinton and J. S. Watson

7.3.1 Introduction

A molten lithium blanket appears to be the most promising means of achieving tritium breeding for Tokamak fusion reactors. Three recovery techniques are currently being considered for the removal of tritium from liquid lithium: (1) extraction with a molten salt, (2) permeation through a niobium window, and (3) sorption on a hydrogen-gettering metal such as yttrium (or possibly zirconium). Tritium sorption from liquid metals is being studied in a batch contactor (volume of 20 cc) using selected metal sorbents. Yttrium, zirconium, uranium, and titanium sorbents have been tested. A sample of the sorbent metal is contacted with a known amount of potassium (potential blanket coolant) containing 620 ppb of tritium for time periods ranging from a few hours to several days at temperatures of 150 to 400°C. After the desired contact time, the potassium is drawn into a sample tube and analyzed for the remaining tritium content. A yttrium sample has shown the only significant tritium removal rate from molten potassium.

7.3.2 Experimental equipment

A schematic diagram of the equipment to contact tritiated liquid metals with selected sorbents is shown in Fig. 7.5. Basically, the equipment consists of a reservoir of tritiated liquid metal, two sampling valves, two contacting chambers, a vacuum source, and an argon supply (less than 5×10^{-4} % total impurities). The tritiated liquid metal

Fig. 7.5. Contacting apparatus for tritium metal sorption studies.

is drawn into the two contacting chambers at a temperature of 150 to 400°C. One chamber contains the metal sorbent, and the second chamber serves as a control. To terminate the experiment, the two liquid-metal samples are removed from the contact chambers and analyzed for tritium.⁴³ To reduce the problems of oxygen contamination, the entire process is enclosed in a glove box with a once-through, dry argon atmosphere. The glove box also serves as a secondary tritium containment, operating at a slight negative pressure and exhausting to a hot off-gas system. Figure 7.6 is a photograph of the liquid-metal--metal-sorbent contacting apparatus as seen through the glove box window.

7.3.3 Results and discussion

The initial data were obtained with tritiated potassium, but preparations are under way to determine the effectiveness of metal sorbents in tritiated lithium. Since the early tests indicated that the removal of tritium by metal sorbents was slow and probably controlled by solid diffusion, a differential bed approach to sorbent materials evaluation was abandoned in favor of a batch contactor.⁴⁴ Two dozen metal sorbent samples were contacted with tritiated potassium at temperatures from 150 to 400°C and time periods extended up to several days before any significant mass transfer of tritium was observed. Although yttrium, uranium, and titanium samples were tried, most of the early metal sorbent tests were made with zirconium. Since a surface film (probably oxide) was thought to be acting as a barrier to mass transfer, the zirconium samples were given different surface treatments to eliminate the possible film resistance.

Fig. 7.6. Tritium--metal sorbent contacting apparatus as viewed through the glove box window.

A yttrium metal sample obtained from Research Chemicals (Division of Nuclear Corporation of America) was the first sorbent material to remove significant quantities of tritium from the molten potassium. Photographs of the yttrium sorbent sample are shown in Fig. 7.7. The sample dimensions are 0.06 in. x .5 in. x 1.5 in. (geometric surface area of 11.2 cm^2); the weight is 3.42 g. The yttrium metal sample appears to have a layered structure and is friable and grayish in color.

The results of the yttrium-tritium sorption experiments in molten potassium are found in Table 7.2. The yttrium sorber was not removed from the contacting chamber until after run 12, and the two subsequent runs (13 and 14) were made with a second yttrium sample of the same dimensions and weight. Runs 7 through 11 were made at 400°C , but the remaining runs were at 300°C . The yttrium-tritiated potassium contact times, t , are given in column 2, while the tritium concentrations remaining in the potassium, C , are shown in column 3. Column 4 tabulates the tritium concentrations in the potassium control samples C_0 . From the 12 control analyses, the average tritium concentration in the potassium feed reservoir was 621 ± 27 ppb. The potassium volumes, V , which were recovered from the sorber contacting chamber, are shown in column 5. Column 6 tabulates the product of yttrium area per unit volume of potassium (A/V) and the contact time (t). The last column lists the ratio of tritium concentration in the yttrium to tritium concentration remaining in the potassium, C_y/C , at the end of each run. The contact area to unit volume of potassium was $0.72 \pm 0.18 \text{ cm}^{-1}$.

Fig. 7.7. Yttrium metal sorbent samples.

Table 7.2. Results of the tritiated potassium-yttrium sorption experiments

Run no.	Contact time, t (hr)	Tritium concentration in potassium		Potassium volume, v (cc)	(At/V) ^a (hr/cm)	(Cy/C) ^b (ppb/ppb)
		sorber, C (ppb)	control, C ₀ (ppb)			
1	51	483	639	18.9	30.2	1.2
2	99	80	579	19.5	56.8	37.1
3	145	30	646	14.6	111	163
4	24	347	590	17.2	15.6	17.2
5	4	620	643	21.0	2.1	9.6
6	48	241	612	12.4	43.3	29.2
7	24	40	618	13.9	19.3	220
8	16	54	611	12.9	13.9	206
9	6	266	637	18.2	3.7	47.3
10	30	22	673	10.0	33.6	631
11	12	319	592	17.5	7.7	47.1
12	72	490	609	18.8	42.9	31.8
13	72	68	---	18.3	44.1	30.6
14	24	257	---	19.8	13.6	13.8

^a A = yttrium geometric surface area, 11.2 cm²,

^b C_y = tritium concentration in yttrium, ppb.

Except for runs 1 and 12, the tritium concentration in the potassium decreased with contact time as a first-order reaction (see Fig. 7.8). The mass transfer resistance in run 1 could be explained by an activation of the yttrium surface; however, a similar yttrium sample in run 13 did not require any extended time for activation. From the bulk tritium concentration in the yttrium (15.6 ppm), the driving force was estimated to be sufficient to reduce the tritium concentration in run 12 (72 hr at 300°C) by more than 21%. However, there was considerable uncertainty in the estimate. The decrease in the effective driving force could indicate a tritium concentration within the yttrium.

By neglecting runs 1 and 12, the time required to reduce the potassium tritium concentration by a factor of 2 with the first yttrium sorber was 32 hr at 300°C and 6 hr at 400°C. Due to a limited supply of tritiated potassium, only two runs were completed with the second yttrium sorber. These two data points predict a potassium-tritium concentration half-life of 22 hr at 300°C. Although the geometric areas of the two yttrium samples were the same (11.2 cm²), the effective sorption rate with the second yttrium sample appears to be about 50% greater than with the first.

The potassium-tritium concentrations for the first yttrium sample are plotted in Fig. 7.9 as a function of the product of the contact area and time per unit volume of potassium. With a surface film driving force that can be approximated by the potassium-tritium concentration at any time, the slope of the data in Fig. 7.9 is equal to a tritium sorption mass transfer coefficient. The mass transfer coefficient

Fig. 7.8. Tritium concentration in potassium vs the contact time.

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Fig. 7.9. Tritium concentration in potassium vs the product of yttrium area and time per unit volume.

increased from 3.0×10^{-2} cm/hr at 300°C to 1.5×10^{-1} cm/hr at 400°C. With these values, the activation energy of the reaction is estimated to be 12 kcal/g-mole.

After runs 12 and 14, the two yttrium samples were analyzed for tritium content by dissolving the metal and counting the tritium in the solution by liquid scintillation. The first yttrium sorber was estimated to have accumulated 0.52 Ci of tritium during the sorption experiments. About 0.42 Ci (81% of the predicted accumulated tritium) was recovered from the first sample. An accident in transferring the second sample from the contacting chamber to the dissolver prevented a quantitative material balance on the second yttrium sample.

Prior to dissolution, the first yttrium sample was cut into three pieces (0.5 in. x 0.5 in. x 0.06 in.), and the second sample was divided into three layered sections (inner layer was about 0.04 in. thick). The tritium concentration was uniform in both directions; however, uncertainties in measurements with the second sample prevent conclusions about the cross-section profiles.

7.3.4 Program status

All of the experimental equipment contaminated with tritiated potassium has been removed from the glove box and transferred to the burial ground. A new contacting system containing lithium is being installed and should be operational by the end of October 1975. The first sorbent materials to be tested in tritiated lithium will be yttrium and zirconium. Since most metal oxides (except yttrium) can be reduced by lithium, the performance of tritium sorbent materials should be improved in the new system.

7.4 Cryosorption Pumping for Fusion Reactors

P. W. Fisher

Significant advances in vacuum technology are required for the development of full-scale fusion power reactors. Advanced vacuum equipment is needed for three reactor subsystems: the plasma recovery system, the neutral beam injection system, and the system for recovering tritium from the blanket. Each of these has separate vacuum systems with different requirements, but in each case cryosorption pumps are the prime candidate.

Table 7.3 shows a compilation of vacuum requirements for Tokamak power reactor reference designs and two EPR reference designs. Estimates of the number and size of vacuum pumps range from six 45,000 liter/sec pumps for the ORNL 1000-MW(t) design to 96 mercury diffusion plus 96 cryosorption pumps for the Wisconsin UWMAK-I (a 5000-MW(t) reference design). The composition of the plasma in these reactors is approximately $D_2:T_2:He = 1:1:0.2$. Therefore, the vacuum system must have the capability for pumping helium as well as the hydrogen isotope mixture. In addition, the system must be clean [i.e., free of high-molecular-weight (high-Z) material which deleteriously affects the plasma]. Vacuum pumps must also be regenerable so that all fuel can be recycled to the reactor. Mercury diffusion pumps proposed in some designs cannot guarantee freedom from high-Z contamination, and they require mechanical forepumps which are difficult to seal for tritium containment. Similar problems are inherent in oil diffusion pumps. Ion pumps and titanium sublimation pumps are not regenerable. Getter pumps and cryocondensation pumps cannot pump helium. Only cryosorption

Table 7.3. Summary of vacuum requirements for fusion reactors

	Full-scale power reactors			EPR's	
	ORNL ^a	Princeton ^b	Wisconsin ^c	ORNL ^d	ANL ^e
Power, MW(t)	1000	5305	5000	450	15-150
Plasma pumping rate, torr - liters/sec	24	494 ^f	1430 ^f	600	704
Total speed of vacuum pumps, liters/sec	2.2×10^5	5.8×10^5	2.3×10^6	1×10^6	8×10^5

^aData taken from ref. 5.

^bData taken from ref. 15.

^cData taken from ref. 45.

^dData taken from ref. 46.

^eData taken from ref. 40.

^fAll plasma scraped off by the divertor was assumed to go to the vacuum system.

pumps appear to meet all criteria for CTR applications; one goal of this program is to confirm their suitability for these systems.

Vacuum pump requirements for neutral beams are different from those for the main plasma vacuum system. The beams are composed entirely of deuterium, therefore, no helium pumping capacity is required. High-Z impurities must still be controlled because they can drift to the plasma, and regenerability is also needed. A unique problem associated with the beam pumping system is possible contamination with cooling water. This could adversely affect vacuum pump operation. Pumping rates for beam lines range from 60 torr-liters/sec for a single TFTR beam module to 120 torr-liters/sec for an EPR module. Present designs call for construction of a neutralization chamber with walls constructed entirely of cryopanel. Since only deuterium has to be pumped, either cryocondensation or cryosorption panels could be utilized. The choice of the optimal system depends on the operating temperature of the panels. If cryosorption panels must be operated near 4.2°K like cryocondensation panels, then the latter system would be chosen for its simplicity. However, if cryosorption panels can be operated at higher temperatures (near 15°K), they would be chosen to obtain significant reductions in cooling capacity.

Many proposed processes for recovering tritium bred in CTR blankets require vacuum pumps. Of prime importance in these systems are high vacuum, capacity, and tritium containment. For example, a permeable metal window for recovering tritium from a 1000-MW(t) reactor operating at 1000°C would require a vacuum pumping rate of about 26 torr-liters/sec at a pressure lower than 3×10^{-5} torr to maintain the tritium

concentration below 10 ppm in a lithium blanket. A similar reactor operated at 500°C would require 16 torr-liters/sec at pressures less than 5×10^{-5} torr. These needs can be fulfilled by either cryosorption pumps or reversible getter pumps. The technology necessary to construct large regenerable getter pumps is not as advanced as that for cryosorption pumps.

Cryosorption pumping emerges as the most likely candidate for these systems; however, this conclusion is based on large extrapolations of current technology. Problems to be addressed in this program include: (1) determination of pumping speeds for selected pure substances at high pressures and high loading, (2) determination of speeds for mixtures similar to those encountered in plasma pumping, (3) study of the effect of contaminants on pumping speed, (4) study of regeneration at low temperatures, and (5) determination of optimal operating temperatures for various CTR applications.

Experimental. To accomplish these goals, an Excalibur Model 1106 cryosorption pump has been fitted to a test stand which is capable of measuring pumping speeds, activation rates, and equilibrium loading pressures. The apparatus⁴⁷ has been modified to incorporate several improvements into the system. The present design is shown in Figs. 7.10 and 7.11. The test stand has two independent means for measuring pumping speed. The feed gas reservoir is a calibrated volume mounted in an air bath and fitted with a large Wallace and Teirnan gauge. This system will provide both the total loading and loading rates. The test chamber attached directly to the pump flange contains an orifice

Fig. 7.10. Equipment for the cryosorption pump evaluation experiment.

PHOTO 2357-75

Fig. 7.11. Photograph of experimental apparatus.

plate of known conductance. Measurement of the pressures on both sides of this plate facilitates calculation of either pumping speed or activation rate. A mass spectrometer is provided for gas analysis. This permits monitoring of impurities (intentionally or unintentionally added) and compositions of various gas mixtures which will be studied. Two Vac-Sorb pumps are used for high-pressure roughing, and a noble-ion pump is used for low-pressure roughing during both bake-out and activation experiments. An automatic liquid nitrogen supply has been added to the system to facilitate operation over extended periods of time. Calrod heaters, heating tape, and insulation have been attached to the outside of the entire pump and test stand for bake-out at 400°C.

Program status. Construction and preliminary tests of the apparatus are complete. Failure of ion gauges, ion gauge readout unit, and 6-in. vacuum flange caused significant delays in the program. These problems have now been corrected.

The initial experiment to be performed by the end of September 1975 will involve the measurement of the equilibrium adsorption isotherm for hydrogen at 77°K. Comparison of our data with existing data by other workers will confirm the amount of adsorbent on the cryopanel (reportedly 30 g of MS-5A). This measurement will also form a datum so irreversible changes in the adsorbent can be detected if they occur.

The most urgent experimental needs at present are associated with design of neutral beams for the TFTR which are to be operational in 1980. Beginning in October 1975 work will be directed toward

this project. First, measurements of pumping speeds for deuterium (especially at high pressure) will be made. Determination of optimal cryopanel temperature and the effect of contaminants will follow.

7.5 Tritium Recovery from Irradiated Li-Al and SAP Alloys

J. B. Talbot

Introduction. A variety of applications have been proposed for lithium alloys with CTRs. Powell³⁴ suggested using a solid Li-Al alloy in the tritium-breeding blanket. This alloy plus its aluminum structure would produce minimum neutron activation and low tritium inventories. However, the usefulness of the material is contingent upon its equilibrium uptake of tritium and its ability to rapidly release tritium of a flush gas such as helium. Tritium sorption in solid equiatomic Li-Al has been examined experimentally. From a previous study,^{26,47} tritium sorption in Li-Al (50-50 at.%) ranged from 10^{-5} to 10^{-6} at. fraction tritium for tritium pressures of 0.03 to 5 torr. This is in agreement with estimates for the blanket-recovery system proposed by Powell. The sorption of tritium in Li-Al appeared to be controlled by surface reaction, slow internal diffusion, or both. An experimental study by Wiswall and Wirsing⁴² tested the removal of tritium from granular forms of Li-Al (an intermetallic compound), LiAlO_2 , and Li_2SiO_3 . The procedure was to flush helium through a bed of irradiated compound at a controlled temperature in the range of 400 to 600°C. Tritium, generated by exposure to thermal neutrons, diffused out of a sample and was carried to a tritium monitor. The data for LiAl were not successfully analyzed in terms of

a physical model. Removal rates were increased both by an increase in helium flow rate, by additions of 1000 ppm hydrogen to the helium, and a decrease in particle size.

The objectives of the present program will be to investigate the tritium removal rates from a Li-Al alloy (~ 0.05 wt % Li) and a Li-SAP (sintered aluminum product) alloy. This experimental effort is more realistic than the previous static equilibrium sorption experiment, since tritium will actually be generated from irradiated lithium in the reactor blanket. This experiment also differs from the previous irradiation studies because it uses samples of Li-Al (with a low lithium content) and SAP, which has not been studied. SAP is a proposed candidate for a structural material; the effect of high oxygen content of SAP will be particularly interesting.

Experimental. Approximately 1-g quantities of ^6Li -doped SAP and ^6Li -doped aluminum in sealed HFIR-HT capsules were irradiated (under the supervision of F. W. Wiffen) to burn much of the lithium. The ^6Li -doped SAP, containing 17.7 wt % Al_2O_3 and 45 wt ppm of lithium, was fabricated from 92 to 96% ^6Li , 1100 grade aluminum, and oxygen picked up from the air during ball-milling of the atomized Al-Li powder. The ^6Li -doped aluminum, containing approximately 500 ppm of lithium, was fabricated from $\sim 92\%$ ^6Li and 99.9999% aluminum (nominal purity). The pieces of Al-O-Li to be irradiated were loaded in standard HFIR hydraulic tube capsules obtained from Isotope Sales. The cavity was filled with the supplied aluminum powder, and the end cap was welded in a helium glove box. The capsules were helium leak-checked by J. W. Woods, and further tests were made by the

Isotopes Division before irradiation. The requested irradiation times were selected from the approximate ${}^6\text{Li} \rightarrow {}^3\text{H}$ burnup curves and the thermal flux values. These times were chosen in order to obtain about 60% conversion of ${}^6\text{Li}$ in the SAP and 95% of the ${}^6\text{Li}$ in the aluminum samples. Table 7.4 gives the requested irradiation times for the above-center HT-7 position in HFIR. The samples were irradiated between May 16, 1975 and June 2, 1975 for the requested times by J. Ratledge.

A schematic of the system to study tritium recovery rates from irradiated LiAl and SAP samples is shown in Fig. 7.12. The design of the system was made principally by David Newell, an ORAU student from Arizona State University. An argon gas line passes into the hot cell, through a preheater, and into the heated sample chamber. The irradiated samples will be heated to 300 to 400°C. The tritium-bearing argon is then cooled and flows to a tritium monitor, Betatec Model 120*. The gas then flows through a bubbler which removes any tritiated water present in the gas. Next the tritium gas from the first bubbler is oxidized in a CuO bed (600°C), cooled, and passed through another water bubbler. Finally, the stream is routed to a molecular sieve bed to remove any remaining tritiated water. The exhaust from this bed is vented to the cell's ventilation system and is released to the ORNL "hot" off-gas stack. Solutions in the water bubblers will be analyzed for tritium by conventional liquid scintillation methods. The irradiated sample will be removed and dissolved so that residual tritium can be determined by liquid scintillation. Material balances to check the released tritium can be made by comparing tritium in the bubblers

* Overhoff and Associates.

Table 7.4. Requested irradiation times for tritium production
in aluminum alloys at irradiation position
HT-7 in the HFIR-HT facility

Sample set	Sample no.	Sample wt (g)	Sample surface area (cm ²)	Requested irradiation time ^a (hr)
SAP 3 - SET 1	1	0.4870	4.3273	133
	2	0.1312	2.7406	
	3	0.1690	3.0012	
	4	<u>0.1440</u>	3.4681	
		0.9312		
SAP 3 - SET 2	5	0.2250	3.7100	133
	6	0.1928	3.3520	
	7	<u>0.3425</u>	3.8971	
		0.7903		
SAP 3 - SET 4	13	1.0486	2.8940	133
6-9 Al - SET 3	11	1.1286	4.5180	13.6
Thermal flux	(2200 m/sec) x 10 ¹⁵ neutrons cm ⁻² sec ⁻¹			2.05

^aTo give 60% burnup of ⁶Li in the SAP samples and 9% burnup of ⁶Li in the 6-9 Al. SAP, 45 wt ppm of ⁶Li = 200 at. ppm of ⁶Li, yields 120 ppm of tritium. Aluminum, ~500 wt ppm of ⁶Li = 2250 at. ppm of ⁶Li, yields 200 ppm of tritium.

Fig. 7.12. System for measuring tritium release rates from irradiated Li-Al and SAP samples.

with an integral of the signal from the tritium monitor. The total tritium content initially present in the sample will be the sum of the released and residual tritium. The irradiation flux for each sample is not known with sufficient accuracy. To check this value, the ^4He content of the sample will be measured by Atomics International. Helium will be formed at the same rate as tritium but is not expected to be released. If the helium content exceeds the released and residual amount of tritium, this indicates that some tritium was released during irradiation and before the sample was heated in the furnace.

The experimental apparatus has been constructed and is presently being tested in Room 210 of Building 4501. Figure 7.13 is a photograph of the equipment. After the system has been installed in Hot Cell "A" of Building 4501, it will be rechecked and calibrated with a standard tritium source. Then the normal studies of tritium release from the irradiated Li-Al and SAP samples will begin. Each sample will contain only about 0.2 Ci of tritium; therefore, operation in a hot cell is not actually necessary. (The experiment could be done in a hood.) However, "hotter" samples are likely to be used later; then experience in using the equipment in a hot cell will be valuable.

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8. COAL CONVERSION PROCESS DEVELOPMENT

Coal conversion development programs include studies of hydrocarbonization, alternate methods for separating solids from coal-liquid products, experimental studies in support of in-situ gasification, and development of methods for analyzing and characterizing liquid and vapor products and effluents from coal conversion plants.

0.1 Analytical Methods in the Characterization of Coal Liquids

J. E. Mrochek

Processes designed to convert coal into either liquid or gaseous products result in the generation of complex mixtures of tars, organic liquids, and aqueous waste streams. Separations methods which include liquid chromatography (LC), gas-liquid chromatography (GLC), and solvent extraction are being utilized to separate these complex samples for subsequent molecular characterization.

8.1.1 Method development for coal-tar analysis

Pyrolysis of single blocks of coal is being utilized to isolate and study certain aspects of in-situ coal gasification. Approximately 5 to 10% of the energy of the coal block is contained in tars which are condensed out of the gases generated during pyrolysis. The tars are separated from the gases by a demister utilizing glass wool and a water-cooled trap. To achieve a good material balance around the block pyrolysis experiment, it is necessary to recover and separate the mixture of tar and water condensed in the trap. As a means of obtaining information about the chemistry of the process, the tars have been separated into saturate and aromatic fractions, and were characterized by LC, GLC, and gas chromatography-mass spectrometry (GC-MS).

Tar fractionation. The first step of the method chosen to separate tars into benzene insolubles, asphaltenes, and heavy oils included Soxhlet extraction with refluxing benzene. Benzene (95%) was removed from the solubles by rotary evaporation at 30°C, and the heavy oils were extracted by Soxhlet extraction using refluxing cyclohexane (95% cyclohexane--5% benzene). The portion of the benzene solubles which was insoluble in refluxing cyclohexane was defined as the asphaltene fraction; the solubles were the heavy oils. The heavy oils were further separated into saturate and aromatic fractions by extracting the aromatic fraction from the cyclohexane solution with nitromethane. Each fraction was gravimetrically determined and reported as weight percent.

Results. Two tar samples recovered from a block pyrolysis experiment (BP-7) were fractionated by the above method. The first sample consisted of tars recovered by centrifugation from the aqueous tar-water mixture in the tar trap. The second sample consisted of tars removed from the glass wool of the demisting filter. In addition to gravimetric analysis of fractions, as described previously, total sulfur and calorific values were measured for each sample. The results, shown in Table 8.1, indicate little difference between the two samples other than a somewhat higher content of aromatics in the tars from the demister.

High-resolution separations studies. A previous report¹ described experiments with a number of adsorbents to determine conditions that would yield a high-resolution separation of complex coal products by liquid chromatography. None of the adsorbent systems tested yielded satisfactory or reproducible resolution. We have recently evaluated the use of spherical silica particles (10- μ m particle size) using dry

Table 8.1. Analytical data for tars recovered
from block pyrolysis experiments^a

	Benzene insoluble (%)	Asphaltenes ^b (%)	Heavy oils ^c (%)	Heavy oil		Sulfur (%)	Calorific (Btu/lb)
				Saturates (%)	Aromatics ^d (%)		
Tars, aqueous phase	4.4	21.1	74.5	34	66	0.44	16,360
Tars, glass wool demister	3.8	21.3	74.9	28	72	0.58	16,456

^aRun BP-7.

^bBenzene solubles which were insoluble in 95% cyclohexane--5% benzene solution.

^cBenzene solubles which were soluble in 95% cyclohexane--5% benzene solution.

^dDetermined by partitioning between cyclohexane and nitromethane.

isooctane as the eluent. The extremely uniform size and shape of these particles permitted longer columns to be used (100- and 300- by 0.22-cm columns were tested) without excessive pressure drop across the columns at reasonable flow rates.

Figure 8.1 illustrates the separation of the aromatic fraction (nitromethane extract) of a coal tar recovered from a block pyrolysis experiment. This LC column, while separating standards into sharp chromatographic peaks according to ring size, nevertheless shows considerable overlap when tested with the complexity of a real sample. This sample contained major amounts of several methyl-substituted homologues of naphthalene, and a number of other polycyclic aromatic hydrocarbons (PAH). Approximately nine peaks and shoulders on peaks were observed.

Illustrated in Fig. 8.2 is the same sample separated by temperature-programmed GLC using a Dexil 300 column (366 by 0.2 cm). Approximately 95 chromatographic peaks were separated, and PAH with boiling points up to 500°C were detected with the flame ionization detector. It is obvious that the LC separation (Fig. 8.1) is inferior to the GLC separation (Fig. 8.2). The utility of LC for complex samples such as this may be its ability to give a relatively rapid classification into ring groups, and quantifying each group based on the average molar absorptivity of model compounds in the group.

8.1.2 Molecular characterization of coal tars from block pyrolysis experiments

Molecular characterization of components in the heavy oil fraction (that portion of the benzene solubles which is soluble in 95% cyclohexane--5% benzene) of coal tars generated in a block pyrolysis experiment (BP-7) was performed by GLC and GC-MS.

Fig. 8.1. Liquid chromatographic separation of polycyclic aromatic hydrocarbons extracted from tars generated by pyrolysis of a coal block. Arrows correspond to elution positions of reference compounds.

Fig. 8.2. Polycyclic aromatic hydrocarbons from coal tars generated by pyrolysis of a coal block. Separation performed by temperature-programmed gas chromatography, and compounds identified by elution position and mass spectrometry.

Illustrated in Fig. 8.3 is the GC separation of the heavy oil fraction prior to any fractionation of this complex liquid. After separating this sample into saturate and aromatic fractions by extracting the latter with nitromethane (described in Sect. 8.1.1), the chromatographic pattern was simplified, as shown in Figs. 8.4 and 8.5. Identification studies were performed using the saturate and aromatic fractions illustrated in Figs. 8.4 and 8.5. These identifications were transferred to Fig. 8.3 by overlaying chromatograms obtained for the two fractions. Peak identification was performed using both mass spectral data and GC retention for reference compounds. Mass spectral data alone are not sufficient for identification because of the similarity in mass spectra between compounds with the same molecular weight; however, when combined with GLC retention data for reference compounds, unequivocal characterization is possible.

It is interesting to note the length of the saturated hydrocarbon chains found in this heavy oil (see Fig. 8.5). Saturated hydrocarbons ($C_n H_{2n+2}$) ranging from C_{12} ($C_{12}H_{26}$) to C_{32} ($C_{32}H_{66}$), and unsaturated hydrocarbons containing one double bond ($C_n H_{2n}$) and the same range of carbon numbers were observed at one-carbon-atom intervals. Concentrations appeared to peak at C_{25} to C_{27} with a gradual increase from C_{12} to C_{25} , but with a sharp decrease from C_{27} to C_{32} . Boiling points of saturated C_{25} to C_{27} hydrocarbons range from 402 to 422°C, and $C_{32}H_{66}$ boils at 467°C.² Chain branching and irregularities would be expected as a result of pyrolyzing cyclic paraffin compounds. However, saturated chains of the length found in these tars have not been associated with the usual structures given for the coal molecule.

Fig. 8.4. Gas chromatographic profile for the saturate fraction of heavy oils from coal tars generated in a block pyrolysis experiment (BP-7). Identifications performed using GC-MS, and verifying elution positions with reference standards of normal hydrocarbons.

Fig. 8.5. Gas chromatographic profile for the polycyclic aromatic hydrocarbon (PAH) fraction of heavy oils from coal tars generated in a block pyrolysis experiment (BP-7). Identifications performed using GC-MS, and verifying elution positions with reference PAH compounds.

8.1.3 Preparative separations of coal liquids

Four coal-derived liquids (COED medium oil, Synthoil, and two different H-Coal product oils) have been separated by liquid chromatography on a preparative scale as a step toward more definitive molecular characterizations. A glass column (200 by 1.9 cm), the upper half of which was filled with fully activated silica and the lower half with fully activated alumina, was eluted with a sequence of increasing-polarity solvents to separate the coal liquids.³ Five step changes in solvent were used to separate these complex samples into saturate, monoaromatic, di- plus triaromatic, and polyaromatic plus polar fractions. The dual silica-alumina column showed no visible evidence of a residue for any samples except Synthoil after the normal five-solvent elution sequence. Further elution with benzene-diethyl ether, and mixtures of these with methanol resulted in the recovery of an additional 15% of the Synthoil sample after completing the normal elution sequence.

The preparative separation results for the four oils, shown in Table 8.2, indicate that the largest fraction for the COED process was the monoaromatic-containing fraction; for Synthoil, it was the polyaromatic plus polar fraction; for the H-Coal process (western subbituminous coal), it was the saturate-containing fraction; and for the H-Coal product derived from eastern bituminous coal, it was the polyaromatic plus polar fraction. The vastly different distribution for the two H-Coal products may reflect compositional differences in the feed. The high viscosity of the liquid product from the Synthoil process was reflected in the large percentage of that product found in the polyaromatic plus polar fractions.

Table 8.2. Preparative separation of several different coal-derived liquid products by liquid chromatography^a

Fraction	COED (wt %)	Synthoil (wt %)	H-Coal ^b (wt %)	H-Coal ^c (wt %)
Saturates	17.8	4.3	45.5	4.2
Monoaromatics	32.8	6.4	18.4	12.4
Di- plus triaromatics	11.8	13.6	12.8	15.8
Polyaromatic plus polar	23.6	76.6	26.8	42.2
Total recovery	86.0	99.9	103.4	74.6

^aFour grams of product loaded on a 200- by 1.9-cm column packed with fully activated silica (-28 +200 mesh) and fully activated alumina (-80 +200 mesh).

^bFrom western subbituminous coal.

^cFrom eastern bituminous coal.

8.1.4 Analysis of aqueous effluents from experimental coal conversion processes

Aqueous samples recovered from coal block pyrolysis experiments and from a water scrubber used in the atmospheric carbonization of coal have been analyzed by high-resolution anion exchange chromatography. The same coal, a western subbituminous grade, was used in experiments in both programs. Major chromatographic peaks observed for aqueous samples from each of the experiments were identical. Identified constituents include phenol; o-,m-,p-cresol; and several isomeric dimethyl phenols. Concentrations of phenol and total cresols were 84 and 108 ppm in the scrubber liquid, and 2350 and 1260 ppm in the aqueous condensate from block pyrolysis. Analytical data of the aqueous condensate from a second block pyrolysis experiment (BP-7), and total organic carbon by silver-catalyzed persulfate oxidation are shown in Table 8.3.

Table 8.3. Analytical data for an aqueous condensate from a block pyrolysis experiment^a

	Density (g/ml)	Phenol (mg/ml)	Total cresols ^b (mg/ml)	Total organic carbon ^c (mg/ml)
Condensate	1.032	3.14	2.11	5.69

^aRun BP-7.

^bBy high-resolution liquid chromatography.

^cBy silver-catalyzed persulfate oxidation.

A preparative anion exchange separation was performed on the aqueous condensate from a block pyrolysis experiment to identify some of the other constituents which were detected by uv monitoring of the LC eluent. Preliminary data indicate that samples collected from the preparative system may all be contaminated with a phthalate ester, possibly from oil in a vacuum pump used to evacuate the experimental system.

8.2 Hydrocarbonization Research

H. D. Cochran, Jr.

Effort on the Coal Hydrocarbonization Project continued toward completion of a bench-scale hydrocarbonization reactor in the areas previously described.¹ The review and evaluation of data from the literature on hydrocarbonization and carbonization processes was published in a summary report.⁴ Experiments at ambient conditions provided design data for the bench-scale experiment in areas such as solids feeding, solids flow measurement, fluidization, fluidized-bed recirculation, cyclone performance, and solids withdrawal. The atmospheric pressure, high-temperature reactor served to test components and concepts for the bench-scale equipment. Four successful experiments were completed, including one in which hydrogen was the fluidizing gas.

Significant progress was made on the bench-scale system. Several building modifications and auxiliary facilities were added, including a fire protection system in the building, a ventilation system in the process area of the building, and a gas supply station outside the building. Pressure vessels for the experiment were designed, approved, fabricated, and installed. Complete instrumentation and controls for the system were designed, procured, and installed. Shakedown testing of the bench-scale hydrocarbonization experiment was begun.

An extensive safety evaluation of the bench-scale hydrocarbonization experiment was undertaken at the request of the Office of Laboratory and Personnel Protection. The evaluation focused on two principal hazards associated with the experiment: the use of appreciable quantities of potentially explosive gases at elevated pressure and temperature, and the production of coal-derived liquids that are known to exhibit carcinogenic and other toxic effects.

8.2.1 Experimental development (F. J. Endelman, R. E. Barker, D. Sellinger and D. E. Spangler)

This work consisted of effort in two areas described below. The first area, ambient mock-ups, involved experiments at ambient conditions to develop components and design data for the bench-scale experiment. The second area, high-temperature studies, centered around experiments with a batch, atmospheric pressure, high-temperature reactor to test components and concepts for the bench-scale system.

Ambient mock-ups. The two-dimensional recirculating fluidized bed mock-up was modified to test a smaller draft tube. Over 30 experimental runs were made using an 0.75-in.-diam draft tube instead of the former 1-in.-diam tube. Results were consistent with previous data from the mock-up.

The modified ball-valve feeder has been tested in conjunction with a pneumatic transport line. Figure 8.6 is a schematic of the system used for the coal feeder tests. In the first experiments, an attempt was made to feed coal from the hopper at atmospheric pressure to the pressurized transport line. Because of blowback into the hopper from the "empty" cup, stable operation was not obtained. The hopper was modified to allow pressure equalization between the hopper and the transport line. With this arrangement, steady coal feeding could be obtained for short periods; however, coal flow would stop occasionally, possibly due to blowback or particle bridging in the hopper. To provide continuous coal feeding for longer periods, the hopper was pressurized to approximately 2 in. of mercury relative to the transport line pressure. This configuration proved satisfactory because it allowed coal to be fed steadily and without interruption.

The coal was deposited in a container mounted on a load cell. A strip-chart record from the load cell was graphically differentiated to obtain the coal flow rate. Figure 8.7 is a plot of coal flow rate vs the shaft speed of the rotary ball-valve feeder with pneumatic transport. The solid line represents data obtained by feeding into an open container (no pneumatic transport); the dashed line is the "least squares" approximation of the data:

$$C = 0.28 S \quad (1)$$

where

C = coal flow rate, lb/hr;

S = shaft speed, rpm.

Fig. 8.6. System for coal feeder tests.

ORNL DWG 75-5338 RI

Fig. 8.7. Coal feeder performance.

The correlation coefficient of 0.99 indicates a good fit of the data. Calibration of the rotary ball-valve feeder under process conditions will be made as soon as the feed hopper and the feeder for the bench-scale system are installed.

After approximately 50 hr of operation, the modified ball-valve feeder developed a leak around the valve stem. The ball valve was subsequently disassembled and inspected. The stem packing washer was worn, allowing gas and coal to escape to the atmosphere. The Teflon valve seats were also permanently damaged, and all seats, washers, O-rings, and packing were replaced. The permanent parts of the valve showed little or no damage.

A prototype solids-mass flowmeter has been designed and built for testing and use on the bench-scale system. The mass flowmeter consists of a section of 1/2 in.-diam SS 304 sched 40 pipe with thermocouples attached upstream and downstream of a 12-in. heated section. Since a coal-gas mixture has a higher heat capacity than gas alone, the temperature difference between upstream and downstream thermocouples will give an indication of the mass flow of coal in the gas stream.

The system shown schematically in Fig. 8.6 was used to test the flowmeter. Measurements of the steady-state temperature rise have been made at several gas and coal flow rates. Figure 8.8 is a graph of the coal flowmeter calibration data. Equation (2) is the "least squares" approximation of the data.

$$\Delta T = 36.0 + 0.23 G - 1.2 C, \quad (2)$$

where

ΔT = temperature rise in the flowmeter, °F,

G = gas flow rate, slpm,

C = coal flow rate, lb/hr.

ORNL DWG 75-5337R1

Fig. 0.0. Calibration data for coal flowmeters.

Figure 8.9 is a plot of Eq. (2) vs the data. The correlation coefficient of 0.91 indicates that Eq. (2) is a good approximation of the data. For use, Eq. (2) is rearranged to allow calculation of coal feed rate, C , as a function of the known gas flow rate, G , and the measured temperature difference, ΔT :

$$C = 30.0 + 0.19 G - 0.83 \Delta T. \quad (3)$$

The estimated standard error in the calculated value of C is $\pm 50\%$. Although this standard error is large, the coal flowmeter appears to be of value for monitoring the coal flow to the bench-scale system, especially as an indicator of flow stoppage. The coal flowmeter will be recalibrated as soon as the necessary bench-scale equipment is installed.

High-temperature studies. Several modifications were made to the atmospheric pressure, high-temperature hydrocarbonization system. These included replacement of the furnaces on the reactor with surface-mounted electrical resistance heaters; insertion of an intercooled condensate recycle on the spray condenser; installation of integral orifice flowmeters in the product gas line; installation of a remotely actuated gas-sampling manifold; installation of a cyclone between the reactor and the spray condenser; and installation of monitoring instrumentation for safe operations with hydrogen.

Chemical analyses of samples from the first run, AHC-1, were completed. The second run, AHC-2, was terminated because of equipment failure. The third and fourth runs, AHC-3 and AHC-4, were completed successfully, and analytical results are available. The first run with hydrogen as the fluidizing gas, AHC-5, was completed successfully, but analytical results are not yet available.

ORNL DWG. 75-8218

Fig. 8.9. Coal flowmeter calibration.

For run AHC-3, argon was used as the inert gas instead of nitrogen to facilitate mass spectrometric analysis for carbon monoxide in the product gas. During this run, 4.4 lb of Wyodak-seam subbituminous coal was carbonized for 5 hr. No abnormal operating conditions or equipment malfunctions were noted. Temperature control of the preheater and reactor exterior walls at 1250°F was excellent. Nine gas samples and several condensate and coal/char samples were obtained.

In run AHC-4, as in AHC-3, 4.4 lb of Wyodak-seam subbituminous coal was carbonized with argon. No abnormal operating conditions were noted during the course of the experiment, except for some initial pressure fluctuations at the inlet to the reactor and the outlet of the spray condenser. These are believed to have been caused by sticking of the check valve downstream of the gas flowmeters. Examination at the completion of the run revealed that the dipleg and bottom cone of the cyclone had filled with solids. Significantly less solids carryover had occurred in this experiment than in previous ones. Ten gas samples and numerous solid and liquid samples were taken for analyses.

Run AHC-5 differed from the previous runs in three significant respects: the scrubber volume was decreased to about 2.5 gal of water (vs 80 gal for run AHC-1 and 25 gal for runs AHC-3 and -4); the coal was predried to about 7.5% moisture; and hydrogen was used as the fluidizing gas during pyrolysis. After preheating the system with argon to 600°F, hydrogen was used continuously for 3 hr at 1000°F to carbonize approximately 4.5 lbs of Wyodak subbituminous coal that had been predried to approximately 7.5% moisture. As usual, gas samples were taken periodically throughout the run, and scrubber liquid and char samples were taken the next day, after cooldown had occurred.

Tables 8.4-8.6 present the analytical results of the solid feed and products from runs AHC-1, AHC-3, and AHC-4, respectively. Tables 8.7-8.9 show the analytical results of the gas samples from runs AHC-1, AHC-3, and AHC-4, respectively. Table 8.10 summarizes the results of these three runs in the form of a carbon balance for each run. Although substantial improvement in carbon recovery is evident, the results show a clear need for further effort in this area.

Table 8.4. Analyses of feed coal and product char from run AHC-1

	Coal	Char
<hr/>		
Proximate analysis (as received, wt %)		
Volatile matter	41.0	31.2
Fixed carbon (by difference)	31.0	57.8
Ash	5.2	8.5
Moisture	22.8	2.5
— Ultimate analysis (maf), wt %		
Carbon	72.6	80.1
Hydrogen	5.4	4.1
Nitrogen	1.0	1.3
Sulfur	0.9	0.6
Oxygen (by difference)	20.1	13.9
Higher heating value (maf), Btu/lb	11,970	13,780
Bulk density, lb/ft ³	37	40
<hr/>		

Table 8.5. Analyses of solid samples from run AHC-3

	Coal	Char	Carryover	
			Piping	Condenser
Proximate analysis (as received), wt %				
Volatile matter	33.7	19.0	62.4	55.4
Fixed carbon (by difference)	34.1	69.9	25.0	32.3
Ash	5.3	9.8	3.0	4.5
Moisture	26.9	1.3	9.6	7.8
Ultimate analysis (maf), wt %				
Carbon	73.9	85.7	84.3	82.0
Hydrogen	5.3	3.3	6.9	6.8
Nitrogen	1.0	1.3	1.2	0.9
Sulfur	1.0	0.7	0.6	0.7
Oxygen (by difference)	18.9	9.1	7.0	9.6
Higher heating value (maf), Btu/lb	12,626	14,132	13,840	14,335
Benzene soluble (as received), wt %	27.5	0.3	57.4	43.8

Table 8.6. Analyses of solid and liquid samples from run AHC-4

	Coal	Char	Cyclone		Condensed liquid
			Cone	Dipleg	
Proximate analysis (as received), wt %					
Volatile matter	33.7	10.7	7.2	20.6	68.8
Fixed carbon (by difference)	34.2	76.7	79.3	63.4	27.6
Ash	5.4	11.0	11.9	9.8	3.6
Moisture	27.5	1.6	1.6	6.2	0
Ultimate analysis (maf), wt %					
Carbon	73.1	87.8	93.2	84.3	73.0
Hydrogen	6.5	2.5	2.0	3.3	7.0
Nitrogen	1.0	1.3	1.2	1.2	0.8
Sulfur	0.9	0.7	0.9	0.8	0.8
Oxygen (by difference)	19.5	7.7	2.8	10.3	18.4
Higher heating value (maf), Btu/lb	12,599	14,656	14,653	13,879	63.4
Benzene soluble (as received), wt %	28.4	< 1	< 1	4.8	63.4

Table 8.7. Mass spectrometric analyses of product gas from run AHC-1

	Sample number									
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Elapsed time, hr	1.2	1.7	2.2	2.8	3.1	3.7	4.2	4.7	5.2	5.7
Reaction temp, °F	700	700	850	1050	920	1030	960	1060	710	390
Volume %										
N ₂ + CO	95.6	91.8	85.0	92.5	93.6	96.2	96.7	96.3	95.6	77.0
H ₂	0.04	0.26	0.14	0.59	0.16	0.08	0.03	0.02	0.01	<0.01
CO ₂	1.66	2.79	0.73	1.59	0.42	0.20	0.14	0.15	0.12	0.13
CH ₄	0.02	1.12	0.16	1.66	0.27	0.11	0.03	0.02	0.01	0.01
C ₂ +	0.06	1.52	0.46	2.00	0.24	0.09	0.03	0.05	0.02	<0.01
H ₂ O	0.58	1.08	0.53	0.76	0.64	0.77	0.71	0.73	0.85	0.27
O ₂	1.52	1.32	12.6	0.79	4.42	2.49	2.21	2.57	3.15	21.7

Table 8.8. Analysis of product gas from run AHC-3

	Sample number								
	G1	G2	G3	G4	G5	G6	G7	G8	G9
Elapsed time, hr	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Reaction temp, °F	410	655	885	935	955	975	960	975	980
Volume %									
CH ₄	tr ^a	0.2	3.6	4.2	2.3	1.5	1.0	1.1	0.7
C ₂ 's	0	0.2	3.3	1.5	0.6	0.3	0.2	0.1	tr
C ₃ 's	0	0.2	2.8	0.9	0.3	0.1	tr	tr	tr
C ₃₊ 's	0	tr	0.9	0.2	0.1	tr	tr	tr	tr
H ₂	tr	0.1	1.7	4.3	3.3	2.7	2.1	2.3	1.9
CO	tr	tr	3.0	1.4	1.2	tr	0.7	0.2	0.2
CO ₂	1.0	2.6	5.7	3.6	2.0	1.3	1.0	0.9	0.7
H ₂ O	1.8	1.9	1.6	1.4	1.8	1.8	2.0	2.1	1.8
Ar	78.2	35.2	61.7	60.0	62.1	67.3	65.2	77.9	76.9
N ₂	14.9	47.0	13.1	18.3	20.9	19.9	21.8	12.3	14.0
O ₂	4.0	12.6	2.6	1.2	5.3	5.0	5.9	3.0	3.8

^atr < 0.1%

Table 8.9. Analysis of product gas from run AHC-4

	Sample number									
	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10
Elapsed time, hr	1.00	1.25	1.55	1.83	2.13	2.42	2.67	3.00	3.25	3.50
Reaction temp, °F	600	825	950	1000	1050	1100	1100	1150	1160	1200
Volume % ^a										
CH ₄	2.4	8.0	10.0	8.1	6.2	4.8	3.4	2.8	2.4	2.0
C ₂ 's	2.2	6.6	4.1	2.1	1.1	0.6	0.2	0.1	tr	tr
C ₃ 's	2.0	5.3	2.8	1.2	0.6	0.3	0.2	tr	tr	tr
C ₄ 's	0.4	0.8	0.4	0.2	tr	tr	0	0	0	0
C ₅ 's	0.1	0.2	0.1	0	tr	0	0	0	0	0
Benzene	tr	0.2	0.1	tr	tr	tr	tr	tr	tr	tr
Toluene	tr	tr	tr	tr	tr	tr	tr	tr	tr	tr
H ₂	0.7	3.2	7.0	8.9	7.8	7.9	8.2	8.8	9.1	9.2
CO	2.4	8.7	7.6	5.5	3.6	3.0	2.9	2.2	3.6	3.1
CO ₂	8.0	9.4	8.3	5.6	4.3	3.5	2.8	2.2	1.6	1.2
N ₂	8.7	3.1	1.9	0.9	0.7	1.2	0.6	1.1	0.6	0.7
O ₂	1.7	0.3	0.2	0.1	tr	tr	tr	0.1	tr	tr
H ₂ O	0.6	0.6	0.7	0.9	0.7	1.2	0.6	1.1	0.6	0.7
Ar	70.8	53.6	56.7	66.5	74.6	77.9	80.8	80.8	82.0	82.7

^atr < 0.1%.

Table 8.10. Carbon balances on runs AHC-1, AHC-3, and AHC-4

	Carbon (g)		
	AHC-1	AHC-3	AHC-4
Input			
Coal feed	948	1002	981
Output			
Solids			
Char	473	584	589
Condenser carryover	9	47	14
Piping carryover	<u>2</u>	<u>a</u>	<u>a</u>
Total	473	645	603
Condensate	14	22	35
Gas	<u>32</u>	<u>91</u>	<u>175</u>
Total Output	519	758	813
% Carbon recovery (output/input)	55	76	83

^aNot recovered.

8.2.2 Bench-scale system (H. D. Cochran, Jr., J. B. Gibson, and G. L. Yoder)

Design and fabrication of the bench-scale hydrocarbonization experiment have been completed; installation is nearly complete. The bench-scale hydrocarbonization experiment located in Building 2528 is an engineering test unit for converting coal into liquid, gas, and solid fuels. This experiment is designed to process coal at a nominal rate of 10 lb/hr at temperatures up to 1250°F and pressures up to 350 psig. Minimal facilities, consistent with the experimental objectives, are provided for coal pretreatment, gas handling, and product recovery. The focus is upon the hydrocarbonization reactor itself.

A simplified flowsheet of the experiment is shown in Fig. 8.10. Coal is fed from a pressurized feed hopper through a metering valve and to the reactor via a pneumatic transport line using a portion of the feed gas for transport. The bulk of the feed gas passes through a packed-bed preheater to the reactor. The solids level in the fluidized bed is set at one of three values by selecting the appropriate solids overflow line. In the reactor, the coal is pyrolyzed under hydrogen pressure to produce liquids, gas, and residual solid char. The liquids, as vapors and fine aerosols, are carried with the gas stream through an internal cyclone for solids removal, and then out the top of the reactor. The solids overflow to the char receiver where they are accumulated. The product stream from the reactor passes through a heated line to a scrubber in which liquids are condensed and the aerosol is knocked down. The cooled product stream then passes through a cold trap to condense the remaining

Fig. 8.10. Simplified flowsheet of bench-scale hydrocarbonization system.

liquids. System pressure is controlled by a throttling valve downstream of the cold trap. After pressure letdown, the gases pass through an absolute filter and are vented from a stack at a point 10 ft above the peak of the building.

This experiment is designed to permit the study of hydrocarbonization over a broad range of operating conditions. The composition of the feed gas will be: (1) pure nitrogen, (2) pure hydrogen, and (3) hydrogen/methane mixtures up to 50 vol % of methane.

The operating temperature will range from ambient (during initial shakedown tests) to 1200°F. Temperatures through the system may range as follows: preheater wall, $\leq 1400^{\circ}\text{F}$; reactor, $\leq 1250^{\circ}\text{F}$; scrubber, 100 to 800°F; cold trap, -110°F to 150°F; feed hopper, ambient to 500°F; char receiver, ambient to 1100°F; and the balance of the system, ambient to 500°F.

The entire system is designed for an operating pressure of 350 psig at maximum operating temperature. Pressures from atmospheric to 300 psig will be explored. There is a significant probability that pressures in excess of 300 psig may be explored later at reduced operating temperatures.

The coal feed rate will be varied from about 5 lb/hr up to maximum capacity, which is expected to be perhaps 40 to 50 lb/hr. A variety of coals may be tested, and a range of coal particle sizes may be employed. Initial experiments will be performed with Wyodak-seam coal with a nominal particle size of 100 mesh.

The feed gas rate (regardless of composition) will be limited to ≤ 70 scfm. About 2 to 4 scfm will be required for transport of

coal to the reactor. The balance will be fed through the preheater. Initial experiments will be conducted at far lower gas feed rates than 70 scfm. The effect of gas feed rate up to 70 scfm will be explored as part of this experiment.

Experiments will generally utilize a coal charge of about 100 lbs. Thus, at the planned feed rates, an experiment might last from 2 to 20 hr.

8.3 Supporting Research and Development on Separations Technology

B. R. Rodgers, S. Katz, P. R. Westmoreland, and R. C. Forrester, III

8.3.1 Solvent fractionation of Char Oil Energy Development (COED) and Solvent Refined Coal (SRC) unfiltered oil

Introduction. A number of solvents were considered for solvent fractionation and agglomeration experiments based on physical properties, chemical structure, and reported solvency for coal-derived liquids. Because of the complicated molecular mixtures that make up coal-derived liquids, there is no "best" solvent. The solvents in these experiments cover a broad range of solvency for coal liquids, from materials which cause an increase in solids content (precipitation), to materials which dissolve nearly all of the organics present in the coal mixtures (extraction). This difference suggests that it is possible to make molecular separations. Thus, if the fractions that contain undesirable contaminants such as sulfur, inorganics (ash), noble metals, and asphaltenes could be identified, they could be removed selectively to a heavy phase; this would leave a contaminant-free light phase. The heavy phase could then be separated by sedimentation, centrifugation, or filtration.

The experimental goals were to explore the possibility of selective precipitation-extraction as a separation technique, and to simultaneously judge the effect of the solvent on agglomeration and separation of the fractionated mixture. As a scouting tool, the experiments showed promise by concentrating sulfur and ash in the heavy phase. Settling and centrifugation downstream of the fractionator may be adequate to effect the desired separation; if filtration is necessary, it will be much easier after fractionation. High filtration rates can be achieved at low pressures (vacuum) and with virtually 100% separation of solids. An in-depth study, which would include minimizing the solvent required, evaluating the effect of temperature and pressure, evaluation of solvent recovery, and preliminary economic process evaluations, is necessary to demonstrate commercial feasibility.

Description of method. Eight grams of unfiltered oil (UFO) was slowly dissolved in 100 cc of solvent at room temperature. The COED-UFO mixture was stirred for 10 min, and the SRC-UFO mixture was stirred for 20 min. The mixtures were transferred immediately to either a settling tube, four 15-cc centrifuge tubes, or the Millipore Vacuum Filter Apparatus described in the ASTM Standards.⁵ Prior to sulfur and ash determinations, the bottoms materials were dried in an oven at 110°C to a constant weight. The separations scheme is shown in Fig. 8.11.

Fractionation-centrifugation of COED-UFO. Results of the centrifugations for COED-UFO are shown in Table 8.11. The solvents are ranked in relation to the amount of material they dissolved. Only small differences could be detected between some of the better solvents.

SOLVENT FRACTIONATION CRITERIA OF SUCCESS

$$\frac{S_H}{S_U} \times 100 = \text{PROPORTION OF SULFUR REMOVED BY CENTRIFUGATION}$$

$$\frac{A_S}{A_U} \times 100 = \text{PROPORTION OF ASH REMOVED BY FILTRATION}$$

$$\text{EFFICIENCY} = \frac{\text{PROPORTION OF CONTAMINANT REMOVED}}{\text{AMOUNT OF SOLIDS HANDLED}}$$

Fig. 8.11. Separations scheme using solvent fractionation.

A_U = ash in unfiltered oil; A_L = ash in heavy phase; A_F = ash in filtrate; A_S = ash in solids; S_U = sulfur in unfiltered oil;

S_L = sulfur in light phase; S_H = sulfur in heavy phase; S_F = sulfur in filtrate, S_S = sulfur in solids.

Table 8.11 Solvent extraction of COED unfiltered oil (UFO)
from Western Kentucky coal

Solvent	Solvency rank	Quantity dissolved (cc-heavy phase/g UFO)
Quinoline	1	0.1
Tetrahydrofuran	2	0.2
Pyridine	3	0.2
Acetone	4	0.2
Toluene	5	0.5
Xylene	6	0.8
Benzene	7	0.8

These data indicate that aromatics containing a nitrogen atom in the ring structure (quinoline, pyridine) and polar molecules containing oxygen (tetrahydrofuran, acetone) are excellent solvents.

Probably the most important consideration in the solvent treatment of coal-derived oils is the distribution of the sulfur and ash. The most desirable result would be a concentration of these materials in the heavy phase and with a minimum solvent holdup. Table 8.12 shows that sulfur and ash concentrate in the centrifuged bottoms (heavy phase) after solvent fractionations.

Fractionation-centrifugation of SRC unfiltered oil. The results of solvent fractionation followed by centrifugation of SRC-UFO are shown in Table 8.13. As with the COED fractionation-centrifugations, the aromatics produced a larger quantity of the heavy phase, and acetone gave an intermediate quantity. The bottoms fractions were significantly larger than in the COED case.

Table 8.12 Sulfur and ash in heavy phase of COED-UFO centrifuged solvent fractions

Solvent	Heavy phase ^a (vol % of mix)	Ash (wt % of HP)	Total sulfur (wt % of HP)	Inorganic sulfur ^b (wt % of HP)
Quinoline	0.3	2.7	2.7	1.5
Pyridine	1.3	14.2	4.3	2.4
Acetone	1.3	16.9	4.8	1.7
Benzene	4.4	11.0	3.7	2.3
Xylene	4.6	6.0	3.7	1.4
Toluene	6.0	6.8	3.6	1.5
UFO, as received		2.1	1.5	0.03

^aHeavy phase = HP.

^bBecause of the small sample size available, inorganic sulfur was estimated indirectly from the ash as FeS₂.

Table 8.13. Sulfur and ash in heavy phase of SRC-UFO
centrifuged solvent fractions

Solvent	Heavy phase ^a (vol % of mix)	Liquid in heavy phase (wt %)	Ash (wt % HP)	Total sulfur (wt % HP)	Iron (wt % HP)
Cresol	0.4	72	54.0	4.3	11.0
Pyridine	0.6	-	48.0	2.9	7.1
Tetrahydrofuran	1.7	-	35.4	3.4	5.5
Acetone	6.7	79	18.0	2.1	3.0
Benzene	9.3	83	9.0	1.4	1.6
Xylene	10.2	79	2.6	0.24	0.41
Toluene	11.0	79	4.0	0.61	0.70
SRC, as received			3.2	0.90	0.47

^aHeavy phase = HP.

The inorganic (ash) and sulfur content in the heavy phase after cresol and pyridine solvent fractionations are especially noteworthy. The aromatic solvents did not separate the ash and sulfur as well as in the COED case, even though they precipitated larger quantities of the heavy phase. Apparently, cresol is very selective in concentrating inorganic ash and sulfur in the heavy phase.

Filtration of solvent fractionated unfiltered oils. The mixtures described previously were filtered through a 0.45- μ Millipore filter for COED-UFO, or a 1.2- μ filter for SRC-UFO, using a standard vacuum procedure similar to the ASTM Standard.⁵ The longer filtration times required for the SRC-UFO resulted in the selection of a slightly more open filter membrane. Apparently, the 0.45- μ filter became plugged with SRC-UFO, but not with COED-UFO.

The results of the membrane filtrations, including analyses of the dried (110°C for 21 hrs) solids, are shown in Table 8.14. A basis for comparison of these results may be obtained by defining the solids concentration of COED-UFO as quinoline insolubles, and the solids collected via precoat filtration as the solids concentration of SRC-UFO. The aromatic solvents (benzene, xylene, and toluene) precipitated additional solids from the UFO of both processes, while cresol, pyridine, and tetrahydrofuran dissolved some of the solids. The aromatic solvents also generally produced greater liquid holdup in the filter cake. There was significant concentration of the contaminants (ash, sulfur, and iron) in the solids from the fractionations.

Table 8.15 shows the results of material balance calculations based on the analysis of solids given in Table 8.14. Since small

ANALYSES OF FILTRATION SOLIDS FROM SOLVENT FRACTIONATED
COED AND SRC OILS

SOLVENT	UNFILTERED OIL	DRY SOLIDS COLLECTED (WT % OF UFO)	LIQUID HOLDUP IN SOLIDS (WT %)	ANALYSIS OF DRY SOLIDS		
				ASH (WT %)	SULFUR (WT %)	IRON (WT %)
BENZENE	COED	14.1	64.6	15.0	4.6	3.1
QUINOLINE	COED	9.7	48.4	20.0	8.3	4.8
TETRAHYDROFURAN	COED	5.9	42.5	21.0	7.2	4.8
PYRIDINE	COED	6.0	62.2	21.0	7.2	4.9
CRESOL	COED	7.7	58.9	21.8	7.4	5.0
XYLENE	COED	14.2	74.4	13.5	4.5	3.3
TOLUENE	COED	13.6	63.0	13.6	4.4	2.8
TOLUENE	SRC	22.1	62.7	14.1	2.3	2.1
BENZENE	SRC	26.2	55.9	13.1	2.1	1.8
CRESOL	SRC	4.0	61.4	50.1	5.7	9.0
XYLENE	SRC	24.4	63.9	12.2	2.1	1.8
DODECANE	SRC	34.0	47.6			
NONE (PRECOAT FILTRATION AT 300°F)	SRC	6.8	35.8	30.6	4.3	4.0
UNFILTERED OIL	COED			2.1	1.5	
UNFILTERED OIL	SRC			3.2	0.9	0.5

Table 8.15. Results of material balance calculations on millipore filtrations

Solvent	Unfiltered oil (UFO)	Contaminant in solids (wt %)	Solids in UFO (wt %)	Contaminant in UFO by analysis (wt %)	Proportion of contaminant in solids (wt %)	Proportion of contaminant in filtrate by difference (wt %)	Contaminant in oil phase after solvent recovery (wt %)	Ratio of contaminants in solids to solids removed
Sulfur								
Benzene	COED	4.3	-14.1	1.49	44	56	0.84	3.1
Quinoline	COED	8.3	9.7	1.49	54	46	0.68	5.6
Tetrahydrofuran	COED	7.2	5.9	1.49	29	71	1.06 ^a	4.9
Pyridine	COED	7.2	6.0	1.49	29	71	1.06 ^a	4.8
Cresol	COED	7.4	7.7	1.49	38	62	0.92	4.9
Xylene	COED	4.5	14.2	1.49	43	57	0.85	3.0
Toluene	COED	4.4	13.6	1.49	40	60	0.89	2.9
Benzene	SRC	2.1	27.3	C.9	64	36	0.33	2.3
Toluene	SRC	2.3	23.7	C.9	60	40	0.36	2.5
Xylene	SRC	2.1	26.7	C.9	62	38	0.34	2.3
Cresol	SRC	5.7	5.8	C.9	37	63	0.57	6.4
None (precoat filtration of SRC at 100°C)		4.3	6.8	C.9	33	67	0.61	4.9
Ash								
Benzene	JOED	15.0	14.1	2.1	100	0	0	7.1
Quinoline	JOED	20.0	9.7	2.1	92	8	0.17	9.5
Tetrahydrofuran	JOED	21.0	5.9	2.1	59	41	0.82 ^a	10.0
Pyridine	JOED	21.0	6.0	2.1	60	40	0.80 ^a	10.0
Cresol	JOED	21.8	7.7	2.1	80	20	0.42 ^a	10.4
Xylene	JOED	13.5	14.2	2.1	91	9	0.19	6.4
Toluene	JOED	13.6	13.5	2.1	88	12	0.21	6.5
Benzene	SRC	13.1	26.2	3.2	107 (100)	0	0	3.8
Toluene	SRC	14.1	22.1	3.2	97	3	0.10	4.4
Xylene	SRC	20.1	4.0	3.2	63	37	1.18 ^a	15.8
None (precoat filtration of SRC at 100°C)		30.6	6.8	3.2	65	35	1.12 ^a	9.6

^aSignificantly exceeds maximum allowable limits as set by EPA.

quantities are involved, and material balances were based on both analytical measurements and experimental filtration measurements, compound errors may be expected. Efforts are currently underway to recover the solvent from the filtrate and to measure contaminant concentration in the remaining oil. However, the trends shown here are believed to be valid, and the more significant results will be verified on the bench-scale equipment under construction (described later in this report).

Table 8.15 also shows that the filtrates from the solvent fractionation separations compare favorably with the EPA sulfur and ash requirements (based on 16,000 Btu per pound of solid product). Quinoline appears to produce the most efficient separation for COED, and cresol produces the most efficient separation for SRC. However, lower contamination values, availability, price, and ease of solvent recovery would seem to favor the aromatic solvents.

Table 8.16 gives the vacuum filtration rates for membrane filtrations of solvent-treated COED-UFO and SRC-UFO. Reported pressurized (50 psi ΔP) rotary drum filtration rates of COED-UFO and SRC-UFO at 350 to 650°F are about 10 gal hr⁻¹ ft⁻². Our laboratory-scale precoat filtration rates for the untreated material at 210°F and 50 psi ΔP are shown for comparison.

These scouting experiments have shown that the solvent fractionation approach to separations is feasible. The filtration problems associated with current processes are reduced or eliminated, and filtrates are obtained which meet most EPA guidelines.

Table E.16 Vacuum filtration rates of solvent treated COED and SRC oils

Solvent	COED (gal hr ⁻¹ ft ⁻²)		SRC (gal hr ⁻¹ ft ⁻²)	
	Average	Highest	Average	Highest
Benzene	58	86	4	12
Toluene	53	70	9	32
Xylene	50	73	6	14
Cresol	13	16	7	14
SP ^a filtration at 100°C with untreated oil			1.8	2.3
SP + 5% body feed filtration at 100°C with untreated oil			4.2	9.1

^aSP = standard precoat.

8.3.2 Precoat filtrations

Introduction. The experimental data showed a strong dependence of filtration rate on temperature and pressure. The thickness of the precoat did not appear to be critical, but the filter cake thickness was important. One would also expect a dependence on the concentration, particle size, and particle shape of the solids, as well as the viscosity-temperature behavior of the mixture.

A number of models correlate filtration data, but the most widely used for cake filtration is that of flow through parallel capillaries, as developed by Carmen from Poiseuille's equation.^{6,7} This relationship, sometimes called the "Filtration Equation," will be used to correlate the data from the precoat filtration of COED and SRC oils. The two parameters of the equation will be evaluated for these two liquids, allowing prediction of behavior at other operating conditions and preliminary scale-up evaluations.

Theory. Precoat filtration proceeds by deposition of an open, porous precoat material, followed by filtration of a concentrated solids-liquid stream. Initially, solids penetrate into the precoat bed, but rapidly bridge pores to produce surface filtration by the filter cake of collected solids. Thus, cake filtration can be described as streamline flow through cake channels by the use of an analogy to Poiseuille's equation:⁸

$$\frac{dV}{Ad\theta} = \frac{\Delta P}{\mu[\alpha(W/A) + r]} , \quad (3)$$

where

V = volume of filtrate collected, gal

A = filter cake surface area, ft²

θ = time, hr

ΔP = pressure drop across cake and media, $\text{lb}_f/\text{in.}^2$

μ = viscosity of filtrate, cP

W = mass of accumulated solids (dry-cake basis), lb_m

α = average specific cake resistance, $\text{hr-ft}^4\text{-gal}^{-1}\text{-cP}^{-1}\text{-in.}^{-2}$

r = resistance per unit area of medium, $\text{hr-lb-ft}^2\text{-in.}^{-2}\text{-gal}^{-1}\text{-cP}^{-1}$.

(This includes all items which contribute to ΔP except the filter cake.)

The cake compressibility and particle size are related to the filtration rate through

$$\alpha = \alpha' (\Delta P)^s, \quad (4)$$

where

α' is a constant dependent on particle size, and s is the cake compressibility.

Integration of Eq. (3) at constant pressure drop gives:

$$\frac{\theta}{V/A} = k_p \frac{V}{A} + C, \quad (5)$$

where

$$k_p = \frac{\mu \alpha w}{2(\Delta P)},$$

$w = W/V$ (dry cake solids per volume of filtrate), and

$$C = \frac{\mu r}{\Delta P}.$$

To evaluate the filtration equation parameters (α and r), k_p and C were determined experimentally. From the solids collected during a standard precoat filtration, w was found to be 0.43 lb/gal. The following viscosity data, provided by Catalytic, Incorporated,⁹ were used:

$$\mu (210^\circ\text{F}) = 6.5 \text{ cP}; \mu (300^\circ\text{F}) = 4.4 \text{ cP}; \mu (390^\circ\text{F}) = 3.4 \text{ cP}.$$

It is recognized that these viscosity values are likely to be slightly high because of vapor losses during the viscosity measurements at these high temperatures. It is essential to obtain high-temperature--high-pressure viscosity data at process operating conditions, and experimentation in this area is planned during the coming year.

Results. Figure 8.12 shows filtration equation plots of SRC-UFO filtrations data at various temperatures and pressures. The values of α and r shown in Table 8.17 were calculated from these data; note that α depends on pressure (i.e., the cakes are compressible). Figure 8.13 shows a logarithmic plot to determine the compressibility factor in Eq. (4). The compressibility factor is 0 for incompressible cakes and 1 for highly compressible cakes (e.g., when the filtration rate is independent of the pressure drop across the cake). From the data shown in Fig. 8.13, the compressibility factor is calculated to be $s = 0.49$, and the particle size constant defined in Eq. (4) is found to be 2.5.

The data in Fig. 8.14 indicate that the bed is incompressible until a pressure near 30 psig is reached. After this, it becomes compressible to a pressure near 60 psig, and then is incompressible to the maximum pressure used in these experiments. These data suggest a definite advantage to using higher pressures (> 60 psig). This possibility will be explored further during future experimentation.

Figure 8.15 illustrates the rapid decrease in flow rate with cake thickness. It also shows that higher temperatures can be used to substantially increase the flow.

Fig. 8.12. The effect of temperature and pressure on standard precoat filtration, SRC-JFO.

Table 8.17. Evaluation of filtration equation parameters

$$\frac{\theta}{V/A} = \left(\frac{\mu \alpha w}{2 \Delta P} \right) \left(\frac{V}{A} \right) + \frac{\mu r}{\Delta P}$$

Condition		$\frac{\mu \alpha w}{2 \Delta P}$	α	$\frac{\mu r}{\Delta P}$	r
ΔP (psi)	T (°F)				
50	210	0.77	27.4	0.50	3.84
50	300	0.37	19.5	0.14	1.59
50	390	0.20	13.6	0.04	0.58
20	300	0.52	10.9	0.28	1.27
40	300	0.38	16.0	0.16	1.45
50	300	0.33	17.4	0.18	2.04
60	300	0.27	17.0	0.20	2.72
80	300	0.24	20.2	0.07	1.27

Calculations based on:

ρ (210°F) = 1.097, experimental measurement

ρ (300, 390°F) = 1.097, assumption

w = 0.432 lb/gal, experimental measurement

μ (210°F) = 6.5 cP, Wilsonville data

μ (300°F) = 4.4 cP, Wilsonville data

μ (390°F) = 3.4 cP, Wilsonville data

Fig. 8.13. Determination of compressibility.

STANDARD FILTRATION FLOW RATE ($\text{gal hr}^{-1}\text{ft}^{-2}$)

ORNL DWG 75-5305R4

Fig. 8.14. Pressure drop vs flow at 150°C.

ORNL DWG 75-5306R3

Fig. 8.15. The effect of cake thickness on flow rate.

Figure 8.16 compares low-pressure drop (~ 12 -psi vacuum), room-temperature filtrations from the solvent fractionations with the standard precoat filtration at 50 psig and 210°F , and precoat filtrations that use coal and diatomaceous earth (D. E.) as body feeds. The fractionated material has much higher filtration rates due to the viscosity and agglomerating effect of the solvents.

Coal as a precoat and body feed - a novel approach to filtration.

It was first suggested in the Phase I report¹⁰ that coal may be useful as a precoat. The initial experiment pursuing this possibility was reported in ref. 11, where coal precoat proved to be at least equivalent to the standard precoat (Johns-Manville "Fibre-Flo") at a differential pressure of 50 psi and 210°F . This encouraging result prompted further experimentation with coals of different particle sizes and at different temperatures. Illinois No. 6 coal was crushed and dry sieved into size distributions as follows: -80 +100 mesh, -100 +120 mesh, -120 +150 mesh, -150 +170 mesh, and -170 +200 mesh. This material was deposited on a 200-mesh screen as a precoat in a 5% by weight slurry before filtration of SRC-UFO. The results shown in Fig. 8.17 indicate that the coal precoat produced higher filtration rates than the Fibre-Flo standard.

Table 8.18 shows that the filtrate from these separations meets or exceeds the EPA stationary power source requirement of 0.96% sulfur and 0.15% ash for a 16,000-Btu solid product.¹² These results, coupled with the availability, cost savings, advantages from downstream processing, elimination of contamination, and elimination of disposal problems, make coal a definite contender for precoat applications. Since a range of different sized coals produced satisfactory results, conventional

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Fig. 8.16. Comparison of coal and diatomaceous earth as precoat and body feed for filtration of SRC and COED liquids.

ORNL-DWG 75-5310R3

Fig. 8.17. The effect of coal precoat particle size on filtration of SRC-UFO.

screens can be used as precoat supports. To obtain an estimate of the effect of temperature on the coal precoat bed, a filtration experiment was made at 390°F and 50 psi. This was followed by a continuous flow of filtered oil through the bed, while the temperature was raised slowly. The flow rate remained constant up to 525°F, at which point a sharp decline of flow rate was noted. Another sharp decline occurred at 560°F, which for all practical purposes plugged the bed. Thus, it is estimated that bituminous coal would have limited utility as a precoat at temperatures above 450°F. Anthracite or devolatilized coals could probably be used where higher temperatures are required.

Table 8.18. Analyses of filtrates from coal precoat filtrations of SRC-UFO

Coal precoat (mesh size)	Filtrate analyses	
	Ash (wt %)	Sulfur (wt %)
-100 +120	0.12	0.69
-120 +150	<0.05	0.61
-150 +170	<0.05	0.65
-170 +200	<0.05	0.66

Figure 8.18 contains preliminary data on body-feed experiments which show that Illinois No. 6 coal produces some improvement when used as a body feed. Further experimentation is planned in this area.

8.3.3 Characterization analyses of coal liquefaction process streams

Chemical and physical properties of COED and SRC unfiltered and filtered oils. Table 8.19 shows the results of physical and chemical property analyses of COED and SRC unfiltered and filtered oils, and includes a duplicate analysis of COED filtered oil as an indication of reproducibility. Nearly all of the inorganics (ash) are removed by

Fig. 8.18. Precoat filtration of SRC unfiltered oil, with body feed.

Table 8.19. Characterization analyses of COED oils from Western Kentucky coal and SRC oils from Illinois No. 6 coal

Property (wt % unless noted)	COED oil			SRC oil	
	Unfiltered	Filtered		Unfiltered	Filtered
		Run 1	Run 2		
Carbon	81.0	82.9	84.0	85.2	89.0
Hydrogen	7.2	7.8	7.8	6.5	7.5
Nitrogen	1.2	1.2	1.2	1.0	0.66
Oxygen			5.5	3.2	2.4
Sulfur	1.5	1.4	1.5	0.90	0.45
Iron		275 ppm		0.47	
Ash	2.1	< 0.05	< 0.05	3.2	< 0.05
Fixed carbon	15.6	10.1	13.2	16.8	3.4
Volatile matter	82.4	89.9	86.8	80.0	96.6
Solids concentration	8.2 ^a	nil	nil	6.8 ^b	nil
Density, g/cc	1.145 @ 20°C	1.109 @ 20°C		1.141 @ 20°C 1.10 @ 100°C	
Average molecular weight	266	290			
Heat content, Btu/lb			16,055	15,815	16,700

^aAs quinoline insoluble.

^bBy precoat filtration at 100°C.

filtration, but a large percentage of the sulfur remains. This indicates that a large percentage of the sulfur is found in organic molecules in the liquid phase; to remove additional sulfur, it will be necessary to precipitate asphaltenes. The experiments carried out previously indicate that most of the sulfur remaining in the liquid phase is precipitated with these asphaltenes.

Table 8.20 shows the results of ASTM distillation of SRC unfiltered and filtered oils.

Table 8.20. Distillation of SRC oils to 250°C

Distilled oils (wt %)	Temperature (°C)	
	SRC-UFO	SRC-FO
5	170	140
10	188	160
20	215	195
30	234	208
40	249	220
50		230
60		236
70		243
80		248

Fifty-six percent of the unfiltered oil distilled above 250°C (480°F) and 15% of the filtered oil distilled above 250°C. The solids removed have a much greater effect on the distilling quality of the oil than would be indicated on a simple weight percent basis.

Viscosity--temperature behavior of COED and SRC unfiltered oils.

Viscosity of COED unfiltered oil was measured over a temperature range of 58 to 145°C (136 to 293°F). The oil was produced from a Western

Kentucky coal, and had been stored in steel cans. A 1500-ml sample was analyzed with a Brookfield LVT viscometer, using various shear rates at each temperature. High and low temperatures were normally alternated.

The effect of temperature on liquid viscosity may be correlated to about 1 to 2% accuracy with the de Guzman-Andradl equation,⁸

$$\begin{aligned}\mu &= b \exp (a/T), \\ \text{or } \log \mu &= a/T + b,\end{aligned}\tag{6}$$

where

μ = viscosity,

T = absolute temperature,

a, b = correlation parameters.

Experimental data on the COED-UFO (see Fig. 8.19) approximately agree with this expression at temperatures less than about 100°C ($1/T > 2.7 \times 10^{-3}$). However, at higher temperatures, the experimental relationship deviates sharply from Eq. (6). Such deviation is expected from the complex mixture of organic compounds and is also exhibited in other published COED viscosity data.

Non-Newtonian characteristics of the oil were investigated by varying the shear rates at constant temperature. Table 8.21 gives examples of the viscosities found at different rotational speeds, but no trend with shear rate was observed. For comparison with other oils and with COED-filtered oil (COED-FO), the variations were ignored. A logarithmic mean of data points was used to produce Fig. 8.19.

Table 8.21. Effect of shear rate on COED-UFO viscosity

	Speed (rpm)	Viscosity (cP)
Spindle 3, 58°C	0.3	62,400
	0.6	60,000 - 70,600
	1.5	50,000 - 62,800
Spindle 1, 144°C	30.0	23.6 - 24.8
	60.0	25.3 - 26.6

Figure 8.20 shows the variation of viscosity of SRC-UFO with temperature, as determined by the standard Saybolt viscosity apparatus. This apparatus was not satisfactory for higher temperatures.

Particle size analyses. One of the most important properties of the unfiltered oils is the distribution of particle sizes. The small particles pack into very tight cakes and cause low filtration rates under process operations. One of the main objectives in the initial particle size determinations was to evaluate the applicability of available particle sizing techniques to colloidal particles from coal liquefaction streams. The H-Coal streams have proved to be among the most difficult to filter; thus, they provided a severe test. In this case, the well-dispersed particles were too small to be measured by either an optical microscope or a Coulter counter. In most cases, the electron microscope proved to be the only applicable instrument.

Figure 8.21 illustrates the difficulty involved in filtering and measuring H-Coal particles. A photomicrograph of the H-Coal particles

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Fig. 8.20. Temperature-viscosity behavior of SRC unfiltered oil.

15,532X

Fig. 8.21. Electron micrograph of H-Coal particles.

is shown at a magnification of 15,523X. Even at this high magnification many of the particles are barely resolvable. Figure 8.22 shows a number and volume percent particle size distribution as determined from the electron micrograph and a Zeiss counter. The colloidal nature of these solids is demonstrated by the fact that one-half of the total number was less than 0.11 μ , and one-half of the total sample volume was less than 0.98 μ .

If only optical techniques were used, it is obvious that considerable error might be involved in particle size determinations of materials similar to the H-Coal sample. As an indication of this error, the Zeiss counter was used to determine the particle size distribution of the 2000X photomicrograph shown in Fig. 8.23. The volume percent distribution was:

Diameter at 90%	8.59 μ
Diameter at 50%	4.16 μ
Diameter at 10%	0.86 μ

These values, when compared to those in Fig. 8.22, show that considerable error occurred because of poor resolution of the very small particles.

The particle size distribution in Figs. 8.24 and 8.25 show that the particles from the SRC process were much smaller than the ones from the COED process, a fact which is reflected in the high filtration rates obtained for COED-UFO throughout these experiments. The particle size effect would predict that H-Coal solids would be more difficult to filter than SRC solids, and that the Synthoil solids (Fig. 8.26) would filter similarly to SRC solids.

Fig. 8.22. Particle size distribution of H-Coal particles.

ORNL DWG 75-861

2000 X

Fig. 8.23. Optical photomicrograph of H-Coal particles.

Fig. 8.24. Particle size distribution of SRC wet filter cake.

Fig. 8.25. Particle size distribution of COED filter cake from PS coal.

Fig. 8.26. Particle size distribution of Synthoil filter cake
as measured by electron microscopy.

Particle size distribution for other coal liquids determined by electron microscope are summarized in Table 8.22. It is possible to compare directly diameters for which 90, 50, and 10 vol % of the particles are smaller.

8.4 Experimental Engineering Support of an In-Situ Gasification Process

R. C. Forrester, III

Early in FY 1975, ORNL was directed to survey several areas of interest to in-situ developmental programs in which it might make significant contributions. At that time, the decision was made to examine on-site surface processing options, including molten-salt scrubbing for sulfur removal, gas cleanup, and methanation. At mid-year, however, the ORNL program was redirected to address information gaps associated with two-dimensional chemical reactivity and fluid-flow patterns that will be encountered underground. The purpose of the existing project is the generation of engineering design data. This work is sponsored by the Division of Petroleum, Natural Gas, and In-Situ Technology of ERDA, whose program management is provided by Dr. Paul R. Wieber, Chief of In-Situ Coal Conversions.

The in-situ gasification of coal entails injection of oxygen or air with or without steam into a coal seam which has been fractured previously by explosives or by high-pressure liquid injection. The presence of injected oxygen permits ignition of the coal and subsequent maintenance of a combustion which moves through the coal bed; this produces an intermediate-Btu product gas which is piped to the surface. One such concept for in-situ gasification is shown in Fig. 8.27. In

COMPARISON OF PARTICLE SIZE DISTRIBUTION

SAMPLE	VOL % LESS THAN D		
	90	50	10
	DIAMETER OF PARTICLE, D		
COED PROCESS UNFILTERED OIL (WK COAL)	9.8	5.2	2.3
COED PROCESS FILTER CAKE (WK COAL)	4.1	2.9	1.2
COED PROCESS FILTER CAKE (PS COAL)	4.1	3.5	1.4
SRC PROCESS UNFILTERED OIL (I6 COAL)	1.6	0.99	0.25
SRC PROCESS DRY FILTER CAKE (I6 COAL)	4.0	3.1	0.96
SRC PROCESS WET FILTER CAKE (I6 COAL)	2.8	2.5	0.88
SRC PROCESS RECYCLE SOLIDS (I6 COAL)	2.0	1.2	0.40
SRC LAB FILTER CAKE (I6 COAL)	2.0	1.6	0.40
SRC LAB USED PRECOAT (FF)	4.0	2.5	0.99
SRC LAB FILTER CAKE (TOLUENE FRAC.) (I6)	2.0	0.88	0.22
SRC LAB FILTER CAKE (CRESOL FRAC.) (I6)	3.2	2.2	0.49
SYNTHOIL PROCESS FILTER CAKE (PS COAL)	3.8	2.6	0.70
H-COAL PROCESS FILTER CAKE (PS COAL)	1.7	0.98	0.34
ILLINOIS NO. 6 COAL (WILSONVILLE GRIND, 11/21/74)	2.7	2.0	1.0
ILLINOIS NO. 6 COAL (WILSONVILLE GRIND, 4/3/75)	3.8	2.2	0.68
PRECOAT - JOHNS-MANVILLE FIBRE-FLO	4.1	2.7	1.2
PRECOAT - JOHNS-MANVILLE SUPER-CEL	4.0	3.3	2.2
FIBERS - FROM JMFF PRECOAT (LENGTHWISE)	3.4	0.92	0.31

KEY: WK = WESTERN KENTUCKY COAL
 PS = PITTSBURG SEAM COAL
 I6 = ILLINOIS NO. 6 COAL
 FF = FIBRE-FLO PRECOAT
 PROCESS = SAMPLE FROM PROCESS
 LAB = SAMPLE FROM LAB EXPERIMENT
 COED = CHAR OIL ENERGY DEVELOPMENT
 SRC = SOLVENT REFINED COAL

Fig. 8.27. LLL in-situ gasification concept.

addition to the Lawrence Livermore Laboratory (LLL) proposed gasification technique shown in Fig. 8.27, ERDA is supporting development of alternative concepts at the Energy Research Centers at Laramie, Wyoming, and Morgantown, West Virginia.

8.4.1 Block pyrolysis studies (R. C. Forrester, III and P. R. Westmoreland)

As mentioned previously, in-situ gasification of coal involves the slow movement of a thermal reaction front through the coal seam. Coal ahead of the thermal wave experiences a slow heating rate under non-oxidizing conditions. Pyrolysis products raise the Btu value of the gas, and condensable liquids are continuously retorted in front of the wave, sometimes causing plugging. This slow pyrolysis is an important part of the in-situ process and affects efficiency and reaction zone control; thus, an important objective of this work has been to develop engineering data on coal pyrolysis and gas flow through coals experiencing simulated in-situ gasification conditions.

At ORNL, experimental simulation of the in-situ gasification process has begun with pyrolysis experiments that utilize large, instrumented blocks of subbituminous coal. Maximum reactor temperatures of approximately 1000°C have been achieved over a 4 to 6-hr time period. Figure 8.28 is a schematic representation of the block pyrolysis apparatus, showing provision for liquid product collection and gas analysis. Heat is supplied by a programmable furnace system capable of maintaining a constant rate of temperature increase of 1 to 10°C/min. The assembled apparatus and its associated control systems are shown in Fig. 8.29.

Heating rate data obtained during the first four tests are shown in Fig. 8.30. A straight line that represents the data, having a slope

Fig. 8.28. Block pyrolyzer schematic.

Fig. 8.29. Block pyrolyzer.

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Fig. 8.30. Heating rate for whole-block pyrolysis of western subbituminous coals.

of approximately $2.5^{\circ}\text{C}/\text{min}$, has been drawn through the points. Reactor temperature was controlled manually by periodically increasing the setpoint of an on-off controller. However, Fig. 8.30 shows that such a procedure is not wholly satisfactory, and a temperature programmer was thus designed and constructed to alleviate this problem.

The observed temperature of the pyrolysis reactor as a function of time for the latest experiments is shown in Fig. 8.31. Slopes of the curves became increasingly constant as experience permitted better tuning of the proportional integral derivative (PID) temperature controller. The heating rate can now be kept nearly constant at $3^{\circ}\text{C}/\text{min}$ between 350° and 1050°C ; however, this range will be increased with further testing. As stated previously, the apparatus is capable of maintaining heating rates of 1 to $10^{\circ}\text{C}/\text{min}$, and other rates will be examined later in the program.

Pyrolysis samples in the form of 6- by 6-in. right circular cylinders are prepared from large blocks of coal and are then instrumented with thermocouples. After positioning within the reactor, the blocks are pyrolyzed under an inert gas atmosphere. Nitrogen purge gas was used in early experiments (BP-1, -2, -3, and -9), but argon was used in all other experiments to simplify gas analysis. Typical thermal profiles produced by pyrolysis experiment No. 3 are shown in Fig. 8.32, and instrumentation for this experiment is shown in Fig. 8.33. Profiles from experiment No. 4 are shown in Fig. 8.34, and instrumentation is shown in Fig. 8.35.

The data presented in Fig. 8.32 describe two separate and distinct profiles. Although this could be the result of anisotropic reactivity

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Fig. 8.31. Programmed heating rates at 3°C/min (nominally).

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Fig. 8.32. Temperature profiles observed during whole-block pyrolysis test BP-3.

Fig. 8.33. Instrumentation details for pyrolysis test block No. 3.

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Fig. 8.34. Temperature profiles observed during whole-block pyrolysis test BP-4.

ORNL DWG 75-5180 R2

Fig. 8.35. Instrumentation details for pyrolysis test block No. 4.

effects, it probably results from inadequate centering of the cylinder within the confines of the reactor. Figure 8.33 shows the radial position of the block itself relative to the reactor walls; the thermocouples on the side of the block nearest the reactor wall measure the highest temperature profile.

To eliminate the problem of centering the block in the reactor, a basket was constructed that permits close alignment of the axes of both the block and the reactor. Figure 8.34 shows the profiles produced by the subsequent test; the spiral emplacement pattern for the thermocouples is shown in Fig. 8.35. An axial pattern was also included near the center of the block. Uneven burning effects are not seen in Fig. 8.34; all probes appear to describe a single thermal profile. In addition, axial probes at the top of the block measure temperatures that fall upon the radial profiles, whereas the bottom of the block does not appear to be heating. We conclude that pyrolysis of the block proceeds inward radially and downward axially, but not upward axially. Convective effects are probably responsible for this behavior.

Early experiments utilized radial, rather than spiral, patterns for thermocouple placement, but the close metal-to-coal spacing resulted in higher effective thermal conductivities near the thermowells. Results shown in Fig. 8.34 indicate the close correlation shown by two different profiles in a single block. Later tests have also demonstrated good reproducibility between experiments (Figs. 8.36 and 8.37).

The heating rate at various points within the block is shown in Fig. 8.38. These curves clearly show an initial period in which the entire block is heated rather uniformly to 100°C, followed by a final

Fig. 8.36. Temperature profiles observed during pyrolysis tests BP-8 and BP-9.

Fig. 8.37. Temperature profiles observed during pyrolysis tests BP-8 and BP-9.

Fig. 8.38. Typical thermal history for a block pyrolyzed at high temperature.

period in which heat transfer is rate-limited by vaporization of water. Temperature overshoot at the sample interior indicates that exothermic reactions dominate high-temperature pyrolysis. These results have been discussed extensively with Livermore researchers¹³ whose modeling efforts include a computer code which may be capable of using our thermal profiles for physical property and kinetic rate determinations.

Coal utilized in these pyrolysis studies came from the Roland and Smith seams at the Wyodak Resources Development Corporation mine near Gillette, Wyoming. Table 8.23 compares the results of our analyses of this coal with analyses performed by other investigators upon coals removed from the same seams. Our procedure for preserving the original condition of samples during storage includes: (1) sealing the sample blocks in plastic at the mine mouth; (2) submersion in water upon receipt at ORNL; and (3) performing all necessary drilling operations under water. The efficacy of these procedures seems to be verified by the agreement shown in Table 8.23.

In an effort to simplify the preparation of cylindrical pyrolysis specimens from coal, a visit was made to the Wyodak mine on April 15, 1975 to test use of a 6-in. coring bit to remove coal cylinders directly from the mine face. This would eliminate the machining step and its associated handling problems. Unfortunately, the shear strength of subbituminous coal is so low that material within the drill bit was completely pulverized. Even coal a foot or more from the cutting edge of the bit was reduced to small fragments.

Table 8.23. Analyses of coal taken from the Roland and Smith seams as received from Wyodak Resources Development Corporation, Gillette, Wyoming

	Wyodak analysis ^a	HRI analysis ^a	Livermore analysis ^b	ORNL Analyses (sample number)					
				2A	3A	3B	7A ^c	11A ^d	11B ^d
Moisture, wt %	21.7	26.2	20.4	27.0	27.9	27.9	29.3	31.9	29.6
Proximate analysis									
Dry, wt %									
Ash	7.9	8.2	-	5.1	4.4	5.2	5.4	5.5	5.7
Volatile matter	45.0	43.6	-	45.2	45.9	49.3	45.8	46.4	49.3
Fixed carbon	47.1	48.2	-	49.7	49.7	45.5	48.8	48.0	44.4
Ultimate analysis									
Dry, wt %									
Carbon	-	69.91	66.7	69.99	69.96	70.79	68.20	68.86	69.76
Hydrogen	-	4.29	5.14	4.47	4.48	5.24	4.68	5.22	5.65
Nitrogen	-	0.65	1.22	1.05	1.07	1.21	0.91	1.06	1.04
Sulfur	-	1.00	1.09	0.38	0.55	0.53	0.61	0.57	0.53
Oxygen (by difference)	-	-	18.35	19.05	19.49	17.06	20.39	19.04	17.60
Ash	-	8.20	7.5	5.06	4.46	5.17	5.15	5.24	5.42
Heating value, Btu/lb dry	8900	-	-	-	8500	9100	8400	8600	9200

^aP. M. Lantz, Process Data for Coal Liquefaction Processes. Part I, ORNL/TM-4789, (May 1975).

^bA. Pasternak et al., Preliminary Coal Gasification Experiments, UCID-16265, (May 1973).

^cSmith seam.

^dRoland seam.

Char samples taken from several of our recent experiments have been analyzed and found to contain 70 to 88% carbon, 0.6 to 4.9% hydrogen, and 9 to 27% ash. Heating values were about 12,000 Btu/lb. Precise analyses of some specific chars are given in Table 8.24. At the suggestion of Dr. Wieber, the reactivity of some of our chars was examined by Argonne National Laboratory as part of their char utilization studies. Surface area and porosity measurements were performed on two samples, one recovered from an experiment having a maximum temperature of 950°C, and the other from a 650°C test. Porosities of the two chars were nearly the same, but the surface areas were different by more than two orders of magnitude. Subsequently, char samples were recovered from various positions within a single test block, and similar determinations of porosity and surface area were made. Table 8.25 presents the results of those tests. No significant difference is observed in either porosity or surface area as a diameter of the char sample is traversed. The external heating rate for this experiment was 3°C/min, and interior heating rates were far smaller as a result of the low temperature maximum. In order to determine the effects of heating rate upon char porosity and/or surface area, detailed char analyses are planned for high-temperature experiments during which interior heating rates are quite large.

Pyrophoric behavior was exhibited by the char produced by one high-temperature experiment (BP-2). After cooling to 70°C, and upon exposure to air, the char self-ignited rapidly, and temperatures greater than 400°C were observed. Two experiments were performed in

Table 8.24. Composition of chars produced by large block pyrolysis experiments

Experiment	Purge gas	Maximum reactor temperature (°C)	Composition (wt %)						Heating value (Btu/lb)
			C	H	N	S	O	Ash	
Experiment No. 11: Center of block, top edge of block.	Ar	~500	70.17	4.88	1.07	0.87	11.28	11.71	14,100
Experiment No. 6: No oxidation.	Ar	~640	79.0	3.56	1.05	0.50	8.37	7.53	13,000
Experiment No. 2: (BP-2) Char was extremely pyrophoric and burned prior to analysis; residue analysis is shown.	Ar	~800	83.3	1.51	1.05	0.35	5.11	8.68	12,600
Experiment No. 8: Simulation of BP-2; average of two char samples.	Ar	~800	81.68	2.68	0.97	0.70	4.92	9.05	14,600
Experiment No. 9: Simulation of BP-2; average of two char samples.	N ₂	~800	78.34	2.97	1.00	0.56	7.69	9.45	14,100
Experiment No. 5: Negligible oxidation.	Ar	~800	87.8	1.13	0.72	0.33	2.52	7.48	13,300
Experiment No. 10.	Ar	~900	85.16	0.86	1.12	0.46	2.09	10.31	14,200
Experiment No. 3: Some oxidation was observed upon exposure to air, but reaction was not so energetic as in test No. 2.	N ₂	~950	70.9	0.62	0.48	0.32	0.88	26.8	10,200
Experiment No. 4: Negligible oxidation.	Ar	~950	76.6	0.60	0.37	0.40	0.87	21.05	11,250
Experiment No. 7.	Ar	~1000	86.60	0.57	0.34	0.64	0.34	11.52	14,400

an attempt to reproduce the conditions observed during the test. Coal blocks were heated at $3^{\circ}\text{C}/\text{min}$ by using argon in run BP-8 and nitrogen in run BP-9 as inert blanket gases. When the reactor temperature reached 800°C , the furnace power was shut off, simulating the abortion of BP-2 at 800°C . Chars were cooled gradually to room temperature and samples were recovered before exposure to air could take place. Subsequent exposure of the remaining chars to air resulted in no perceptible temperature increases. A sample of BP-8 char was placed in a water bath and heated in air, but for temperatures up to 95°C , no pyrophoric behavior was noted. Time has not permitted further exploration of this phenomenon.

Table 8.25. Porosity and surface area variations within the char block of pyrolysis experiment BP-11 (maximum center temperature approximately 340°C ; external temperature, 500°C)

Sample position (r/R_o)	Porosity ^a (%)	Surface area (m^2/gm)
1.0	7.6	1.2
0.6	10.0	0.6
0.3	6.9	1.3
0	7.2	3.4
0.3	7.9	1.8
0.6	7.0	1.8
1.0	8.6	1.0

^aDetermined by mercury porosimetry at 15,000 psi.

The overall distribution of solid, liquid, and gaseous products for some of the early pyrolysis experiments is tabulated in Table 8.26; typical variations of gas production rate and composition with elapsed time are shown in Fig. 8.39. Pertinent details of experiment BP-3, for which the gaseous product analysis is given in Fig. 8.39, include: (1) a maximum reactor temperature of 950°C, (2) an initial coal block weight of 3.973 kg, and (3) a nitrogen-gas purge stream. Insufficient data are available to permit generalizations regarding composition of product gases from block pyrolysis.

Table 8.26. Distribution of products produced by large block pyrolysis of western subbituminous coals

Experiment No.	Temperature (°C)	Product distribution (wt %)		
		Char	Liquid	Gas
6	650	50	37	13
2	800	27	53	20
5	800	38	37	25
3	950	43	28	29
4	950	39	28	33
7	1000	32	30	38

Quantitative and qualitative analyses of liquid products from the pyrolysis studies have been performed at ORNL by J. E. Mrochek as part of a program to develop methods for characterizing coal conversion process liquids. Contents of the pyrolyzer tar trap were allowed to separate into two phases: a nominally aqueous phase, and a tar. The tar was first extracted with cyclohexane, and the extract was contacted with nitromethane to concentrate polycyclic aromatics. Both the stripped

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Fig. 8.39. Evolution rate and composition of gases produced by whole block pyrolysis experiment BP-3.

cyclohexane and the nitromethane fractions were examined by gas chromatography, and major polycyclic aromatic constituents were identified. Figure 8.2, for example, shows the high resolution made possible by using advanced gas-liquid chromatographic techniques. Compounds shown include nitromethane-soluble aromatic hydrocarbons with boiling points as high as 550 to 600°C. Table 8.27 lists a few of these compounds and their boiling points.

Table 8.27. Compounds tentatively identified in the organic phase of tar-trap liquids recovered during whole-block pyrolysis studies

Compound identified	Boiling point (°C at 760 mm Hg)
Naphthalene	218
2-Methylnaphthalene	241
1-Methylnaphthalene	245
Biphenyl	255
Dimethylnaphthalene	268
Acenaphthalene	270
Dibenzofuran	285
Fluorene	297
Phenanthrene	340
3-Methylphenanthrene	351
Dimethylphenanthrene	363
Fluoranthene	393

8.4.2 Coal-derived aerosols: Disengagement techniques (C. J. Golden, P. R. Westmoreland, and R. C. Forrester, III)

Rapid cooling of condensible organic vapors produced by pyrolysis of coal leads to the formation of aerosols which are extremely difficult to separate from the product gas stream. As a result, some quick scouting

tests of traditional disengagement techniques were performed to improve the pyrolysis experiment and to provide recommendations for others in the ORNL Coal Technology Program who have aerosol disengagement problems. Qualitative evaluations of systems tested to date are shown in Table 8.28.

Table 8.28. Qualitative evaluation of some traditional disengagement techniques for trapping coal-derived aerosols

Technique	Evaluation and comments
Spray towers	Ineffective ^a
Bubble columns	Ineffective ^a
Packed towers	Very effective ^a
Cartridge-type string filters	Very effective
Electrostatic precipitation	Partially effective
Glass-wool demisters	Partially effective
Internal cooling coils and glass wool	Effective

^aThese were one-stage devices using water as the working fluid. Repeat tests using a low vapor pressure organic solvent (such as methylnaphthalene) are planned.

Figures 8.40 and 8.41 show the apparatus used in these tests and a few of the disengagement systems.

8.4.3 Underground Coal Gasification Symposium (P. R. Westmoreland and R. C. Forrester, III)

The Laramie Energy Research Center (LERC) sponsored a "First Annual Underground Coal Gasification Symposium" (UCG) at Laramie, Wyoming, July 28 to August 1, 1975. Topics covered included government policies, previous UCG experiences, current ERDA programs for in-situ gasification process development, laboratory support of UCG, mathematical modeling,

Fig. 8.40. Aerosol disengagement scoping studies: schematic diagram of apparatus.

Fig. 8.41. Aerosol trapping techniques evaluated by disengagement scoping studies.

economics, and other related projects. LERC's Hanna site, the only underground gasification field experiment operating in the United States, was toured as part of the symposium.

ORNL block pyrolysis research efforts were described to individuals through informal discussions. The results we achieved seemed to be of interest and of value to those involved in underground gasification development, and several requests for further information were received. Several field observations were noted which agreed with our laboratory observations of very steep temperature profiles. These steep profiles result from absorption of relatively large amounts of heat in vaporizing water trapped inside the coal block.

8.4.4 Evaluation of hazards posed by coal-derived liquids (R. C. Forrester, III)

Many processing techniques for the liquefaction or gasification of coal are being developed at ORNL and elsewhere. Although different in many other respects, all coal conversion processes produce a liquid effluent consisting of a complex mixture of hydrocarbons. Such mixtures invariably contain significant amounts of polynuclear aromatic compounds, some of which are known to be highly active carcinogens.

Earlier this year, Coal Technology Program staff members inspected facilities at the Union Carbide Corporation Technical Center, South Charleston, West Virginia, to obtain information regarding the control and safe handling of carcinogenic coal-derived liquids. As a result of the visit, the experimentalists working in the program became increasingly aware of the health hazard posed by these materials. The extent of this hazard has been evaluated, and a preliminary report has been issued to interested members of the ORNL staff.

This document was prepared primarily for use in the Experimental Engineering Section of the Chemical Technology Division, and is meant to serve only as a preliminary operating procedure. The underlying philosophy that has been adopted for the protection of personnel involved in experimental coal processing operations is defined, and procedures that have been instituted to govern the conduct of such experimental work and handling of associated coal-derived liquids are detailed.

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