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TECHNICAL PROGRESS REPORT

ON

FUNDAMENTAL STUDIES OF SEPARATION PROCESSES

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ABSTRACT

Good progress has been made in three different major areas. First, using high-precision gas chromatography, a mathematical technique, principal component analysis, has been shown to be a promising means for analyzing chromatographic peak shapes. In addition, a study of electrostatic effects on gas chromatograms obtained using liquid-crystal stationary phases appears to have uncovered a new type of diffusion-limited phenomenon. A projected study, using high-precision digital data, should serve to broaden the general understanding of diffusion phenomena and also that of the fine structure of liquid crystals. Second, studies of micellar and ion-exchange equilibria have been shown to be feasible, using gels as column packings, at pressures up to 50,000 psi. Third, using a relatively inexpensive quadrupole mass spectrometer as a detector for a gas chromatograph, enrichment of the C-13/C-12 ratio in carbon dioxide, after passage through a short column of silica, has been confirmed. Currently, the enrichment is being examined under different conditions in the hope of achieving a sufficient separation to allow analyses to be made without using a mass spectrometer.

INTRODUCTION

One year ago, we had just started the gigantic task of converting the on-line computer-controlled high-precision gas chromatograph from a Hewlett-Packard 2115 to a Digital Equipment Corporation PDP 11/20. The interfacing hardware, the assembly-language control routines, and the Fortran data-processing routines had to be redone completely. (Only BASIC was available for the 11/20 until recently.) Except for a few utility routines, such as writing alphanumerics using the plotter, all service programs have now been completed.

In addition, other major programs have been completed. First, programs for data acquisition and processing for the mass spectrometer permit the recording of either normalized mass spectra or intensities of selected masses as a function of time. The latter is used in our fractionation studies of carbon dioxide where changes in masses 44 ($^{12}\text{C}^{16}\text{O}_2$), 45 ($^{13}\text{C}^{16}\text{O}_2$), and 46 ($^{12}\text{C}^{16}\text{O}^{18}\text{O}$) are being followed as a function of time. Second, a new program has been written in BASIC for principal-component analyses (PCA) of chromatographic peaks. PCA has been shown to be quite sensitive to changes in peak shape so further applications are planned. Third, a modest amount of effort has been directed toward the writing of general utility programs and toward successful calibration of a flow transducer

at a level of better than 0.1% in accuracy and reproducibility. The recent improvement in reproducibility by a factor of four over our earlier work will permit substantially better studies of optimization to be made. Both the PCA work and the transducer calibration were undertaken jointly with an NSF-funded study because both programs needed the results.

As indicated in last year's report (C00-1222-50), three young faculty members from Wabash College, Kent State University, and Memphis State University worked part-time for two months last summer. Two of them already had experience in interfacing and programming small computers while the third had a background in Fortran. Hence, they provided much-needed help to us in both hardware and software while gaining the experience they desired with a new generation computer and with our special chromatographic capabilities.

The topics in the present report will follow the order in last year's proposal. In that way, it should be easier for the reader to compare our plans with our accomplishments.

HIGH-PRECISION GAS CHROMATOGRAPHY

In the past, our high-precision gas-chromatographic data have been obtained in two ways. In one, a digital controller

and a voltage-to-frequency converter (for digitizing the data) were combined with a paper-tape punch to obtain data in a form that could be processed by an off-line computer. The second method employed an on-line 2115 Hewlett-Packard computer, loaned by Professor Perone, for data acquisition and processing. In May 1971, the changeover in software and interface hardware to the PDP 11/20 of Digital Equipment Corporation began. Meanwhile use of the off-line equipment continued, but the PDP-11/20 was employed for data processing. The 11/20 is now on-line.

The Van Deemter plot of the Height Equivalent to a Theoretical Plate vs the linear flow rate of the carrier fluid is widely used to report changes in column efficiency. Usually, the HETP passes through a minimum at slow-to-moderate flow rates, and the steepness of the rise beyond the minimum increases with molecular weight in a homologous series. In a study of the resolution of pairs of n-alkanes, we discovered that, at a fixed temperature, the steepness of the slope passed through a maximum (C00-1222-54). Using an entirely different apparatus, we have confirmed the earlier finding. There is no question that slow diffusion is the source of the slope reversal. However, from a study of the changes with flow rate of the peak-tailing and retention volume, we should be able to determine whether the deviations from ideal behavior get larger at faster flow rates (1, 2) or at slower flow rates

(3, 4). Further study of the diffusion effect (as well as the proposed automated optimization program) was postponed until very recently so that the flow transducer could be calibrated.

In the past, the accuracy and reproducibility of the flow measurement have severely limited our ability to repeat measurements at different times and to compare results obtained at different flow rates. While results at a given flow rate could be reproduced to better than 0.1%, the flowrate could, at best, be measured with a standard deviation of about 0.4%.

We have now succeeded in calibrating a commercially available flow meter, based upon thermal conductance, against a soap bubble meter to better than 0.1%. This was accomplished by placing not only the flow controller but also the bubble meter (and all of the connecting gas lines) in a large cabinet thermostated to about 0.1 C°. In addition, except for reading the bubble meter, all timing was done by the computer. We had expected to have to resort to photoelectric or electrical detection of the bubble, rather than visual, but that proved to be unnecessary. As a result, we have resumed the diffusion study mentioned above and will follow it with the optimization study. Later, the proposed characterization of one or more pure polymeric phases will be started. These could serve as much-needed reference compounds for laboratory-to-laboratory comparisons of gas chromatographic data (5).

Soon after delivery of the new computer, a program was written for exploring the capabilities of principal-component analysis (PCA) for characterizing peak shapes (C00-1222-53). Changes in shape with sample size and length of the hydrocarbon chain of an n-alkane were found to require a maximum of two vectors, after normalization of the peak heights and retention times. We believe that PCA represents a promising supplement to moment analysis. Furthermore, it appears to be less sensitive to noise than the higher moments. For that reason, PCA and moments will both be used in our on-line studies so that comparisons can be made under a variety of conditions.

HIGH-PRESSURE CHROMATOGRAPHY

Liquid. The original plan had been to make a study of the effects of pressure on the adsorptions of systematic series of organic compounds by silica gel at pressures up to 50,000 psi. However, it later seemed wiser to evaluate in a broad way the capability of the apparatus for modifying different types of equilibria. By demonstrating the feasibility of using swollen gels, rather than rigid solids, as column packings, steric-exclusion studies of conformational changes using the customary polydextrans and polyacrylamide, has been opened up to biochemists. In addition, selectivity changes of conventional

ion-exchange resins can now be examined.

In the first study, aqueous solutions of sodium dodecyl sulfate (SDS), a typical micelle-forming compound, were studied at different pressures. Progress was slow at first because two different suppliers of "greater than 95% pure" and "99% pure" SDS actually had products which we found contained close to 30% impurities, mostly C_{14} and C_{16} sulfates. Those impurities were confirmed by hydrolysis of the sulfates to form alcohols, followed by gas-chromatographic comparisons with known alcohols.

Finally, a pure preparation of SDS was located. Both G-10 and G-25 Sephadex (highly cross-linked dextrans) operated at 20,000 psi without showing any change from the high or low exclusion limits measured at close to atmospheric pressure using Carbowax 20M, a polyethylene glycol, and ethylene glycol itself. However, at 50,000 psi, the beds appeared to compress even though the pressure-drop across each column was maintained the same as in the runs at atmospheric pressure by using a valve at the end of the column to maintain the same flow rate of eluent. Later, using Corning CPA-10-240, a rigid porous silica, the micelles eluted at the same volume over the entire range of 10,000 - 50,000 psi. Hence, the size of the micelles appears to have been unaffected by pressure.

Preliminary experiments using an anion exchanger, Bio-Rad AG1-X4, have indicated that Br^- exchange with NO_3^- is, as expected,

rather insensitive to pressure. (On the other hand, we expect bromide complexes of metal ions to be shifted by high pressures.) Unfortunately, the background signal^{at 50,000 psi} for the refractive index detector was so high and variable that the bromide in the element had to be located visually using silver ion to form a precipitate. After 24 hours of use, bed "compression" appeared to have occurred. However, we now suspect that the observed "compression" was actually loss of resin by dissolution or peptization, for both the anion resin and the Sephadex. The question of solubility is now being checked. In case that proves to be the reason for the decrease of column height, anion resins of higher cross-linking are available for testing. Hopefully, they will be sufficiently less soluble to permit valid data to be obtained.

Gas. Gas chromatography, at pressures near (usually above) the critical point, has been shown by several groups of investigators to be a promising approach for separations of large molecules. Our studies have two long-term goals:

- (a) To explore the effects of operating variables on different types of polymers, especially those that appear to be useful as reference phases for conventional gas-liquid chromatography;
- (b) To evaluate and minimize the effect of decompression on the measured column efficiency.

The experiments of Sie et al (6, 7) on the squalane-propane system using high-pressure CO₂ as the carrier have

been successfully duplicated. In addition, using CO_2 at 1800 psi and a silicone oil, SE-30, exploratory experiments have shown that ethylene glycol can be isolated in a very short time from Carbowax 200. Hence, the apparatus is functioning well and a separation of higher polyethylene glycols looks promising.

The recent work of Jentoft and Gouw (8) has already demonstrated the power of supercritical-fluid chromatography for separations of polymers and other high-molecular-weight species. Thus, it appears to be a much more powerful technique than steric-exclusion chromatography for separating such species, providing that they are stable to solvolysis and decomposition at the temperatures in question. (Chelates, as demonstrated by the results of Klesper, Corwin and Turner (12) offer many interesting possibilities.)

A system with which we plan to work is the phenyl-methyl silicones. These silicone phases are widely used in conventional gas chromatography, so the availability of one or more pure species would be highly advantageous. In addition, because the phenyl derivatives absorb strongly in the ultraviolet, another goal can be accomplished by those same experiments. That goal is to examine effects of depressurization on the separation. Therefore, we have designed and built an ultraviolet-transmitting cell for a detection system that can be used at column pressures. Column efficiency can be

determined both before and after depressurization so as to evaluate both the hardware and the techniques for depressurization. Complete (9) and partial (9) depressurization has been employed with CO_2 , but no comparisons of efficiency losses have been found in the literature.

Another variation has also been explored briefly. The supercritical-fluid chromatograph has also been tested in a form used by Phifer and plummer (10) close to atmospheric pressure. Using moist nitrogen at high pressures, considerably enhanced volatility of the dimethoxy ethers of the polyethylene glycols was obtained. Although we decided to drop this particular study after a paper appeared on steam chromatography (11), our apparatus can be adapted relatively easily for mixing any two phases in known amounts. Hence, the possibility exists for studies that would involve quickly changing the composition of the eluent so as to modify the elution behavior.

ISOTOPIC SEPARATIONS

As stated in last year's proposal, our long-term goal is to employ stable isotopes, usually in their natural abundances, for chromatographic studies of physical and chemical interactions. In the course of these studies, optimal conditions will be sought

for the interactions that look most promising for analytical and/or preparative purposes. At present, the carbon (and oxygen) isotopes in carbon dioxide are being studied after contact with different adsorbents. The ^{13}C - ^{12}C separation should be useful for ^{13}C tracer studies involving humans. It would be particularly useful if the separations were large enough that a mass (or infrared) spectrometer would not be needed for the analyses.

During the past year, two quadrupole mass filters were tested extensively, especially for reproducibility. By early May, acceptance tests had been completed on our own instrument, a Model C quadrupole system from UTHE Technology Inc.

In our first attempt, the enrichment of $^{13}\text{CO}_2$ in the leading edge of a peak eluted from a silica column was confirmed (13). After passage through a 12-foot column of silica at 50°C , the peak for mass 44 was two seconds later (out of a total of 10 minutes) than the peak for mass 45. On a 26-foot column at room temperature, an 8-sec difference was found in a total of 30 minutes. (As yet, the smaller separation reported for ^{18}O has not been detected.) Currently, the separation by silica is being examined under a variety of conditions using packed columns at room temperature and above, so as to determine the optimal conditions for the separation.

Future plans include the assembly of a simple sub-ambient chromatograph as well as the testing of other stationary phases.

Once the approximate optimal conditions have been found, long packed (or coated) capillary columns will be employed in an attempt to obtain resolved peaks for masses 44 and 45. In addition, a brief comparison with carbon monoxide may be made (14).

Prior to the acquisition of a mass spectrometer, a more nearly classical study of isotopic effects on separations was carried out on deuterated toluenes (C00-1222-52). Using aqueous silver nitrate as the stationary phase, deuterium in the methyl group was found to have too small an effect to be detected. However, each deuterium on the aromatic ring changed the distribution ratio almost exactly 1%. The latter value was also found in a brief study of benzene itself.

ELECTROSTATICALLY-CHARGED COLUMNS

In an earlier report (C00-1222-47), changes in retention behavior were shown for a variety of volatile solutes in contact with a liquid-crystal stationary phase when an electrostatic field was imposed. Extension of that study to a number of cholesteryl esters of n-alkanoic acids between C_3 and C_{14} have uncovered some unusual findings: (a) The change in retention time with applied potential is relatively larger for the shorter retention times, (b) is greater for faster

flow rates, and (c) is in the direction of the limiting maximal retention volume attained at very slow flow rates. Furthermore, starting with the propionoate, the changes go through a maximum before reaching a minimum at nonoate; then, they pass through another maximum at undecanoate. Furthermore, the changes in retention volume with flow rate are characteristic of a diffusion-limited process. Hence, the undecanoate, which exhibited the largest changes, and the nonoate, which occasionally showed small changes in the opposite direction, will be examined in detail using the high-precision apparatus. If time permits, a brief examination will be made of another series of liquid crystals in the hope of shedding light on the structural factors that influence the magnitude of the diffusion phenomenon.

FINAL REMARKS

The computer system provided through an NSF grant made it possible to confirm easily and quickly the fractionation of the carbon isotopes. Similarly, the real-time BASIC developed for the NSF study, made it possible to write data-acquisition and plotting programs for the mass spectrometer in a matter of hours. Using those programs, a series of mass

spectra can be obtained from which chromatograms for individual masses can be calculated and plotted.

As rank amateurs in the realm of vacuum systems and mass spectrometry, we received considerable help on many occasions from Messrs. J. W. Amy, W. E. Baitinger and R. M. Caprioli in the Departmental Mass Spectroscopy Center. In addition, Mr. Klaus Ernest of Lawrence Livermore Laboratory was extremely helpful in discussing specifications and performance tests for the mass spectrometer. In addition, he provided data on the performance of one of his systems to illustrate how the tests might best be made.

The Principal Investigator has devoted, and will continue to do so, at least 20 percent of his time to this contract.

PUBLICATIONS AND MANUSCRIPTS

Publications

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2. T. A. Maldacker and L. B. Rogers, "Effect of Loading on Separation Efficiency Using Steric Exclusion Chromatography", Separation Sci. 6, 747 (1971).
3. P. J. Taylor, R. A. Culp, C. H. Lochmüller, L. B. Rogers and E. M. Barrall, II, "Effect of an Electric Field on Gas Chromatographic Retentions by Liquid Crystals", Separation Sci. 6, 841 (1971).
4. L. J. Lorenz and L. B. Rogers, "Specification of Gas Chromatographic Behavior Using Kovats Indices and Rohrschneider Constants". Anal. Chem., 43, 1593 (1971).
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6. B. A. Bidlingmeyer and L. B. Rogers, "Investigation of Pressure-Induced Changes in the Chromatographic Selectivity of Methyl and Ethyl Orange on Silica Gel", Separation Sci., 7, 131 (1972).

Manuscripts

1. C00-1222-52, D. Macnaughtan, Jr. and L. B. Rogers, "Effect of Position of Substitution on the Separation Behavior of Deuterated Toluene". (Accepted by Separation Science).
2. C00-1222-53. D. Macnaughtan, Jr., L. B. Rogers and Grant Wernimont, "Principal-Component Analysis Applied to Chromatographic Data" (Accepted by Analytical Chemistry).
3. C00-1222-54, R. S. Swingle and L. B. Rogers, "Predicting Gas-Chromatographic Resolution for Pairs of n-Alkane Homologs (Accepted by Analytical Chemistry).

PERSONNEL

July 1, 1971 to June 30, 1972

Senior Staff

L. B. Rogers, Principal Investigator, 20% Academic Year.
P. J. Taylor, Postdoctoral Appointment, 100% Entire Year.
R. A. Culp, Jr., Postdoctoral Appointment (50% Summer 1971).
G. Higgins, Postdoctoral Appointment (50% Summer 1971).
J. Zimmerman, Postdoctoral Appointment (40% Summer 1971).

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D. L. Kemp
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K. A. Mueller
J. A. Nieman
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