Pacific Northwest National Laboratory

Operated by Battelle for the
U.S. Department of Energy

Science to Support DOE Site Cleanup: The Pacific Northwest National Laboratory Environmental Management Science Program Awards

Fiscal Year 1997 Mid-Year Progress Report

June 1997

Prepared for the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

PNNL-11589

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor Battelle Memorial Institute, nor any of their employees, makes **any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights.** Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or Battelle Memorial Institute. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

PACIFIC NORTHWEST NATIONAL LABORATORY
operated by
BATTELLE
for the
UNITED STATES DEPARTMENT OF ENERGY
under Contract DE-AC06-76RLO 1830

This document was printed on recycled paper.

Science to Support DOE Site Cleanup: The Pacific Northwest National Laboratory Environmental Management Science Program Awards

Pacific Northwest National Laboratory
June 1, 1997

The Pacific Northwest National Laboratory was awarded ten (10) Environmental Management Science Program (EMSP) research grants in Fiscal Year 1996. This section gives a summary of how each grant is addressing significant DOE cleanup issues, including those at the Hanford Site. The technical progress made to date in each of these research projects is addressed in more detail in the individual progress reports contained in this document.

This research is primarily focused in three areas-Tank Waste Remediation, Soil and Groundwater Cleanup, and Health Effects.

Tank Waste Remediation

Over three-hundred underground storage tanks containing about 350,000 cubic meters (92 million gallons) of mixed chemical and radioactive waste are located at the Hanford Site (Washington), Oak Ridge Reservation (Tennessee), Savannah River Site (South Carolina), Idaho National Engineering and Environmental Laboratory (Idaho), and West Valley Site (New York). The liquids, sludges, saltcake, and calcined solids (at Idaho) in these tanks contain about 70% (720 million curies) of the man-made radioactivity existing in the DOE complex. Remediation of this tank waste is one of the most technically complex, scientifically challenging, and potentially expensive problems facing DOE.

Hanford contains 60% of the waste volume and 30% of the radioactivity for all DOE high-level waste tanks. At Hanford there are 177 underground storage tanks containing 210,000 cubic meters (55 million gallons) of high-level waste. The current baseline approach to remediating this tank waste involves retrieving the waste followed by a solid-liquid separation. The liquid, low-activity stream will have cesium (Cs) and other radionuclides removed and then be immobilized as low-level radioactive and chemical waste. The solid, high-activity stream will be pretreated to reduce its volume (mainly through the removal of non-radioactive inorganic components such as chromium, phosphorus, and aluminum) and then immobilized as high-level radioactive waste.

The presence of sub-micron, colloidal particles in the waste complicates nearly every aspect of waste processing. They make the separation of solid and liquid waste streams difficult by forming colloidal suspensions, which may take months to settle, if at all. These suspensions can also form sediments or gels, clogging pipes and interrupting waste transfer operations.

Colloidal particles can also contaminate the liquid waste, complicating downstream processing. Radionuclides can adsorb to the particles' surface and radioactively contaminate the low-level liquid waste stream, forcing it to be treated as high-activity waste, thereby increasing treatment costs. In other cases, the presence of colloidal particles interferes with separations processes; for example, plugging ion exchange columns causing schedule delays and increased costs.

The EMSP project **Colloidal Agglomerates in Tank Sludge: Impact on Waste Processing** (Page 1) is conducting experiments to understand and identify the chemical conditions which control the formation and agglomeration of colloidal particles. In this way, the impact that agglomerate structures have on the rheology and sedimentation properties of the waste can be quantified, and methods for manipulating agglomerate structures to optimize tank waste transfer and processing conditions can be developed. It is important to note that the new knowledge and data generated by this project will also be of benefit to the cleanup of N-Basin liquids at Hanford, and of the tank wastes at the Oak Ridge Reservation and the Savannah River Site.

The removal of radionuclides to prepare the liquid waste stream for immobilization as low-level waste also presents a series of challenges. The removal of cesium appears to be straightforward, with technologies capable of meeting the performance criteria. In some tanks, however, there may be a need to remove the lanthanides and actinides (because of their long half-lives of 10^2 - 10^6 years) and/or Sr, which has been complexed by organic complexing agents present in the waste.

Unfortunately, there are few technologies for the selective removal of the *f*-block metals (lanthanides and actinides). The EMSP project **Architectural Design Criteria for *f*-Block Metal Sequestering Agents** (Page 15) is using computational and experimental methods to optimize ligand architecture for *f*-block metal recognition. By developing an accurate set of criteria for ligand architecture design, ligand structures can be targeted for synthesis, dramatically reducing the time and cost associated with metal-specific ligand development. This provides cleanup projects with more cost-effective and efficient separations agents that can reduce schedules and save money, especially the costs associated with the regeneration of separations materials and/or the disposal of spent separations materials.

Turning to the issue of Sr, one way to remove complexed Sr is to first destroy the organic complexants. The EMSP project **Ionizing Radiation Induced Catalysis on Metal Oxide Particles** (Page 23) is developing a novel approach for organic destruction utilizing the redox chemistry resulting from the radiation already present in the tanks. This approach has the advantages of being non-thermal and emission-free. Moreover, it is applicable to any organic-containing waste stream, and can also make use of the currently unused Cs and Sr capsules at Hanford as radiation sources.

This chemistry, which is initiated by radiation in the wastes, can also cause tank farm operations and waste storage problems. These processes produce flammable gases, degrade organics, change organic fuel and oxidant concentrations, and alter the surface chemistry of insoluble colloids influencing sedimentation and gas/solid interactions. The EMSP project

Interfacial Radiolysis Effects in Tank Waste Speciation (Page 29) is focused on understanding the radiolytic processes in these mixed-phase systems to determine the radiation-induced physical and chemical changes occurring in the wastes, which impact tank safety and waste storage and processing.

Current strategies for reducing the volume of the solid, high-activity waste stream involve the development of methods to selectively dissolve and remove nonradioactive elements such as aluminum, phosphorus, and chromium while retaining the radioactive elements in the sludges. This partitioning is fundamentally dependent upon the chemical speciation of the elements present in the processing solutions. The EMSP project **Chemical Speciation of Strontium, Americium, and Curium in High Level Waste: Predictive Modeling of Phase Partitioning During Tank Processing** (Page 37) addresses the problem of trivalent actinide (americium/cesium) and Sr speciation and solubility in tank liquids. The resulting data will be used to develop thermodynamic models to predict changes in chemical speciation and solubility as a result of changes in tank processing conditions. In this way, existing processes can be optimized and new processes can be developed.

Once the high-activity stream has been reduced in volume, the remaining waste, with its concentrated radionuclides, will be immobilized. The impact of internal radiation on the long-term performance of these immobilized forms is unclear. And given the time frames involved (*ca.* 10,000 years) the data will not be gathered from simple experimentation. The EMSP project **Radiation Effects in Nuclear Waste Materials** (Page 43) is focused on developing a fundamental understanding of radiation effects in glass and ceramic waste forms. This understanding and associated data will improve models assessing the long-term performance of these waste forms. It is also important to note that these studies will benefit the assessment of waste forms proposed for the immobilization and disposal of plutonium residues and scrap, and excess weapons plutonium.

Soil and Groundwater Cleanup

Contaminant plumes, contaminated soils, and landfills account for some 4,800 waste sites on DOE property located in 31 states (DOE 1995). Approximately 35 million cubic meters of soil are contaminated with low-level and mixed low-level waste (ORNL 1994). Another 1.2 million cubic meters of soil is contaminated with transuranic and mixed transuranic waste. Landfills of buried waste are estimated to contain 3 million cubic meters of low-level waste. This includes 105,000 cubic meters of retrievably stored transuranic waste. Several hundred square miles of groundwater are contaminated with a variety of radionuclides and hazardous chemicals in concentrations above drinking water standards and DOE's concentration guidelines.

Nearly 150 square miles of groundwater contamination underlies Hanford. The radioactive and hazardous wastes are dispersed through large volumes of soil and groundwater making potential treatment by conventional soil excavation and groundwater cleanup technologies ineffective and costly. In addition, some 650,000 metric tons of solid waste is buried at Hanford.

The current baseline approach for treating contaminated groundwaters is to pump out the groundwater, treat it *ex-situ* and then return it to the subsurface ("pump and treat"). For contaminated soils the plan is simple: dig it up and dispose in a controlled disposal unit. At Hanford, that unit is the Environmental Restoration Disposal Facility (ERDF).

ERDF, centrally located on the 200 Area plateau, is receiving contaminated soils and solid waste from the 100 Area. Currently, there are two cells, each with a capacity of approximately 1.2 million cubic yards, in operation, with the potential for 8 new cells depending upon future needs. These cells, classified as RCRA (Resource Conservation and Recovery Act) landfills, have a design life of approximately 20-30 years, which may be extended through the use of caps and/or engineered barriers.

While *ex-situ* options may be available and effective over the short term, they are more expensive, labor intensive, and have a greater potential for worker and environmental risk than *in-situ* methods. Consequently there are many drivers for the development of *in-situ* treatment, monitoring, and characterization solutions.

One class of *in-situ* treatment approaches holding great promise are processes that use biological organisms to effect the cleanup (bioremediation). The EMSP project **Dynamics of Coupled Contaminant and Microbial Transport in Heterogeneous Porous Media** (Page 53) is providing fundamental data on the attachment/detachment dynamics of anaerobic bacteria in porous media under growth and growth-limiting conditions. This understanding of coupled contaminant and bacterial transport in actual subsurface media is critical to the design, evaluation, and success of any bioremediation or biobarrier strategy.

Another critical factor controlling the success of bioremediation efforts is the response of the subsurface microbial community and individual bacteria to the stresses encountered at contaminated sites. These stresses include low nutrient concentrations, low oxygen concentrations, and the presence of pollutants. Defining bacterial responses to these stresses presents an opportunity for improving bioremediation strategies, both with indigenous populations and genetically engineered microbes, and will reduce the cost and time required to achieve cleanup goals.

The EMSP project **Genetic Analysis of Stress Responses in Soil Bacteria for Enhanced Bioremediation of Mixed Contaminants** (Page 61) is focused on identifying the stress-inducible genes from soil bacteria with unique degradative capabilities, and then demonstrating how the manipulation and expression of these genes will allow the degradation of trichloroethylene under low-nutrient conditions.

Non-biologically controlled processes can also contribute to the *in-situ* treatment of subsurface contaminants. For example, many of the inorganic and organic contaminants present in the soils and groundwater at Hanford and other DOE sites can be altered, immobilized, or destroyed by reduction and oxidation reactions occurring at mineral surfaces. The EMSP project **Molecular-Level Processes Governing the Interaction of Contaminants with Iron and Manganese Oxides** (Page 67) is a combined experimental and theoretical study developing a detailed understanding of specific mineral surface (iron and manganese oxides) and contaminant (chromate and chlorinated

solvents) reactivities that will substantially improve models of contaminant fate and transport in geologic systems, and optimize the manipulation of geochemical reduction and oxidation processes for improved subsurface treatment.

Health Effects

Future land uses, regulatory requirements, stakeholder and tribal concerns, and the potential health impacts from hazardous waste and radionuclide contamination will dictate the acceptable treatment approaches and cleanup levels at Hanford and other DOE sites. Trichloroethylene (TCE), a chlorinated solvent, is one of the most common chlorinated organic contaminants at DOE sites (including Hanford). Cleanup costs for chlorinated solvents are most frequently driven by TCE because it is both widespread and is generally present at the highest concentrations on DOE sites. The EMSP project **Mechanism Involved in Trichloroethylene-Induced Liver Cancer: Importance to Environmental Cleanup** (Page 75) is developing critical data for updating the human health and safety risk-based cleanup standards for trichloroethylene (TCE) that were developed 20 years ago. These data could potentially allow changes in risk-based standards for TCE that could reduce complex-wide cleanup costs by several billions of dollars while still protecting humans and the environment.

References

1. DOE (U.S. Department of Energy). March 1995. *Estimating the Cold War Mortgage: The 1995 Baseline Environmental Management Report*, DOE/EM-0232 (2 Vols.), U.S. Department of Energy, Washington, D.C.
2. ORNL (Oak Ridge National Laboratory). December 1994. *Integrated Data Base Report-1993: U.S. Spent Nuclear Fuel and Radioactive Waste Inventories, Projections, and Characteristics*, DOE/RW-0006, Rev. 10, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

For more information contact:

Dr. Rod K. Quinn
Manager, Environmental Science and Technology Programs
Pacific Northwest National Laboratory
(509) 375-6625
rod.quinn@pnl.gov

Dr. John P. LaFemina
Manager, Advanced Technology Development
Environmental Science And Technology Programs
Pacific Northwest National Laboratory
(509) 375-6895
john.lafemina@pnl.gov

or,

Mr. Roy E. Gephart
Program Manager
Environmental and Health Sciences Division
Pacific Northwest National Laboratory
(509) 375-6754
re_gephart@pnl.gov

Contents

Tank Waste Remediation

Colloidal Agglomerates in Tank Sludge: Impact on Waste Processing (J.W. Virden, PI*).....	1
Architectural Design Criteria for <i>f</i> -Block Metal Sequestering Agents (B.P. Hay, PI).....	15
Ionizing Radiation Induced Catalysis on Metal Oxide Particles (T.A. Fryberger, PI).....	23
Interfacial Radiolysis Effects in Tank Waste Speciation (T.M. Orlando, PI).....	29
Chemical Speciation of Strontium, Americium, and Curium in High Level Waste: Predictive Modeling of Phase Partitioning During Tank Processing (A.R. Felmy, PI).....	37
Radiation Effects in Nuclear Waste Materials (W.J. Weber, PI).....	43

Soil and Groundwater Cleanup

Dynamics of Coupled Contaminant and Microbial Transport in Heterogeneous Porous Media (T.R. Ginn, PI).....	53
Genetic Analysis of Stress Responses in Soil Bacteria for Enhanced Bioremediation of Mixed Contaminants (K.K. Wong, PI).....	61
Molecular-Level Processes Governing the Interaction of Contaminants with Iron and Manganese Oxides (S.A. Chambers, PI).....	67

Health Effects

Mechanism Involved in Trichloroethylene-Induced Liver Cancer: Importance to Environmental Cleanup (R.J. Bull, PI).....	75
---	----

* PI = Principal Investigator

Contents

1	Colloid A, Components in Tank Sludge Impact on Waste Processing (W. J. Lee, R. L. ...)
15	Artificial Design Criteria for Blast Mitigation Against (R. H. ...)
23	Longing Radiation Induced Changes on Metal Oxide Particles (T. ...)
30	Interfacial Hydrophobic Effects in Tank Waste Specimens (T. ...)
35	Chemical Speciation of Chromium, Arsenic, and Cadmium in High Level Waste (T. ...)
45	Feasibility of Using Partitioning During Tank Processing (A. ...)
48	Radiation Effects in High Level Waste Materials (W. B. Weber, ...)
53	Soil and Groundwater Cleanup (R. ...)
57	Impact of Organic Contaminants and Microbial Activity in Hydrogenation (R. ...)
61	Genetic Analysis of Spore Response in Soil Bacteria for Enhanced Bioremediation (R. ...)
67	Molecular Level Probes for the Interaction of Contaminants with Iron and Manganese Oxides (S. A. ...)
75	Microbial Involvement in Trichloroethylene Induced Liver Cancer: Implications for Environmental Cleanup (R. ...)

Tank Waste Remediation

Colloidal Agglomerates in Tank Sludge: Impact on Waste Processing

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

Dr. J.W. Virden

(509) 375-6512 (Phone)

(509) 375-2186 (Fax)

jw_virden@pnl.gov

Pacific Northwest National Laboratory

PO Box 999, MSIN K2-44

Richland, WA 99352

Contributors and Collaborators

J. Berg (UW)

B.C. Bunker (PNNL)

L. Frank (SNL)

K.D. Keefer (PNNL)

J. Martin (PNNL)

D.R. Rector (PNNL)

P.A. Smith (PNNL)

Research Objective

Disposal of millions of gallons of existing radioactive wastes is a major remediation problem for the Department of Energy (DOE). Although radionuclides are the most hazardous waste constituents, the components of greatest concern from a waste processing standpoint are insoluble sludges consisting of submicron colloidal particles. Depending on processing conditions, these colloidal particles can form agglomerate networks that could clog transfer lines or interfere with solid-liquid separations such as settle-decant operations. Under different conditions, the particles can be dispersed to form very fine suspended particles that will not create sediment in settle-decant steps and that can foul and contaminate downstream treatment components including ion exchangers or filtrations systems. Given the wide range of tank chemistries present at Hanford and other DOE sites, it is impractical to measure the properties of all potential processing conditions to design effective treatment procedures. Instead, a framework needs to be established to allow sludge property trends to be predicted on a sound scientific basis. The scientific principles of greatest utility in characterizing, understanding, and controlling the physical properties of sludge fall in the realm of colloid chemistry. The objectives of this work are to accomplish the following:

- understand the factors controlling the nature and extent of colloidal agglomeration under expected waste processing conditions
- determine how agglomeration phenomena influence physical properties relevant to waste processing including rheology, sedimentation, and filtration
- develop strategies for optimizing processing conditions via control of agglomeration phenomena.

Research Statement

Technical Approach

The technical approach is shown schematically below. This project will use characterization data obtained from *real* Hanford tank sludge samples to guide and validate all model studies. The physical characterization data obtained on real tank sludge will be utilized in three areas: model colloid systems, predictive modeling, and agglomerate structure characterization. Model colloidal systems will investigate the effect of particle size, solid loadings, agglomerate structure, and particle composition on dispersion rheology and sedimentation behavior as a function of proposed processing conditions. Agglomerate structure will utilize Small Angle Neutron Scattering (SANS, Oak Ridge National Laboratory [ORNL]), Small Angle X-ray Scattering (SAXS, Pacific Northwest National Laboratory [PNNL]), and light scattering (Sandia National Laboratory [SNL]) to characterize agglomerate structure. The information discovered in model colloid systems and agglomerate structure will serve as input into the predictive modeling task. The predictive modeling task will be compared to such measurements as viscosity and sedimentation rate on Hanford tank waste.

Research Progress

Project Status

This project was initiated in FY97 and is being performed cooperatively with PNNL, SNL, and the University of Washington. The small angle neutron scattering facility at ORNL is being utilized for sample characterization.

Progress in the first six months has focused on characterization and modeling of colloidal systems similar to those found in Hanford tank waste. Experimental and modeling work is ongoing at PNNL and the University of Washington. Initial results have revealed a restabilization of colloidal dispersions at high salt concentrations. These results are very interesting and will require further study to fully explain. A series of computer programs are being developed based on the Brownian dynamics method to simulate the formation of colloidal gels and determine their physical properties. Brownian dynamics simulations is proving to be a useful tool for understanding the link between microstructure and the physical properties of a suspension or gel.

In the second half of FY97, SNL will have received their funding and will be focusing on modeling and validating colloidal agglomerate structures under a variety of conditions. Results from SANS will be interpreted and used to correlate agglomerate structure for colloidal dispersion under different solution conditions. The SAXS equipment at PNNL will be operational and

will investigate the relationship between agglomerate structure and colloidal dispersions. Work on model colloidal systems and the modeling dispersion behavior will continue. Specific results are described in detail below.

Technical Progress

Model Colloid Systems

The primary emphasis of the colloidal studies work has been the investigation of binary colloid systems at solution pH (pH=12-13.5) and salt concentrations (2-5M NaNO₃) analogous to conditions existing in Hanford waste storage tanks. Sedimentation, particle size determination, and rheological measurements were used to probe the agglomeration, particle packing, and flow behavior of Al- and Fe- hydroxide mixtures in addition to aluminum hydroxide suspensions containing varying ratios of large (1.3 mm) and small (50 nm) particles. Initial experiments were also conducted to investigate the effect of high electrolyte concentration (2-5M) on the sedimentation and rheological properties of nanometer- and micron-sized particles at high solution pH.

Gibbsite: Ferrihydrite Binary Mixtures

Previous work¹ has shown that the presence of very small quantities (<1 volume %) of nanometer-sized particles can have dramatic effects on the sedimentation, filtration, rheological, and consolidation behavior of sediments and suspensions. Any condition such as dissolution of larger particles, separation of hard agglomerates, or dissolution/reprecipitation reactions that increase the volume fraction of fines in suspension or decrease the overall particle size could significantly affect the colloidal behavior of the system. Of particular concern are possible reactions between alumina or silica and other colloids existing in the tanks upon mixing and/or transport, because the solubility of Al³⁺ is high at pH 13 (>10⁻²M). Elemental chemical analysis and Transmission Electron Microscopy/Energy Dispersive X-Ray Spectroscopy (TEM/EDS) investigations performed on core tank samples have shown aluminum and iron to be major components in the insoluble fraction of many tank sludges. For this reason, we are investigating the sedimentation, agglomeration, and viscosity behavior of binary mixtures containing Al and Fe hydroxides.

For these studies, a commercially available gibbsite (Al(OH)₃) powder (Alcoa S-3) with mean particle size of 1.3 mm was mixed with a hydrated ferrihydrite (Fe(OH)₃) slurry (Noah) containing hard agglomerates (1.6 mm) of nanometer-sized primary particles (approximately 50 nm). 1 volume % (total solids loading) suspensions were prepared with varying ferrihydrite:gibbsite (Fe:Al) ratios and aged at 22°C and 40°C. Figure 1 shows the sedimentation behavior of the Fe:Al mixtures as a function of time at ratios from 0:100 to 100:0 volume percent. The sediment cake volume increases in proportion to the concentration of nanometer-sized ferrihydrite in the mixture with little or no effect of time or temperature on this trend. The introduction of small particles typically leads to lower packing densities in sediments due to the open, ramified nature of the agglomerates formed. The trend measured in the sedimentation experiments was also seen using viscosity measurements. Figure 2 shows the viscosity measured

on 1 volume % Fe:Al slurries after 5 days of stirring at room temperature. As the concentration of fine particles in suspensions increases, the viscosity increases and the particle-packing density decreases, consistent with an increasing number of loosely packed aggregates in suspension. Future efforts will focus on measuring agglomerate structure of these dispersions and relating structure to measured properties.

Figure 1. Sediment Volume Measured for Binary Ferrihydrite: Gibbsite Mixtures. 1 volume % total solids loadings, 10 ml total sample volume.

Figure 2. Viscosity of 1 volume % Ferrihydrite: Gibbsite Binary Mixtures at 5M NaNO₃ and pH 12. Particle concentrations are expressed as percentages of the total solids loading.

Salt Effects

One novel aspect of tank conditions is the extremely high salt (electrolyte) concentration. At counterion concentrations of 2-5M, existing colloidal interaction theories (DLVO) predict complete collapse of any electrostatic repulsion between particles, and strong aggregation of particles due to long range van der Waals attractive forces. More recently, the existence of very short range (1-5 nm) hydration and/or steric forces, resulting from surface/cation hydration or

surface reactions, are being suggested to explain particle-particle interactions observed at very small separation distances (<10 nm). Velamankani² and Lange³ have conclusively shown that electrostatically dispersed systems that have been "salted out" (coagulated) will pack to higher green densities than agglomerated (electrostatically attractive) suspensions of colloidal particles. The "coagulated" systems of Velamankani are produced at salt concentrations of 1M or less. To our knowledge, there are no studies of colloidal behavior at electrolyte concentrations approaching those relevant to tank waste (5M).

For this reason, sedimentation experiments were conducted on boehmite (AlOOH), ferrihydrite ($\text{Fe}(\text{OH})_3$), and gibbsite ($\text{Al}(\text{OH})_3$) particles at NaNO_3 concentrations ranging from 0.01M to 5M. The sedimentation results for 1.3mm gibbsite particles at pH 12 are shown in Figure 3. Interestingly, the particle-packing densities do not simply reach some minimal value due to particle-particle attraction into the primary minimum. The improved packing densities (lower cake volumes) at 0.01M NaNO_3 can be explained by the presence of weak electrostatic repulsion arising from the negative surface charge on the aluminum hydroxide particles at this pH, but at 1M salt, particle surface charges are effectively screened (neutralized) within nanometers of the particle surfaces. This fact would suggest that once the electrostatic contribution is effectively screened, particles would attract (aggregate) strongly due to the strength of van der Waals forces at these small separation distances. The data in Figure 3 follow this trend between 0.01-1M NaNO_3 , but deviate at higher salt concentrations. Particle-packing densities at 5M salt are higher than those at 0.1M and 1M. To further substantiate this finding, 10 volume % suspensions were prepared at pH=12 and stirred for 2 hours and 5 days. Suspension viscosities were then measured using the Bohlin VOR rheometer. The results are plotted in Figure 4 for shear rates of 11.6 and 116 s^{-1} . The viscosity data show identical trends to the sedimentation results with the highest viscosity suspensions (lowest packing densities) at approximately 1.0M NaNO_3 . At this time, the exact mechanisms for the restabilization of the dispersions at high salt concentrations are not fully understood. Future efforts will concentrate on understanding the mechanisms responsible for the observed behavior.

Figure 3. Sedimentation Volume for 1 volume % Gibbsite Slurries Prepared at pH 12 and Varying NaNO_3 Concentrations. 10 ml total suspension volume.

Figure 4. Viscosity of 10 volume % Gibbsite Suspensions versus NaNO_3 Concentration at pH 12. Gibbsite mean particle size of $1.3 \mu\text{m}$.

Rheological Studies - Binary Systems

The ultimate objective of this work is the ability to predict the rheological properties of tank sludges if given accurate measurements of particle size and size distributions, solution pH, salt concentration, and total insoluble solids loading. In essence, we hope to use a standard set of readily measurable slurry parameters to predict the relative viscosity, sedimentation, and filtration behavior of actual tank waste and simulate this behavior using simple 1, 2, or 3 component systems. Figure 5 shows the complex rheological behavior measured for tank 241-C-107 (hereinafter referred to as C-107) compared with rheological measurements on ferrihydrite slurries at 0.5, 1.0, and 2.0 volume % at pH 13.5 and 5M NaNO_3 . One can readily see that the viscosity behavior of C-107 can be simulated by colloidal slurries of fine iron hydroxide particles (particle size approximately 50 nm). Figures 6 and 7 show similar measurements made using 50 nm boehmite (AlOOH) and 1.3 mm gibbsite ($\text{Al}(\text{OH})_3$). A striking feature of the C-107 viscosity data is the existence of a large discontinuous region between approximately 0.1 s^{-1} and 1.0 s^{-1} . This feature is due to the highly attractive state of the particles in suspension, and the existence of a 3-dimensional gel network in suspension. In essence, the network structure of particle agglomerates in the suspension is capable of partially reforming at a rate comparable to network disruption caused by the shear forces induced in the rheometer. Note that this region is clearly present in suspensions prepared from nanometer-sized iron and aluminum oxides, but is absent in suspensions of the larger gibbsite particles. We found that in the gibbsite system (1.3 mm) the observed discontinuity was only observed at much higher solids loadings (10 volume %) and at viscosities several orders of magnitude higher than that measured for C-107. These measurements support previous TEM micrographs that show the existence of nanometer-sized particles in the C-107 sludges, and that these fine particles play a significant role in the colloidal behavior of the system.

Figure 5. Viscosity as a Function of Shear Rate for Agglomerated Ferrihydrite (50 nm) Suspensions Compared to the Rheological Behavior of C-107 Tank Sludge. Suspensions pH=13.5 electrolyte concentration was 5M NaNO₃.

Figure 6. Viscosity as a Function of Shear Rate for Agglomerated Boehmite (50 nm) Suspensions Compared to the Rheological Behavior of C-107 Tank Sludge. Suspensions pH=13.5 electrolyte concentration was 5M NaNO₃.

Though the single component, fine particles seemed to simulate the rheological properties of C-107 sludges, the low solids loadings in the simulants (0.5-1.2 volume %) are nowhere near the values measured on actual core samples. Though the value reported for total solids in tank C-107 varies in different reports, and different measuring techniques, Thermal Gravimetric Analysis (TGA) techniques generally report solids loading of approximately 40-50 volume %. Such measurements would, by necessity, include the soluble as well as insoluble solids. In careful measurements performed by PA Smith and DR Rector in 1996 on C-107 samples, the solids loading for *insoluble* particles was measured at 6.8 volume %. In addition, TEM micrographs of the same C-107 sample showed the presence of fine (10-50 nm) Al-containing

Figure 7. Viscosity as a Function of Shear Rate for Agglomerated Gibbsite (1.3 μm) Suspensions Compared to the Rheological Behavior of C-107 Tank Sludge. Suspensions electrolyte concentration was 5M NaNO_3 .

particles, many 1-10 micron-sized particles of AlOOH or $\text{Al}(\text{OH})_3$, with some larger particles ($>50\text{nm}$). Based on this information, we initiated a series of rheological studies on binary systems containing large (1.3 mm gibbsite) and small (50 nm boehmite) particles in an attempt to simulate the viscosity behavior of C-107 samples using particles of similar size, composition, and total solids loadings as those measured in the actual sample. In addition, solution conditions of $\text{pH}=13.5$ and 5M NaNO_3 were used to simulate tank conditions. Our goal is to reproduce the complex viscosity behavior (particularly the discontinuity) with mixtures of micron- and nanometer-sized particles at total solids loadings near those measured in C-107.

Figure 8a shows the viscosity versus shear rate data for a fixed boehmite concentration of 0.5 volume % (fine particles) and gibbsite concentrations ranging from 0-7 volume % (large particles). In all cases the viscosity of the binary simulant exceeds that of C-107. Figure 8b is the same data replotted to emphasize the region of discontinuity ($0.1\text{-}1\text{ s}^{-1}$). The effect of the larger particles is clearly seen in disrupting the network formed by agglomerated fine particles as the restructured region lessens in magnitude and shifts towards lower shear rate upon increasing additions of gibbsite particles. By lowering the initial boehmite concentration to 0.3 volume %, the rheological behavior of C-107 could be closely approximated with gibbsite:boehmite mixture ratios of 3.0:0.3 and 5.0:0.3. These findings hold promise that binary or ternary systems of particles that match the approximate size distribution and solids loadings may serve as colloidal properties simulants for engineering studies.

Figure 8. a) Viscosity Versus Shear Rate for Binary Mixtures of Gibbsite and Boehmite Particles. Ratios are expressed in volume percent. Suspension pH=13.5 with 5M NaNO_3 Electrolyte concentration. b) Enlarged Region of Figure 8a Emphasizing Shear Rate Region Between 0.1 s^{-1} and 10 s^{-1} for Binary Mixtures of Gibbsite and Boehmite Particles.

Predictive Modeling

A large fraction of the insoluble solids that exists in Hanford tank waste is in the form of sub-micron-sized particles. Under the high pH and salt concentration conditions that exist in the tanks, these colloidal particles tend to attract each other to form porous aggregates and gels. Recent work related to tank waste processing indicates that the aggregation of these particles to form colloidal gels can have an enormous impact on tank waste processing. For example:

- The efficiency of solid-liquid separation through sedimentation depends on how effectively the particles in the final sediment pack. The formation of a colloidal gel can reduce or even eliminate the degree of settling that occurs during a pretreatment wash or leach procedure.
- The retrieval and transport of tank sludge depends on the viscosity of the suspension. The formation of a colloidal gel can change a low-viscosity Newtonian suspension into a high-viscosity shear-thinning fluid.

The objective of this task is to use computer simulation to model the aggregate or gel microstructure formation based on the known interactions between colloidal particles, and to predict the physical properties resulting from the microstructure. The primary physical properties of interest are the compressive yield stress for sedimentation and rheological properties for tank waste mobilization and transport.

Method

The physical properties of a colloidal gel depend both on the microstructure of the gel and the interaction potential between particles. The microstructure, in turn, depends on the Brownian motion and aggregation history of the individual particles. This transient aggregating behavior is modeled using the Brownian dynamics method.

The first part of this year has been spent on developing a series of computer programs based on the Brownian dynamics method to simulate the formation of colloidal gels and determine their physical properties. In Brownian dynamics, the motion of individual particles are determined based on the random forces exerted by the surrounding fluid and particle-particle potential interactions. The translational motion is described by the Langevin equation

$$m \frac{dv_i(t)}{dt} = \zeta v_i(t) + F_i(t) + F_i^R(t)$$

where ζ is the Stokes friction coefficient, $F_i(t)$ are the interparticle forces, and $F_i^R(t)$ is a random component with Gaussian distribution. For solutions with a high salt concentration, such as those in tank waste, the repulsive electrostatic double-layer collapses resulting in a van der Waals attraction potential. The momenta decay rapidly and the update algorithm can be written as

$$r_i(t + \Delta t) = r_i(t) + [F_i(t) + R_i(t, \Delta t)](\Delta t / \zeta)$$

where R_i is the random force. The motions of the individual particles are integrated over time until the particles have aggregated to form a continuous network. Simulations of several thousand particles are typical.

Two different Brownian dynamics algorithms have been developed to model colloidal aggregation and gel formation. One algorithm tracks individual particles until they contact another particle or cluster of particles. The new cluster is then treated like a rigid body with the appropriate Brownian and cluster-cluster interactions. This algorithm is useful for efficiently creating gels for diffusion-limited aggregation systems. The cluster size distribution as a function of time and the fractal dimension of the clusters compare favorably with values in the literature.

Another Brownian dynamics algorithm has been developed that connects contacting particles with a center potential, which allows each particle to rotate around the surface of the connected particle. Using this method, the fractal agglomerates are formed with truss-like structures rather than individual rigid bonds.

A colloidal gel is formed by running the Brownian dynamics transient until all particles and clusters connect to form a single structure. Information about the structure of a suspension or gel is obtained using the particle-particle correlation functions, $g(r)$, and the structure factor, $S(q)$. To evaluate $g(r)$, one momentarily stops the aggregation process at predetermined times and

obtains a histogram giving the number of pairs of particles that are found within the spherical shells of radii r and $r+dr$, irrespective of whether the particles belong to the same cluster or different ones. The pair correlation function is calculated from this histogram with the formula

$$g(r) = \frac{\text{density of pairs in } (r, r + \delta r)}{\text{average density of pairs}}$$

The scattered intensity, $S(q)$, of a macroscopic system containing identical particles with number density ρ , assuming single scattering, is given by

$$S(q) = 1 + \frac{4\pi\rho}{q} \int_0^\infty r \sin(qr) (g(r) - 1) dr$$

The structure factor, $S(q)$, can be related to the intensity results from neutron and x-ray scattering experiments.

The physical properties of a colloidal gel are determined by applying forces to the simulated gel network and measuring the response. For example, the compressive yield stress is defined as the relative weight per unit area of sediment that can be supported by a colloidal gel as a function of solids volume fraction. This is simulated by forming a colloidal gel between two parallel walls, one of which remains fixed while the other acts as a freely moving piston. A force is applied to the moving wall and the system is allowed to compress until it reaches a new equilibrium value. By repeating this procedure, the compressive yield stress vs. solid fraction is determined. A similar approach can be taken to determine rheological properties.

Preliminary Results

The methods described in the previous section will be used to examine a variety of homogenous and heterogenous particle systems in support of the experimental efforts of this project. The first system to be modeled is a mixture of large and small particles to understand the properties of the suspensions as a function of solids loading and particle size distribution.

The gel structure of binary particle mixtures was modeled using Brownian dynamics simulations. The simulation begins with a suspension of large and small spherical particles with a 10:1 diameter ratio. The solids loading is 2 vol%, divided evenly between the large and small particles, using a total of 10,010 particles. Before starting the transient, the particles are randomly placed on a lattice and then the system is relaxed using a series of Monte Carlo moves. The particles combine based on diffusion-limited aggregation.

After a specified period of time, the pair distribution function between large and small particles, $g(2,1)$, is determined and is presented in Figures 9 and 10. The small particle density is normalized with respect to the bulk small particle number density. The radial distance is in terms of the small particle diameter, $s(1)$. Note that there is a peak near $5.5 s(1)$, which

Figure 9. Pair Distribution Function Between Large and Small Particles, Normalized to Small Particle Density, $s(2)/s(1)=10$, $N=10010$, $f(1)=f(2)=0.01$.

Figure 10. Pair Distribution Function Between Large and Small Particles, Normalized to Small Particle Density, $s(2)/s(1)=10$, $N=10010$, $f(1)=f(2)=0.01$. (Expanded to show detail.)

represents the small particles adsorbed on the surface of the large particle. Then there appears to be a depletion region between 5.5-7.5 $s(1)$ where the small particle density is less than the bulk average. For greater distances, the density is equal to that of the bulk density. These results appear to be relatively insensitive to the concentration of large particles but very sensitive to the small particle number density, which determines the mean path of a small particle before it becomes part of the gel.

These results indicate that the adsorbed particles are removed from the depletion region, but that the presence of the large particles is felt only a few small particle diameters into the gel. Beyond this distance, the gel is essentially the same as that for a pure small particle gel with the same bulk number density. The small particle structure appears to dominate the properties of the gel, and the addition of large particles at these concentrations appear to perturb the structure slightly. The effect on the strength of the gel will be determined by performing a series of compressive yield stress simulations for both the monotonic and binary systems and comparing results.

Conclusions

Brownian dynamics simulations provide a useful tool for understanding the link between the microstructure and the physical properties of a suspension or gel. The preliminary results for a binary system of large and small particles indicates that the small particles dominate the structure of the gel. However, this must be verified by determining the effect on the strength of the gel by performing a series of compressive yield stress simulations for both the monotonic and binary systems.

References

1. Bell, K.E., J.D. Franklin, R.H. Stephens, R.G. Hale, C. McDonald, D. Lauhala, and M. Umphrey. 1995. *Tank Characterization Report for Single-Shell Tank 241-C-107*, WHC-SD-WM-ER-474 Rev 0, Westinghouse Hanford Company, Richland, WA.
2. Velamankani, B.V., J.C. Chang, F.F. Lange, and D.S. Pearson. 1990. "New Method for Efficient Collodial Particle Packing via Modulation of Repulsive Lubricating Hydration Forces." *Langmuir*, 6:1323-1325.
3. Chang, J.C., F.F. Lange, and D.S. Pearson. "Viscosity and Yield Stress of Alumina Slurries Containing Large Concentrations of Electrolyte," *J. Am. Ceram. Soc.*, 77[1]:19-26, 1994.

Architectural Design Criteria for f-Block Metal Sequestering Agents

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

Dr. B.P. Hay
(509) 372-6239 (Phone)
bp_hay@pnl.gov
Pacific Northwest National Laboratory
PO Box 999, MSIN K9-77
Richland, WA 99352

PNNL Contributors

Dr. M. Alnajjar
Dr. D.A. Dixon
Dr. G.J. Lumetta
Dr. L. Rao
Dr. B.M. Rapko

External Collaborators

Prof. R.T. Paine
(505) 277-1661 (Phone)
rtpaine@unm.edu
Department of Chemistry
University of New Mexico
Albuquerque, NM 87131

Prof. K.N. Raymond
(510) 642-7219 (Phone)
raymond@garnet.berkeley.edu
Department of Chemistry
University of California at Berkeley
Berkeley, CA 94720

Prof. D.M. Roundhill
(806) 742-3067 (Phone)
uldmr@ttacs.ttu.edu
Department of Chemistry
Texas Tech University
Lubbock, TX 79409-1061

AWU Contributors

Dr. O. Clement
Dr. B.K. McNamara
Dr. G. Sandrone
Dr. P.L. Zanonato

Research Objective

The objective of this project is to provide the means to optimize ligand architecture for *f*-block metal recognition. Our strategy builds on an innovative and successful molecular modeling approach in developing polyether ligand design criteria for the alkali and alkaline earth cations. The hypothesis underlying this proposal is that differences in metal ion binding with multidentate ligands bearing the same number and type of donor groups are primarily attributable to intramolecular steric factors. We propose quantifying these steric factors through the application of molecular mechanics models.

The proposed research involves close integration of theoretical and experimental chemistry. The experimental work entails synthesizing novel ligands and experimentally determining structures and binding constants for metal ion complexation by series of ligands in which architecture is systematically varied. The theoretical work entails using electronic structure calculations to parameterize a molecular mechanics force field for a range of metal ions and ligand types. The resulting molecular mechanics force field will be used to predict low-energy structures for unidentate, bidentate, and multidentate ligands and their metal complexes through conformational searches. Results will be analyzed to assess the relative importance of several steric factors including optimal M-L length, optimal geometry at the metal center, optimal geometry at the donor atoms (complementarity), and conformation prior to binding (preorganization). An accurate set of criteria for the design of ligand architecture will be obtained from these results. These criteria will enable researchers to target ligand structures for synthesis and thereby dramatically reduce the time and cost associated with metal-specific ligand development.

Research Statement

Critical tasks in the cleanup of U.S. Department of Energy (DOE) sites include processing radioactive wastes for disposal in long-term storage, remediation/restoration of environmental sites resulting from radioactive contamination, and decontamination/decommissioning of nuclear facilities. Because the radioactive components, most of which are metals, are typically present in very low concentrations, it is desirable to remove them from the bulk of the contaminated source (process waste stream, groundwater, soil) and concentrate them to minimize the volume of radioactive material destined for permanent subsurface disposal and thus minimize further waste management and disposal costs. One group of radionuclides, the actinides, which are pervasive throughout the DOE complex, is of special concern. In particular, thorium, uranium, neptunium, plutonium, americium, and curium with half-lives ranging from 10^2 to 10^6 years can all be found in various DOE wastes, contaminated soils and groundwaters, and contaminated facilities. Lanthanide elements are also of concern because they form an important group of fission products that can persist in radioactive wastes for decades (e.g., $^{152,154,155}\text{Eu}$).

Organic ligands with a high degree of metal ion specificity are essential components for developing separations processes for metal ions. Over the past 50 years, much research has focused on the discovery of selective ligands for *f*-block metal separations; both neutral and ionic ligands have been examined.

Despite past success in the discovery of ligands that exhibit some degree of specificity for the *f*-block metal ions, the ability to further control binding affinity and selectivity remains a significant challenge to the synthetic chemist. The approach for developing these ligands has involved lengthy and costly experimental programs of organic synthesis and testing, which, in the absence of reliable methods for screening compounds before synthesis, requires an extensive research effort. Criteria for accurately selecting target ligands would result in much more effective use of resources.

Research Progress

This project uses a combined theoretical and experimental approach to develop ligand design criteria for selective complexation of *f*-block metal ions. These criteria will address how to best connect donor functionality to achieve metal ion recognition in multidentate ligands. For the *f*-block metal ions, donor groups of interest include amides, phosphine oxides, pyridine N-oxides, and catechols. In FY97 our research has focused on amide ligands with 35% of our resources allocated for theory and 65% of our resources allocated for experiment. Ligands containing the amide functional group are currently used in DIAMEX (actinide separation process under testing for treatment of commercial reprocessing wastes) and TRUEX (actinide separation process under testing for use in tank waste cleanup at Idaho National Engineering and Environmental Laboratory (INEEL)).

Our approach will use molecular mechanics models to relate ligand structure to metal ion binding affinity. To provide a firm foundation for this study, an exhaustive review on the application of molecular mechanics to obtain structure-function relationships in coordination chemistry has been written and accepted for publication as a chapter in the Encyclopedia of Computation Chemistry. This review covers all molecular mechanics methods that have been successful in correlating ligand structure with reactivity toward metal ions.

The use of a molecular mechanics model requires the development of an extended force field to handle metal-amide complexes. This will be accomplished using both experimental data (crystal structures and vibrational spectra) and electronic structure calculations. *Ab initio* DFT and molecular orbital theory calculations will be used to examine the structure and bonding of the various metal ions to simple unidentate and bidentate ligands. Because the design of selectivity involves both an affinity for the target metal and a rejection of competing metal ions, the MM3 model needs to be applicable not only to *f*-block metals but to other metals. For this reason, our model is being designed to evaluate amide complexes with metal ions throughout the periodic table.

Force field development for metal-amide complexes is well underway. An exhaustive survey of the Cambridge Structural Database has been conducted. A review article on structural aspects of metal-amide interactions has been written and will be submitted for publication. The database survey yielded 60 metal amide crystal structures that have been used in parameter development. A preliminary set of MM3 parameters have been developed and will be refined on completion of electronic structure calculations (*vide infra*). The structures used in this parameterization contained a variety of metal ions including lanthanides and actinides. Parameter correlations with metal size and charge are under development and will be used to extend the model to metals not covered by the existing experimental data. An abstract covering the results of this study has been submitted for presentation at the Fall National ACS Meeting.

Electronic structure calculations on metal-amide complexes are in progress. These calculations will yield ground state geometries and potential energy surfaces that will be used to validate and refine the MM3 parameters obtained through empirical fitting to crystal structure data described above. Because of their expense, the electronic structure calculations are focused on simple complexes of representative metal ions selected to cover a range of charge, size, and metal electronic configuration. Density functional theory has been used to calculate the structures of a variety of amides and metal ion complexes at the local and nonlocal levels with at least polarized valence double zeta basis sets. Frequencies have been calculated for all species to determine if they are minima. The calculations have been done with the computer codes NWChem and UniChem. This work has provided extensive testing of the new code NWChem being developed at the Environmental Molecular Sciences Laboratory (EMSL), Pacific Northwest National Laboratory (PNNL).

The first set of DFT calculations centered on optimizing the structure of the simple amides N,N-dimethylacetamide (DMA), malonamide, tetramethylmalonamide and its enol, and tetramethylsuccinamide. Calculations have also been done on the complex of DMA with the ions Mg^{2+} , Ca^{2+} , Sr^{2+} , Cu^{2+} , and Zn^{2+} . The results of binding the naked ions showed that the $\text{C}(\text{O})\cdots\text{M}^{2+}$ distances were too short as compared to experiment and that there were problems with the Zn^{2+} basis set for this type of structure. Attention is now focused on $[\text{M}(\text{DMA})(\text{OH}_2)_5]^{n+}$ species. These complexes were generated from optimized structures of hexa-aquo complexes for the dications above as well as Mn^{2+} , Fe^{3+} and K^+ . For Li^+ and Na^+ , also under examination, the number of H_2O molecules in the aquo complex is 4 and 5, respectively. Comparison to experiment or the MP2 calculations of Feller show good agreement for the pure aquo clusters. The $\text{C}(\text{O})\cdots\text{M}^{2+}$ distances are in much better agreement with the values from the crystal structures. Geometries have been optimized for the penta-aquo dication metal complexes with DMA. Potential curves for the $\text{C}(\text{O})\cdots\text{M}$ stretch, the C-O-M bend, and the torsion about the C-O bond have been calculated for the Mg^{2+} derivative and are in progress for the other four dications. When these calculations are complete, we will do the same for the three alkali ions noted above, the trications Fe^{3+} , Sc^{3+} , La^{3+} , and Lu^{3+} , and the tetracations Ti^{4+} , Zr^{4+} , and Th^{4+} . The DFT calculations on the *f*-block metal ion species will be done with effective core potentials, calculations that can only be done with our new program, NWChem.

Experimental structure-stability data is needed to validate predictions from theoretical models. Such sets of data would satisfy the following criteria: 1) the ligand series should differ

only in the placement of the donor atoms, 2) the coordination environment of the metal-ligand complexes must be known, and 3) relative binding affinities should be determined under identical experimental conditions of solvent, counter ion, and temperature. A literature search was performed to locate any information regarding single-phase stability constants for such amide complexes of lanthanides or actinides and solution structural information on such complexes. This search revealed that not only are there no existing structure-stability data sets, but that there are no stability-constant data for *f*-block metal ions with pure amide ligands. Given that amide group is neutral over a wide pH range, this situation reflects the general difficulty of determining stability constants for non-ionizable ligands. The goal of this year's experimental effort is to generate structure-stability data sets for series of ligands bearing only the amide functionality with selected *f*-block metal ions. To achieve this goal, experimental effort has focused on (1) amide ligand synthesis and (2) evaluation of known methods for the determination of metal-amide binding constants.

A series of twelve diamides based on derivatives of malonamide and succinamide has been targeted for synthesis at PNNL. These ligand structures have been chosen to examine the influence of the spacing between the two amide groups, alkyl substitution on the methylene or ethylene spacer, and N-alkyl substitution. Five of the twelve diamides have been prepared in tens of gram quantities in 99+% purity. Purity and composition have been determined by proton and carbon NMR, GC-MS, and FTIR. The remaining seven diamides are scheduled to be completed by the end of FY97. In addition, a subcontract has been placed with Professor D. Max Roundhill at Texas Tech University to prepare series of calix[4]- and calix[6]-arene amides. Synthetic routes to four compounds have been demonstrated and scale-up to multi-gram quantities is in progress. Synthetic routes to other compounds in these series are under development.

A variety of methods for the determination of metal-amide binding constants are under investigation. These methods include electronic spectroscopy, IR spectroscopy, use of ion selective electrodes, calorimetry, and solvent extraction. Of these methods, only solvent extraction remains to be tried.

Electronic spectroscopy has been used to monitor the complexation of DEHBA (N,N-(di-2-ethylhexyl)butanamide) and the DIAMEX ligand (N,N'-dimethyl-N,N'-dibutyl-2-tetradecylpropane-1,3-diamide) with Eu(III) and Nd(III) in acetonitrile. This method is not applicable for these metal ions because there is very little shift in absorption bands on amide complexation. Another electronic spectroscopy approach is to monitor competition of the amide ligand with a ligand that absorbs strongly in the visible region, e.g., murexide. However, this approach failed in the case of TMM (N,N,N',N'-tetramethylmalonamide) with Eu(III). Although neither approach appears suitable for lanthanides, the possibility of using electronic spectroscopy methods for actinides remains to be investigated. The amide ligands are known to extract the tetravalent and hexavalent actinides better than the trivalent lanthanides, which suggests stronger binding of the amides to the former metal ions. Thus, it is expected that amide binding the tetravalent and hexavalent actinides will result in significant shifts in the electronic spectra of these ions.

The frequency of the intense carbonyl stretch is known to be sensitive to metal ion complexation. Therefore, FTIR spectroscopy was used to examine the complexation of Eu(III) with DEHBA and the DIAMEX ligand. Although shifts in the carbonyl frequency of 40 - 50 cm^{-1} were observed in the presence of Eu(III), the spectra are complex. The FTIR spectra indicate the formation of two or more different Eu/amide complexes depending on the Eu/amide ratio. The presence of multiple species vitiates the determination of binding constants by the continuous variation method. It remains to be seen whether the calixarene/amide systems will also form multiple species when reacted with lanthanide and actinide ions.

Because ion-selective electrodes specific for *f*-elements are not available, the measurement of metal-amide binding constants by potentiometry requires the use of a competitor, i.e., another metal ion in solution that competes with the *f*-block metal ion and whose concentration can be determined by a reversible electrode. Such electrodes are available for Ag(I), Cd(II), and Pb(II). Potentiometric measurements of Ag(I) in absence and presence of the DIAMEX ligand indicate that the interaction between this metal ion and the ligand are too weak to be observed in acetonitrile solvent. The possibility of using Cd(II) or Pb(II) as a competitor remains to be investigated.

To date the most promising approach has been calorimetry. The complexation of Eu(III) by both the TMM and the DIAMEX ligands have been investigated in a number of pure and mixed solvents. In a solvent that coordinates the metal ion strongly (e.g., DMSO), no complexation is detected. In a solvent that coordinates the metal ion weakly (e.g., acetonitrile or propylene carbonate), complexation is too strong ($\log K$ greater than 5) to be measured by this method. We have demonstrated that mixtures of weak and strong solvents allow binding constants to be measured by this method. For example, we have been able to determine a 1:1 binding constant ($\log K = 1.2$) and a 1:2 binding constant ($\log K = 2.3$) for complexation of Eu(III) with TMM in 90% acetonitrile:10% DMSO. To our knowledge this is the first determination of a lanthanide diamide binding constant. The data demonstrate that the solvent can be adjusted to bring binding constants within the range that can be measured by this method. Alternative solvent systems, with metal coordinating properties intermediate to the extremes investigated to date, are under investigation. We are also exploring the possibility of measuring the heats of formation for the metal/amide complexes directly by using thermochemical methods such as thermogravimetric analysis, differential scanning calorimetry, and bomb calorimetry. This latter approach has the advantage that solvent effects are eliminated.

Although our major emphasis has been on amides, research also has begun on other classes of ligand within the scope of this project. These include mixed amide-phosphine oxide, mixed phosphine oxide-pyridine N-oxide, and catechol ligands.

A solvent extraction method has been used to determine single-phase binding constants of the TRUEX ligand ((octyl)(methyl)-N,N-diisobutyl-carbamoylmethylphosphine oxide) and NOPOPO ((2,6-bis(diphenylphosphino)methylpyridine N,P,P trioxide) with Am(III) and Eu(III). This is the first measurement of a binding constant for the TRUEX ligand. The results of this study show NOPOPO is a much more potent actinide extractant than the TRUEX ligand and demonstrate that the degree of actinide extraction correlates with single-phase binding constants.

A manuscript on the binding constant measurements has been written and will be submitted for publication. A second manuscript on the distribution of Am(III), Eu(III), and UO₂(II) with NOPOPO is in preparation.

We have recently received a set of structure-stability data for Fe(III) complexation with a series of tris-catecholate ligands compiled by Prof. Kenneth N. Raymond at the University of California - Berkeley. This data will be useful in the parameterization and validation of MM3 force field parameters for metal-catechol complexes.

Presentations

Rapko, B.M. "Extraction of *f*-Elements by Phosphine Oxide/Pyridine N-Oxide Ligands." *213th ACS National Meeting*, San Francisco, CA, April 13 - 17th, 1997.

Lumetta, G.J., B.K. McNamara, and I.E. Burgeson. "Amide Complexes of *f*-Block Elements." Abstract submitted for the *21st Annual Actinide Separations Conference*, Charleston, SC, June 23-26, 1997.

Rao, L., Y. Xia, B.M. Rapko, and P.L. Martin. "Synergistic Extraction of Eu(III) and Am(III) by TTA and the Neutral Donor Extractants CMPO and NOPOPO." Abstract submitted for the *21st Annual Actinide Separations Conference*, Charleston, SC, June 23-26, 1997.

Zanonato, P.L., and L. Rao. "Complexation of Eu(III) by N, N, N', N'-tetraalkyldiamides." Abstract submitted for the *214th ACS National Meeting*, Las Vegas, NV, September 7-11, 1997.

Clement, O., G. Sandrone, D.A. Dixon, and B.P. Hay. "A MM3(96) Force Field for Metal-Amide Complexes." Abstract submitted for the *214th ACS National Meeting*, Las Vegas, NV, September 7-11, 1997.

Rapko, B.M., G.J. Lumetta, B.K. McNamara, L. Rao, and P.L. Zanonato. "Determination of Actinide and Lanthanide Binding Constants with Amides and Diamides." Abstract submitted for the *Tenth Symposium on Separation Science and Technology for Energy Applications*, October 20-24, Gatlinburg, TN.

Publications

Hay, B.P., and O. Clement. "Metal Complexes" in *Encyclopedia of Computational Chemistry*, P. von Rague Schleyer, Editor-in-Chief, John Wiley and Sons, London, 1997, *in press*.

Clement, O., and B.P. Hay. "Structural Aspects of Metal-Amide Complexes." Manuscript in preparation for *Coordination Chemistry Reviews*.

Dixon, D.A., and G. Sandrone. "The Structures and Energetics of Aquo-Metal Dication Complexes from Density Functional Theory." Manuscript in preparation for publication in *Journal of Physical Chemistry*.

Sandrone, G., O. Clement, B.P. Hay, and D.A. Dixon. "The Structures and Energetics of Metal Dication-Amide Complexes from Density Functional Theory." Manuscript in preparation for publication in *Journal of Physical Chemistry*.

Rao, L., Y. Xia, B.M. Rapko, and P.F. Martin. "Synergistic Extraction of Eu(III) and Am(III) by Thenoyltrifluoroacetone and Neutral Donor Extractants: Octyl(Phenyl)-N,N-Diisobutylcarbonyl-methylphosphine Oxide and 2,6-Bis(Diphenylphosphino)N-methyl Pyridine N,P,P Trioxide." Manuscript in preparation for publication in *Solvent Extraction and Ion Exchange*.

Rapko, B.M., G.J. Lumetta, B.K. McNamara, L. Rao, and P.L. Zanonato. "Determination of Actinide and Lanthanide Binding Constants with Amides and Diamides." Manuscript in preparation for publication in *Separation Science and Technology*.

Ionizing Radiation Induced Catalysis on Metal Oxide Particles

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

T.A. Fryberger

(509) 375-2709 (Phone)

ta_fryberger@pnl.gov

Pacific Northwest National Laboratory

P.O. Box 999, MSIN K2-20

Richland, WA 99352

Contributors and Collaborators

Prof. A. Janata

(404) 894-4828 (Phone)

jiri.janata@chemistry.gatech.edu

School of Chemistry and Biochemistry

Georgia Institute of Technology

Atlanta, GA 30332-0400

Prof. M. Alam

(505) 835-5831 (Phone)

alam@nmt.edu

Department of Materials Engineering

New Mexico Tech

Socorro, NM 87801

Research Objective

This project focusses on a novel approach for destroying organics found in high-level mixed waste prevalent at DOE sites. In this project we propose that organics can be destroyed by utilizing reduction/oxidation (redox) chemistry resulting from electron-hole (e^-/h^+) pairs generated in stable, wide bandgap semiconductors via interactions with ionizing radiation (α, β, γ). Conceptually this process is an extension of visible and near-UV photocatalytic processes known to occur at the interfaces of narrow bandgap semiconductors in both solution and gas phases. In these processes, an electron is excited across the energy gap between the filled and empty states in the semiconductor. The excited electron does reductive chemistry and the hole (the point from which the electron was excited) does oxidative chemistry. The energy separation between the hole and the excited electron reflects the redox capability of the e^-/h^+ pair, and is dictated by the energy of the absorbed photon and the bandgap of the material. The use of ionizing radiation has advantages in that it i) overcomes optical transparency limitations associated with visible and near-UV illumination (γ -rays penetrate much farther into a solution than UV/Vis light), and ii) permits the use of wider bandgap materials (such as ZrO_2), which possess potentially greater redox capabilities than those with narrow bandgap materials.

Planned experiments are aimed at extending the body of knowledge about e^-/h^+ pair chemistry of semiconducting metal oxide (MO) materials by examining the influence of surface structure, defects, and dopants on the photocatalytic activity of narrow bandgap materials (TiO_2), and by expanding these studies to wider bandgap materials (ZrO_2) that are virtually unexplored in terms of their e^-/h^+ pair chemistry. Experiments are being conducted in three areas: 1) g-radiocatalysis of reactant-colloidal metal oxide solutions, 2) photoelectrochemical studies at model MO electrodes, and 3) photochemical studies in ultra-high vacuum (UHV) and high pressures on model MO surfaces. An outcome of this proposed work will be a more thorough evaluation of the use of ionizing radiation in the catalytic remediation of organics (and other problem species) in high-level mixed waste.

Research Statement

High-level radioactive waste storage tanks within DOE sites contain significant amounts of organic components (solid and liquid phases) in the form of solvents, extractants, complexing agents, process chemicals, cleaning agents, and a variety of miscellaneous compounds. These organics pose several safety and pretreatment concerns, particularly for the Hanford tank waste. All currently proposed tank waste cleanup alternatives encounter potential safety and/or pretreatment problems associated with organics. Remediation technologies are needed that significantly reduce the amounts of problem organics without resulting in toxic or flammable gas emissions, and without requiring thermal treatments. These restrictions pose serious technological barriers for current organic destruction methods, which utilize oxidation achieved by thermal or chemical activation.

Research Progress

Progress from September 1996 through May 1997 is discussed below in each of the three research areas of the program. The main conclusions thus far are the following:

1. Proof-of-principle results have been reproduced at a different γ -facility. These results show a significant radiocatalytic effect for the destruction of EDTA in the presence of colloidal TiO_2 .
2. An electron scavenger, such as oxygen, is necessary to prevent charge buildup on the TiO_2 particles during organic oxidation. However, the inherent dissolved oxygen in solution is of sufficient concentration to preclude the need to bubble oxygen in the solution.
3. As expected, TiO_2 is a better oxidative radiocatalyst than ZrO_2 , but supported noble metals on either oxide do not increase EDTA destruction. This unexpected but fortuitous result indicates that expensive metals may not be necessary in scale-up applications.
4. Electrochemical studies indicate that defects created in the colloidal particle during irradiation may adversely influence charge carrier dynamics in the particle.
5. Vacuum studies indicate that surface defects may promote oxidative photochemistry by bending the semiconductor bands upward, but inhibit reductive photochemistry. However, these same defects may also promote thermal reductive processes.

Details of these and other findings are discussed to a greater extent below.

γ -Radiocatalysis of Reactant-Colloidal Metal Oxide Solutions

The proof-of-principle experiments cited in the EMSP proposal for this program were reproduced using a different γ -facility and an improved experimental setup. These experiments involve the radiocatalytic oxidation of ethylene diamine tetraacidic acid (EDTA), which is a complexing agent found in many of the Hanford waste tanks. Essentially, the current results agree with proof-of-principles studies. An increase in the G-value (defined as the number of events per 100 V of absorbed energy) for the radiolytic destruction of EDTA was observed in the presence of colloidal TiO_2 and O_2 over that occurring in aqueous solution (from 2.4 to 3.7). The enhancement is obtained only when both TiO_2 and O_2 are present in solution.

EDTA Radiocatalysis Studies on TiO_2

- **O_2 Dependence:** The estimated G-values for EDTA radiolysis on TiO_2 in the absence of O_2 (solution and headspace purged with dry N_2) are the same as the aqueous solution value (2.4). This indicates that the presence of an electron scavenger (O_2 in this case) is essential for organic (EDTA) radiocatalytic oxidation. However, special measures for introducing O_2 , such as bubbling, were not necessary in these experiments because the amount of O_2 dissolved in the solution and in the headspace were sufficient for the test batch reactors used.

- **TiO₂ Powder Concentration:** The G-value for EDTA destruction in colloidal TiO₂ solutions is dependent upon the TiO₂ powder concentration. This is logical because the absorption of radiation is partitioned between the solution, with its G-value of 2.4 for EDTA destruction, and the TiO₂ particles. The overall G-value increases from 3.1 to 3.7 when the TiO₂ concentration is increased from 0.05 to 0.10 g/ml. With a 0.02 M EDTA and 0.10 g/ml TiO₂ solution, we estimate that 91.5% of the radiation is absorbed by water, 7.8% by TiO₂, and 0.6% by EDTA. Therefore, the G-value for EDTA destruction from radiation absorbed by TiO₂ is probably considerably higher than the values we estimated based on the overall radiation absorbed.
- **Noble Metal Catalysts:** Findings indicate that impregnation of the TiO₂ catalyst with either a 1% (by weight) Pd or Pt salt, followed by reduction, had little or no effect on the rate of EDTA destruction versus that obtained in the absence of the metal. In fact, the rate decreased for the Pt case suggesting that the metal's presence may inhibit destruction, either by blocking sites or by trapping holes. No thermal decomposition of EDTA was observed at room temperature in the presence of the metal. This result indicates that expensive noble metals are not necessary for organic oxidation on TiO₂ by radiocatalysis.
- **Studies with Colloidal ZrO₂:** Examination of the destruction of EDTA over colloidal ZrO₂ and Pt/ZrO₂ catalysts was begun. Preliminary results indicate that, as expected based on the flat band positions of ZrO₂ relative to those of TiO₂, ZrO₂ is not as effective as an oxidation catalyst, presumably because valence band holes in ZrO₂ are higher lying (less oxidizing). The rate of EDTA destruction on ZrO₂ was independent of the presence of Pt, and found to be about 10 - 20% less than that of TiO₂.

Tetraphenylborate Radiocatalysis Studies

In an effort to determine the source of benzene emission during Cs extraction from Savannah River tank wastes, a series of experiments were conducted involving sodium tetraphenylborate (TPB) radiolysis on colloidal TiO₂. Because titanates are used in the sorption of Sr from these wastes, it was thought that radiocatalysis could be a cause of the decomposition of tetraphenylborate to produce benzene. The findings are as follows:

- The rate of TPB destruction by γ -irradiation is the same with or without TiO₂, and there is no enhancement in the presence of O₂. These results suggest that an e⁻/h⁺ pair mechanism is not involved in benzene generation, in agreement with results from Savannah River and Georgia Tech.
- TPB thermally decomposes at room temperature in the presence of either a TiO₂- or ZrO₂-supported Pt catalyst. The product yields (benzene and biphenyl) differ somewhat in the presence or absence of γ -radiation, suggesting radiolysis of the thermal products may be taking place.

Scale-up and Demonstration

For the radiocatalysis process to be of practical use, catalytic destruction of organics must be demonstrated on a larger scale than the batch reactors used so far. Initial contacts were made with external sources for scale-up reactor work. In particular, informal discussions with Prof. I. Z. Ismagilov of the Boreskov Institute of Catalysis, Novosibirsk, Russian Federation, and with Alcoa, Inc. have begun. The latter have identified this process as a possible remediation technology for one of their industrial process streams.

Photoelectrochemical and UHV Studies at Model Metal Oxide Electrodes

To develop an understanding of the similarities and differences between radiolytic chemistry and photochemistry on metal oxide semiconductors, both photoelectrochemical and UHV studies are being carried out on model surfaces. These results will be correlated with the solution studies described above.

Photoelectrochemical Studies

The effect of Ar⁺ sputter damage on the electrochemical response of TiO₂(110) has been examined. Sputter damage, in some sense, may mimic material damage occurring in high radiation fields.

- In agreement with literature results, we have found that reoxidation of sputtered TiO₂(110) with O₂ in UHV is straightforward and easily monitored by XPS.
- Mott-Shottky analysis of sputtered and reoxidized TiO₂(110) transferred to solution shows that UHV oxidation still leaves carrier states in the space charge region (200 - 1000 Å). These states may have a significant effect on the efficiency of e⁻/h⁺ pair separation.

UHV Studies of Chromium Redox Chemistry on TiO₂

Radiocatalysis should not be limited to organic destruction, but should apply to metal redox reactions as well. For this reason we have begun to investigate the chemistry of CrO₂Cl₂ on TiO₂(110). The CrO₂Cl₂, having Cr in the 6+ oxidation state, may be a suitable analog for probing the chemistry of Cr(VI) species found in Hanford tank wastes.

- CrO₂Cl₂ is molecularly adsorbed on TiO₂(110), presumably in a bidentate structure. Work function change measurements indicate the oxygen atoms are bound to the surface and the chlorine atoms extend into vacuum.
- If isolated oxygen vacancies are present on the surface, charge transfer between CrO₂Cl₂ and these sites bends the TiO₂ bands upward. This effect should drive photo-excited conduction

band electrons from the surface to the bulk during radiolysis, thus hindering reductive precipitation of Cr(III) on TiO_2 . However, the isolated vacancies do not decompose CrO_2Cl_2 .

- CrO_2Cl_2 thermally decomposes on $\text{TiO}_2(110)$ above 500 K. The decomposition process involves thermally induced reduction of Cr(VI) to Cr(III) via multiple oxygen vacancy sites that are created during O_2 desorption, the onset of which is at 500 K.
- Reduced Cr species are stable on TiO_2 , and diffuse into the bulk above 750 K.

A study of the reaction of CrO_2Cl_2 with water on $\text{TiO}_2(110)$ has been completed. These results are currently being analyzed. However, it appears that water reacts with CrO_2Cl_2 at 110 K to eliminate HCl and form a Cr(VI)-containing oxyanion species on the surface. This species is also reduced by the defects in the oxide, but at a different rate than that of the parent species. The oxyanion chemistry (in the absence of Cl) may be a better model than CrO_2Cl_2 for probing Cr(VI)-Cr(III) chemistry at metal oxide materials.

References

Su, Y., Y. Wang, J.L. Daschbach, T.B. Fryberger, M.A. Henderson, J. Janata, and C.H.F. Peden. "Gamma-Ray Destruction of EDTA Catalyzed by Titania." *J. Adv. Oxid. Technol.*, submitted.

Alam, M., M.A. Henderson, P.D. Kaviratna, G.S. Herman, and C.H.F. Peden. "Chromyl Chloride Chemistry at the $\text{TiO}_2(110)$ Surface." *J. Phys. Chem. B*, submitted.

Interfacial Radiolysis Effects in Tank Waste Speciation

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

T.M. Orlando

(509) 375-6404 (Phone)

(509) 375-6442 (Fax)

tm_orlando@pnl.gov

Pacific Northwest National Laboratory

PO Box 999, MSIN K2-14

Richland, WA 99352

Contributors and Collaborators

D.M. Camaioni (PNNL)

K. Knutsen (AWU - Post Doctoral Fellow)

D. Meisel (ANL)

Research Objective

The purpose of this program is to deliver pertinent, fundamental information that can be used to make technically defensible decisions on safety issues and processing strategies associated with mixed chemical and radioactive waste cleanup. In particular, an understanding of radiolysis in mixed-phase systems typical of U.S. Department of Energy (DOE) heterogeneous, radioactive/chemical wastes will be established. This is an important scientific concern with respect to understanding tank waste chemistry issues; it has received relatively little attention. The importance of understanding solid-state radiolysis, secondary electron interactions, charge-transfer dynamics, and the general effect of heterogeneous solids (interface and particulate surface chemistry) on tank waste radiation processes will be demonstrated. In particular, we will investigate i) the role of solid-state and interfacial radiolysis in the generation of gases, ii) the mechanisms of organic compound degradation, iii) scientific issues underlying safe interim storage, and iv) the effects of colloid surface-chemical properties on waste chemistry.

Research Statement

The radioactive and chemical wastes present in DOE underground storage tanks contain complex mixtures of sludges, salts, and supernatant liquids. These "mixtures," which contain a wide variety of oxide materials, aqueous solvents, and organic components, are constantly bombarded with energetic particles produced via the decay of radioactive ^{137}Cs and ^{90}Sr . Currently, there is a vital need to understand radiolysis of organic and inorganic species present in mixed waste tanks because these processes:

- produce mixtures of toxic, flammable, and potentially explosive gases (i.e., H_2 , N_2O and volatile organics)
- degrade organics, possibly to gas-generating organic fragments, even as the degradation reduces the hazards associated with nitrate-organic mixtures
- alter the surface chemistry of insoluble colloids in tank sludge, influencing sedimentation and the gas/solid interactions that may lead to gas entrapment phenomena.

Unfortunately, the mixed chemical and radiolytic nature of the problem greatly complicates the interactions and the information available on radiolytic processes in multiphase heterogeneous systems is insufficient to develop either accurate safety margins or radiolysis models that adequately describe DOE tank waste chemistry to support technically sound decision making.

Research Progress

Controlled radiolysis studies of NaNO_3 solids and SiO_2 particles were carried out using pulsed, low- (5-150 eV) and high- (3 MeV) energy electron-beams at Pacific Northwest National Laboratory (PNNL) and at Argonne National Laboratory (ANL), respectively. The pulsed, low-energy electron beams probe the inelastic scattering and secondary cascading effects produced by high-energy beta and gamma particles. Pulsed radiolysis allows time-resolved measurements of the high-energy processes induced by these particles.

Using low-energy (10-75 eV) electron-beam irradiation of "nominally" dry NaNO_3 solution-grown and melt-grown single crystals, we observed H^+ , Na^+ , O^+ , NO^+ , NO , NO_2 , O_2 , and $\text{O}(^3\text{P})$ desorption signals. Our threshold measurements and yields indicate that the degradation proceeds mainly via destruction of the nitrate moiety. The H^+ and Na^+ yields are primarily related to the presence of water and Na metal, Na hydrides and oxides, or other defect sites on the salt surface. The water is due to diffusion from the bulk of solution-grown crystals and controlled water adsorption on melt-grown crystals. The metallization and/or metal hydride/oxide build-up is a result of the very large electron-beam degradation cross-section ($\geq 10^{-16} \text{ cm}^2$) of NaNO_3 .¹ The build-up of alkali-metal colloids during the irradiation of alkali-halide materials is well known² and is expected for other alkali salts such as NaNO_3 . Figure 1 shows the Na^+ desorption yield as a function of incident electron energy. The signal below the 33 eV is all due to Na buildup and the break seems to be associated with charge build-up, band-bending, and then charge release. Charge trapping and metallization is reduced at temperatures above 420 K,^{1,3} a temperature higher than typically found in high-level liquid waste (HLLW) tanks.

Figure 1. The Na^+ desorption yield as a function of incident electron energy. The data were obtained from an electron-beam damaged NaNO_3 crystal at room temperature.

The HLLW tanks contain a large fraction of solid components (colloids and salt cakes with enormous interfacial surface areas) and radiation-induced metallization may occur in the bulk. It is well known that the reaction of metallic sodium with water produces hydrogen gas and heat. We do not yet know whether mechanisms leading to sodium metallization at crystal-vacuum interfaces occur at crystal-water interfaces, but alternative processes involving charge transfer or energy transfer from crystal to interfacial water molecules still may provide pathways for hydrogen gas production. Thus, further work is underway to investigate the amount of molecular hydrogen produced during direct radiolysis of nitrate salts containing adsorbed and interstitial water.

Previous work³ at PNNL has also demonstrated that excitation of NaNO_3 interfaces with 193 nm photons (6.4 eV) results in the stimulated desorption (PSD) of predominantly $\text{NO}(^2\Pi)$ and $\text{O}(^3\text{P}_1)$. The 193 nm (6.4 eV) valence band excitation in NaNO_3 solids/interfaces has been correlated with the $\text{NO}_3^- \pi^* \leftarrow \pi$ and, and is below the nominal bandgap energy of ~ 8 eV. Excitations of this band create localized excitons, which couple to phonons and trap at surface and/or defect sites. Decomposition of these excitons results in the direct desorption of both thermal and non-thermal $\text{NO}(^2\Pi)$ and $\text{O}(^3\text{P})$. We favor a mechanism in which surface excitons decay to produce $\text{NO}(\text{g}) + \text{O}(\text{g}) + \text{O}^-(\text{sf})$, either in one step, or via an NO_2^* - intermediate. This intermediate is consistent with the observation of NO_2^- as a primary bulk radiolysis product.^{4,5} We have extended these studies under the EMSP program using low-energy electron-beam irradiation, and again observe $\text{NO}(^2\Pi)$ and $\text{O}(^3\text{P}_1)$ as the primary direct neutral desorption products. Figure 2 shows that the electron energy thresholds for O and NO production are between ~ 6 -8 eV, which corresponds to the $\pi^* \leftarrow \pi$ band, but also overlaps the charge-transfer/conduction band-transition in NaNO_3 . In addition to the above-mentioned exciton dissociation channels, removal of an electron from the NO_3^- valence band results in the production of NO_3^* ,

Figure 2. The threshold incident electron energies for the stimulated production and desorption of NO and O from NaNO_3 interfaces.

which can then dissociate to the neutral products detected. At incident energies above the conduction band, localization of secondary electrons at nitrate defect sites could result in the production of an excited species NO_3^{*2-} which, though stable in the bulk, can decay at the surface via a process known as dissociative electron-attachment to form $\text{NO}_2^{*-} + \text{O} + \text{e}$ and $\text{NO} + \text{O}_2^{*-} + \text{e}$. The NO_2^{*-} and O_2^{*-} can further dissociate to form O^- , which can trap at the surface or in the bulk.

In addition to NO and O, some $\text{O}_2(\text{g})$ and $\text{NO}_2(\text{g})$ are produced from both electron stimulated desorption (ESD) and 193 nm PSD of NaNO_3 . The relative time-dependent yields for NO, O_2 , and NO_2 are displayed in Figure 3. Unlike NO and O, we believe these latter species are produced indirectly and do not represent direct products of NaNO_3 decomposition. In fact, the NO_2 data are well fit by a Maxwell-Boltzmann distribution at the NaNO_3 surface temperature of 423 K, indicating interaction with the surface prior to desorption. Neither NO_2 nor O_2 shows the "hot" temperature component we observe in both NO and $\text{O}(^3\text{P})$, but both show a 1-electron dependence upon electron fluence. Thus, they may result from ESD processes at defect sites (such as $\text{NO}_2^- + \text{e} \rightarrow \text{NO}_2 + 2\text{e}$), or from secondary reactions of the primary products (such as $\text{O} + \text{NO}_3^- \rightarrow \text{O}_2 + \text{NO}_2^-$). They are most likely not formed from recombination of the direct NO and O desorption products.

Figure 3. The NO, O_2 , and NO_2 desorption yields from 100 eV ESD of NaNO_3 . All signals have been adjusted for cracking in the quadrupole.

Comparison of 100 eV ESD and 193 nm PSD data shows that the velocity and state distributions of the neutral desorption products are very similar. The NO is clearly the predominant desorption product from both of these processes, with O_2 yields typically a factor of 10 smaller. The NO_2/NO yield ratio, however, is a factor of ~ 2 higher from ESD ($\sim 1/6$) as opposed to PSD ($\sim 1/12$). Our results clearly show that interfacial radiolysis leads to the production of several reactants and oxidants, particularly Na, NO, and oxygen, which are difficult

to rationalize by homogeneous radiolytic models and may provide ready explanations to some observations in the tank.

Earlier studies at ANL on Hanford grout samples raise the possibility of gas generation from water in the pores by radiation that is absorbed by the solid concrete.⁶ Recent studies at PNNL on tank waste simulants addressed this issue, but the results were not conclusive.⁷ Therefore, pulse radiolysis studies were initiated at ANL to address this question: can energy absorbed by solid particles dispersed in the aqueous phase escape the solid and lead to gas generation from the aqueous solution? Pulse radiolysis studies at the silica/water interface indicate that absorption of the radiation in the solid may lead to the appearance of charge at the liquid phase. This is demonstrated in Figure 4, which shows the absorbance by hydrated electrons (thus, by definition, are in the aqueous phase) following pulse irradiation of a sample containing 0% or 50% by weight SiO_2 particles. The decrease in the amplitude of the solvated electrons absorption is minimal despite the fact that the solids fraction increases considerably. These preliminary results seem to indicate that the yield of electrons in the liquid phase depends on both the solid and liquid fraction and does not necessarily decrease with decreasing water content. To date up to 60% weight of silica (in contact with glycerin to obtain refractive-index matching) was found to induce only a small reduction in the yield of solvated electrons. (These are thermal electrons that are stabilized by their hydration shell. In practical terms, they may generate hydrogen atoms or initiate other fuel-generating reduction processes.) Furthermore, our preliminary results indicate that this phenomenon may depend on particle size. It may be recognized that grout (or saltstone) may contain as much as 30% by weight water and our simulant comes close to this environment.

Figure 4. Histograms (absorbance vs time) of the decay of the solvated electrons in suspensions of SiO_2 . Solid points - No silica. Open points - 50 wt. % silica.

In summary, the primary direct radiolysis products of NaNO_3 solid surfaces are NO , O , O^- , and Na . Secondary (indirect) radiolysis products such as O_2 , NO_2 , NO_2^- , NO_3^{2-} , NaO , NaH , and NaOH can also form in the bulk. The O_2 and NO_2 can then diffuse and react with interfacial

species. High energy, pulse radiolysis studies at the silica/water interface produce solvated electrons and the yield seems independent of the water fraction. The solvated electrons and ionic and neutral desorption products are very reactive in the aqueous environment and are important in solution phase and interfacial reactions. As pointed out in an earlier study at ANL,⁸ it is likely that NO reactions with organic radicals lead to the production of $N_2O(g)$ and $NH_3(g)$ in Hanford tank wastes. Thus, the electron-stimulated reactions of the desorption products, such as NO, NO_2 , O, O_2 , and Na-metal with water and organic radicals at salt interfaces are currently under further investigation.

References

1. Knutsen, K., and T.M. Orlando. 1996. *Surf. Sci.* 348:146.
2. Weerkamp, J.R.W, J.C. Groote, J. Seinen, and H.W. den Hartog. 1994. *Phys. Rev. B.* 50:9781.
3. Knutsen, K., and T.M. Orlando. 1997. In press, *Phys. Rev. B.*, May 15, 1997 issue.
4. Cunningham, J., in: "Radical Ions," eds. E. T. Kaiser and L. Kevan (*Interscience*, New York, 1968) pp. 475-523.
5. Cunningham, J., and L.R. Steele. 1962. *Phys. Rev. Lett.* 9:47.
6. Jonah, C.D., S. Kapoor, M.S. Matheson, W.A. Mulac, and D. Meisel. 1994. *Gas Generation from Hanford Grout Samples*, ANL-94/7, Argonne National Laboratory, Argonne, Illinois.
7. Bryan, S.A., and L.R. Pederson. 1995. *Thermal and Combined Thermal and Radiolytic Reactions Involving Nitrous Oxide, Hydrogen, and Nitrogen in the Gas Phase; Comparison of Gas Generation Rates in Supernate and Solid Fractions of Tank 241-SY-101 Simulated Waste*, PNL-10490, Pacific Northwest Laboratory, Richland, Washington.
8. Miesel, D., C.D. Jonah, S. Kapoor, S. Matheson, and M.C. J. Sauer. 1993. *Radiolytic and Radiolytically Induced Generation Gases from Synthetic Wastes*, ANL-93/43, Argonne National Laboratory, Argonne, Illinois.

species. High energy, pulse radiolysis studies of the electron transfer reactions of organic radicals and ions in aqueous solution have been reported. The electron transfer reactions of organic radicals and ions in aqueous solution are very reactive in the aqueous environment and are important in radiation damage and chemical reactions. As pointed out in our earlier work at ARL, it is likely that NO reactions with organic radicals lead to the production of NO_2 and HNO_2 and that the electron transfer reactions of the decomposition products, such as NO_2 , NO , O_2 , and H_2O_2 with water and organic radicals at all interfaces are currently under investigation.

References

1. Kuntze, K., and T.M. O'Leary, 1993, *Proc. 2nd Int. Conf. on Radiolysis*, p. 348-352.
2. Warkentin, R.W., J.E. Crooks, J. Searles, and H.W. van den Hulst, 1994, *Proc. 2nd Int. Conf. on Radiolysis*, p. 353-357.
3. Kuntze, K., and T.M. O'Leary, 1993, *Proc. 2nd Int. Conf. on Radiolysis*, p. 358-362.
4. Cunningham, J., in "Radiolysis of Organic Compounds", ed. E. J. Kuntze and J. Searles, (Interscience, New York, 1992), p. 453-473.
5. Cunningham, J., and E. J. Searles, 1992, *Proc. 2nd Int. Conf. on Radiolysis*, p. 363-367.
6. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
7. Bryan, S.A., and J. R. Kuntze, 1992, *Proc. 2nd Int. Conf. on Radiolysis*, p. 368-372.
8. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
9. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
10. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
11. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
12. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
13. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
14. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
15. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
16. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
17. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
18. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
19. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.
20. Joseph, C.D., S. Kapoor, M. S. Matheson, W.A. Mahood, and D. Marshall, 1994, *Gas Phase Chemistry of Organic Compounds*, ARL-94-1, Air Force Research Laboratory, Dayton, Ohio.

Chemical Speciation of Strontium, Americium, and Curium in High-Level Waste: Predictive Modeling of Phase Partitioning During Tank Processing

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

Dr. A.R. Felmy
(509) 376-4079 (Phone)
(509) 372-6328 (Fax)
ar_felmy@pnl.gov
Pacific Northwest Laboratory
P.O. Box 999, MSIN K9-77
Richland, WA 99352

Co-Investigator

Dr. G. Choppin
(904) 644-3875 (Phone)
(904) 644-8281 (Fax)
Choppin@chem.fsu.edu
The Florida State University
Department of Chemistry, B-164
Tallahassee, FL 32306-3006

PNNL Contributors

J.A. Campbell
D.A. Dixon

Research Objective

In this research program, Pacific Northwest National Laboratory (PNNL) and Florida State University (FSU) are investigating the speciation of Sr and Am/Cm in the presence of selected organic chelating agents (ethylenediaminetetraacetic acid (EDTA), N-(2-hydroxyethyl) ethylenediaminetriacetic acid (HEDTA), nitrilotriacetic acid (NTA), and iminodiacetic acid (IDA) over ranges of hydroxide, carbonate, and ionic strength conditions present in high-level waste tanks. This fundamental understanding of chemical speciation reactions is essential to develop methodologies for removal of Sr and Am/Cm from the chelates, via competition, displacement or other reactions, without the need for the development of costly and potentially hazardous organic destruction technologies. These studies specifically focus on the effects of hydrolysis, carbonate concentration, ionic strength, and selected cation competition on the removal of Sr and Am/Cm from the organic chelates.

Research Statement

On a project and scientific basis the project is divided into research tasks: chemical speciation studies, development of molecular models for solution complexes, and development of thermodynamic data and models. This information on chemical speciation is used, in conjunction with certain necessary thermodynamic measurements, to develop thermodynamic models that will allow the prediction of changes in chemical speciation and solubility that can occur in response to changes in tank processing conditions. In this way we can simultaneously develop new approaches that address fundamental problems in aqueous speciation and provide useful and practical information needed for tank processing. All of these studies are designed to address key scientific issues involved in chemical speciation studies.

- Current analytical methods for examining the speciation of actinide elements in simple solutions, such as UV-VIS, Raman, etc., develop difficulties in interpreting or analyzing spectral features when multiple complexes are present and contribute to the observed spectra. In this project we are developing molecular models to analyze spectral features and analytical methods, principally based upon mass spectrometry, capable of identifying species present in mixed metal-ligand systems.
- The solubility of several alkaline earth and trivalent actinide compounds under high base conditions is too low for direct chemical speciation measurements. Determining the aqueous speciation in such solutions is currently done largely by curve-fitting solubility data to hypothesized speciation schemes. In this project we are developing molecular-based models to better constrain the possible solution species considered.
- Activity coefficients for aqueous species can change by orders of magnitude in going from dilute solution to the concentrated solutions typical of tank supernatants. Such changes in activity coefficients can dramatically affect the solubility of Sr and trivalent actinide

compounds. In this project we are utilizing the equations of Pitzer to develop such highly accurate models.

The following section gives a brief description on the progress to date of the PNNL studies. The research at FSU, which is currently focused on the speciation of Cu under similar hydroxide and carbonate conditions, will be reported by Professor Choppin in a separate report. The final section summarizes the major accomplishments.

Research Progress

In fiscal year 1997 (FY97) studies at PNNL have been focused on the competitive effects of hydrolysis and carbonate complexation of Sr with the organic chelates. Only initial experiments have been performed with trivalent actinide analogs. Plans call for completing the major portions of the work on Sr in FY97 with the work on the trivalent actinides beginning in FY97 and extending into FY98 and FY99.

Chemical Speciation Studies

Extensive experimental studies have already been conducted on the chemical speciation and solubility of Sr in high base and high carbonate conditions. These studies are summarized as follows.

- Completion of the studies on the hydrolysis and carbonate complexation of Sr and selected alkaline earth cations under high base and high carbonate conditions. These studies have not only identified new chemical species, such as the strong $\text{Sr}(\text{CO}_3)_2^{2-}$ complex at high carbonate concentration, but have also found consistent trends in the stability of alkaline earth hydrolysis and carbonate complexes as predicted from both thermodynamic and molecular models. A manuscript on the results of this research is currently in preparation (see below).
- Completion of the studies on the competitive effects of hydrolysis and carbonate complexation on the displacement of Sr from the EDTA chelate. Interestingly, high base concentration (up to 5M) resulted in little displacement of Sr from the EDTA chelate, owing to the relatively weak hydrolysis of Sr relative to other alkaline earth cations such as Mg^{2+} , but Sr was displaced by high carbonate concentration. An abstract on these results has been accepted for presentation at the next ACS National Meeting, see below, preparation of a manuscript will begin this FY.
- Research on the effects of hydrolysis and carbonate complexation on the displacement of HEDTA, NTA, and IDA have also been initiated. Though not complete, sufficient data are available to contrast the stability of the different chelates at high base and carbonate conditions to allow the submittal of an abstract on these results (see below); the effects of other competing cations, specifically Ca^{2+} , will also be addressed in this presentation.

Progress has also been made on the development of analytical methods capable of determining chemical speciation in complex mixed metal-ligand systems, principally using mass spectrometry (e.g., matrix-assisted laser desorption ionization/time-of-flight mass spectrometry (MALDI/TOF) and electrospray ionization mass spectrometry (ESI-MS). Matrix-assisted laser desorption ionization (MALDI) was designed to provide a simple method for introducing high molecular weight species directly into the gas phase in both neutral and ionic form. Electrospray ionization, a relatively new ionization technique for mass spectrometry, is an effective method for analyzing nonvolatile analytes in solution by mass spectrometry without prior sample alteration (e.g., derivatization). One major advantage of the ESI-MS is that it is a sufficiently gentle ionization technique that noncovalent, weak associations present in solution can be transferred to into the gas phase. The gentleness of the ESI process allows for the preservation of solution associations such as metal-chelator complexes and weak noncovalent associations during the ionization process. Several EDTA solutions under basic conditions have been examined and complexes identified utilizing these methods, including relatively weakly bound sodium ions with the EDTA chelate (i.e., $(\text{EDTA} + 2\text{Na})^{2-}$ at m/z 167)). High concentrations of salt have a tendency to suppress the ion signal. Additional efforts are currently underway using ion exchange to reduce the salt contribution and increase the ion signal.

Molecular Modeling Studies

The initial molecular modeling studies of the Sr-OH, and Sr-CO₃ system have also been completed. Specifically, *ab initio* electronic structure calculations at the density functional theory level with at least a polarized double zeta basis set have been performed. Calculations on the carbonate complexes of Ca²⁺ and Sr²⁺ have been completed for 1, 2, and 3 carbonate groups. Calculations on Ca²⁺(H₂O)₆, Ca²⁺(H₂O)₅, and Ca⁺(H₂O)₅(OH) have also been done. One interesting aspect of these results is that although the proton binding energies for the hydrated Ca and Sr complexes are similar and correlate well with our speciation and thermodynamic measurements, the molecular modeling results indicate quite different microscopic explanations for the observed hydrolysis behavior. The calculations show that the acidity of Ca²⁺(H₂O)₆ is 168 kcal/mol at the nonlocal DFT level and that extensive hydrogen bonding of the H₂O molecules to the OH⁻ in the isolated complex occurs. The structure of Sr⁺(H₂O)₇(OH) shows a completely different structure with the OH⁻ dissociating from the complex and hydrogen bonded only to three complexed H₂O molecules. The acidity of the octavalent Sr complex is 166 kcal/mol. The nmr chemical shifts for the Ca and Sr complexes have also been calculated. These studies provide unique insight into possible chemical speciation effects, especially in solutions where it was not possible to experimentally determine the speciation directly. These results are currently being summarized in a paper being prepared for the Journal of Solution Chemistry (see below).

The structures of NTA, IDA, and EDTA and different anions of these acids have also been optimized and vibrational spectra calculated. These results will be used to calculate the various acidities of the complexing agents. An important result is the need to treat hydrogen bonding between the acids and the carboxylate groups in these species. We will complete the above calculations and optimize complexes of Sr²⁺ with EDTA and the other complexing agents during the remaining part of the FY.

Thermodynamic Modeling Studies

Early this FY a preliminary thermodynamic model was developed, based upon the equations of Pitzer, to describe the changes in chemical speciation and solubility of Sr that can occur in tank processing solutions with changes in base concentration, carbonate complexation, and EDTA concentration. The specific chemical system addressed was the Na-Ca-Mg-Sr-OH-CO₃-EDTA-NO₃-H₂O system at 25°C. This model was developed as an initial guide to help design specific experimental studies on chemical speciation and was based upon the extensive amount of experimental data in the literature on EDTA complexation. Unfortunately, although an extensive amount of reliable experimental data are available to parameterize the model at low ionic strength and low base concentration (i.e., <0.1M NaOH), this did not prove to be the case at higher ionic strength and high base concentration. As a result, the predictions of this initial model did not prove to be quantitatively accurate when compared to the new experimental data obtained in this study at high ionic strength. The key model deficiency proved to be the interactions of the bulk cations and anions (i.e., Na⁺, NO₃⁻, OH⁻...) with the highly charged EDTA⁴⁻ species, which dominates at pH values greater than 12. The necessary thermodynamic measurements, specifically osmotic coefficient measurements, are currently being conducted to obtain the necessary data to improve the accuracy of the model. Although delays in opening the new Environmental and Molecular Sciences Laboratory has hampered progress on these measurements, sufficient data have been obtained to submit an abstract on the EDTA⁴⁻-NaOH system (see below). In addition, a small effort was initiated to upgrade an existing chemical equilibrium model to better incorporate all of the thermodynamic data available for the organic chelates and develop thermodynamically consistent equations of state. An abstract on this effort has also been prepared (see below).

Summary of Accomplishments

- The studies on the hydrolysis and carbonate complexation of Sr and selected alkaline earth cations under high base and high carbonate conditions has been completed. A manuscript is being prepared.
- The studies on the competitive effects of hydrolysis and carbonate complexation on the displacement of Sr from the EDTA chelate has been completed. An abstract has been submitted.
- An abstract has been submitted on research progress on the displacement reactions of Sr and Ca complexes with HEDTA, NTA, and IDA at high ionic strengths.
- Molecular modeling studies on alkaline earth cations hydrolysis and carbonate complexation has been completed. Studies on organic chelate structure and complexation has been initiated.
- An initial thermodynamic model for the Na-Ca-Mg-Sr-OH-CO₃-EDTA-NO₃-H₂O system at 25°C has been completed. Experimental studies to develop key needed parameters have also been initiated.

- A meeting has taken place with the Co-PI, Professor Choppin, in February to discuss progress on the project and coordination of research efforts.

Papers and Abstracts

Felmy, A.R., D.A. Dixon, J.A. Campbell, and M.J. Mason. 1997. "The Effects of OH, CO₃, and Ca on the Displacement of Sr from the EDTA Chelate: Implications for Waste Processing." Abstracted accepted for the *ACS National Meeting*, Las Vegas NV, September 7-11, 1997.

Felmy, A.R., D.A. Dixon, and M.J. Mason. 1997. "The Complexation of Alkaline Earth Cations by Organic Chelates at High Ionic Strength: Competitive Effects of Hydrolysis and Carbonate Complexation." Abstract accepted for the *52nd Calorimetry Conference*, Asilomar, CA August 4-8, 1997.

Felmy, A.R., D.A. Dixon, and M.J. Mason. "Hydrolysis and Carbonate Complexation of Alkaline Earth Cations: Consistency of Thermodynamic and Molecular Modeling Results." To be submitted to the *Journal of Solution Chemistry*.

Oakes, C.S. and A.R. Felmy. 1997. "Thermodynamics of [Na₄EDTA+NaOH]{aq}, Including New Isopiestic Measurements to 373K." Abstract accepted for the *52nd Calorimetry Conference*, Asilomar, CA August 4-8, 1997.

Sterner, S.M., A.R. Felmy, J.R. Rustad, and K.S. Pitzer. 1997. "Thermodynamic Analysis of Aqueous Solutions Using 'Insight'." Abstract accepted for the *International Symposium on Hydrothermal Reactions*, Gatlinberg, TN July 22-24, 1997.

Radiation Effects in Nuclear Waste Materials

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Project Manager and Principal Investigator

Dr. W.J. Weber

(509) 375-2299 (Phone)

(509) 375-2186 (Fax)

wj_weber@pnl.gov

Pacific Northwest National Laboratory

P.O. Box 999, MSIN K2-44

Richland, WA 99352

Co-Investigator

Dr. L.R. Corrales

(509) 375-6410 (Phone)

(509) 375-6631 (Fax)

lr_corrales@pnl.gov

Pacific Northwest National Laboratory

P.O. Box 999, MSIN K1-96

Richland, WA 99352

Contributors and Collaborators

R.E. Williford (PNNL)

H.L. Heinisch (PNNL)

J.D. Vienna (PNNL)

G.D. Maupin (PNNL)

M.J. Schweiger (PNNL)

J. Song (AWU - Post Doctoral Fellow)

R. Devanathan (AWU - Post Doctoral Fellow)

W. Jiang (AWU - Post Doctoral Fellow)

R.C. Ewing (University of New Mexico)

R.B. Bircher (ANL)

M. Nastasi (LANL)

S.D. Conradson (LANL)

J. Tesmer (LANL)

Research Objective

The objective of this multidisciplinary, multi-institutional research effort is to develop a fundamental understanding at the atomic, microscopic, and macroscopic levels of radiation effects in glass and ceramics. This research will provide the underpinning science and models for evaluation and performance assessments of glass and ceramic waste forms for the immobilization and disposal of high-level tank waste, plutonium residues and scrap, and excess weapons plutonium. Studies will focus on the effects of ionization and elastic collision interactions on defect production, defect interactions, diffusion, solid-state phase transformations, and gas accumulation using actinide-containing materials, gamma irradiation, ion-beam irradiation, and electron-beam irradiation to simulate the effects of α -decay and β -decay on nuclear waste glasses and ceramics. This program will exploit a variety of structural, optical, and spectroscopic probes to characterize the nature and behavior of the defects, defect aggregates, and phase transformations. Computer simulation techniques will be used to determine defect production, calculate defect stability, defect energies, damage processes within an α -recoil cascade, and defect/gas diffusion and interactions. A number of irradiation facilities and capabilities will be used, including user facilities at several national laboratories, to study the effects of irradiation under different conditions.

Research Statement

A significant challenge facing the environmental management and restoration activities at DOE facilities is the stabilization and immobilization of high-level tank waste, high-level sludge in the fuel storage basins, and Pu residues/scrap. In addition, the immobilization and disposal of surplus weapons-grade Pu is becoming a growing technological and political issue that is historically and politically tied to environmental management and restoration activities. These high-level wastes will be converted to glass or glass-ceramic waste forms for permanent disposal in a geologic repository. A key challenge is to develop predictive strategies and models, based on sound scientific understanding, to fully assess long-term performance. Self-radiation effects from α -decay and β -decay can significantly impact long-term performance. Waste forms for the disposal of Pu residues/scrap, surplus weapons-grade Pu, and other high-actinide waste streams may differ significantly in composition from the waste forms for high-level tank wastes and will experience significantly higher radiation doses and He production. The current baseline data and scientific understanding of radiation effects in glass, glass-ceramics, and crystalline phases are critically lacking. Without a fundamental understanding of radiation effects at the molecular, microscopic, and macroscopic levels, meaningful predictions of performance are not possible. This lack of understanding of the radiation-damage processes that occur also makes it impossible to extrapolate the limited existing data bases to larger doses, lower dose rates, different temperature regimes, or different compositions.

Research Progress

Work to date under this project has resulted in two manuscripts that have been submitted to journals for publication. These manuscripts are: W.J. Weber, R.C. Ewing, and A. Meldrum, "The Kinetics of Alpha-Decay-Induced Amorphization in Zircon and Apatite Containing Weapon-Grade Plutonium or Other Actinides," submitted to Journal of Nuclear Materials; and N.J. Hess, W.J. Weber, and S.D. Conradson, "Initial Results from Zr, U, and Pu XAS on Self-Radiation Effects in Glass and Ceramics," submitted to Journal of Materials Research. Other highlights of research progress are discussed below.

Theory, Simulations, and Modeling

Molecular Dynamics Simulation of Exciton Propagation in Network Materials

A semiempirical exciton propagation model that is applicable to covalently bonded networked systems has been developed. The model has been integrated into a molecular dynamics semiempirical methodology utilized at Pacific Northwest National Laboratory (PNNL). The exciton propagation algorithm is being tested on a model crystalline Si system that presents the simplest case and can be compared to other theoretical, simulation, and experimental results. The approach is a modification of the tight-binding checkerboard method of Hall and Whaley.¹

Initial tests have included propagating an exciton in a perfect lattice and in a distorted lattice consisting of eight atoms in a periodic diamond lattice. The exciton transfer probabilities are determined from the off-diagonal elements of the one electron Hamiltonian. The hole transfer probabilities are determined from the off-diagonal elements corresponding to the $3sp^3$ hybrid orbital overlaps between neighboring atoms. The electron transfer probabilities are determined from the off-diagonal elements for two distinct cases: 1) the electron transfers along the conduction band via the overlap between the $4s$ orbital of one Si atom and the $3sp^3$ hybrid orbital of the neighboring atom; and 2) the transfer occurs via the overlap of the $4s$ orbitals between neighboring atoms. Based on these tests, the former case is the preferred electron transfer mechanism in a perfect lattice, while the latter case is the preferred electron transfer mechanism for a distorted lattice. Current results indicate that the transfer of the excited electron utilizes both transfer pathways.

The density distribution of both the electron and the hole for a fixed lattice configuration has been calculated. In the perfect lattice, the exciton density distributes itself in groups of two unit cells and fluctuates between groups of atoms. In the distorted lattice, the exciton density is localized at the defect. The energetics of these two states indicate that trapping of the exciton at a distorted (defect) site is preferred. Thus, the exciton force contribution to the ions should yield to distortion from a perfect lattice state.

These initial studies have utilized a small periodic system to test, debug, and optimize the methodology. Due to constraints of *ab initio* and tight-binding methods on computer memory, other theoretical and simulation activities have focused on small molecular clusters or nanoparticles systems (~200 atoms) with surfaces. The current approach for exciton propagation uses a deterministic set of equations with matrices that require memory of the order of N^2 ; consequently, computations are currently constrained to small systems. To expand this approach to significantly larger systems (i.e., >1000 atoms), a Monte Carlo approach to simulate the exciton propagation in the network will be implemented. Application of this methodology to amorphous silica and other glasses will begin this summer.

Defect Energies in Crystalline Ceramics

Activities under this task are focused on using computational methodologies to determine fundamental defect energies in complex ceramics relevant to nuclear waste applications. Such defect energies are often difficult to determine experimentally, particularly for multi-cation ceramics. Two fundamental energy parameters important to radiation effects are the threshold displacement energies for the various cations and anions and the migration energies of radiation-induced defects (e.g., interstitials and vacancies). Both energy minimization methods and molecular dynamic simulations can be used in determining these energies. Initial work under this project has utilized energy minimization methodology, as described below. Computational-intensive molecular dynamic (MD) simulations, which are currently being developed and applied to SiC under a BES-sponsored project at PNNL, will be applied to ceramics relevant to nuclear waste applications later this summer. The initial MD simulations will focus on zircon (ZrSiO_4) using new interatomic potentials developed by R.W. Grimes at Imperial College, UK. (The MD simulations will also be used to study primary displacement processes in energetic displacement cascades.)

Displacement energies, E_d , and defect migration energies, E_m , are fundamental parameters controlling defect production, defect migration, and the kinetics of microstructure growth and phase transformations. These energies are not easily determined experimentally for many ceramic materials; however, advances in computational methodologies and their application to ceramics materials provide a means to determine these energies for a number of ceramic materials of interest. Although computationally intensive MD methods can be used to determine these energy parameters, energy minimization methods, such as utilized in the GULP code (General Lattice Utility Program, by J.D. Gale, Imperial College, London, UK), can be used to determine E_d and E_m for a number of ceramics. Calculations of E_d and E_m for several ceramics have been performed using GULP and compared to experimental values where available.

GULP uses a Mott-Littleton approximation to simulate isolated defects in extended solids. Two of its many options are particularly useful in the present work. For determining E_d , the ion "translate" option permits the movement of a selected ion to a point along a prescribed vector, after which the lattice is relaxed to determine if the ion returns to its original position, or assumes a new equilibrium position, such as an interstitial site. For determining defect migration energies, GULP contains a useful "transition" state option.

The validity of using GULP for these computations was established by calculating E_d and E_m in several ceramic materials where these energies are reasonably well known. The computational results show excellent agreement with accepted experimental values. For example, the calculated $E_d(\text{Al})$ and $E_d(\text{O})$ in Al_2O_3 were 19.4 and 56.1 eV, respectively, in good agreement with accepted experimental values (20 and 50 eV, respectively). The cation migration energy for MgO was calculated to be 2.4 eV, also in good agreement with accepted values (2.0-2.3 eV). Additional results for oxide ceramics with good supporting data bases (i.e., Al_2O_3 , MgO, ZnO) have been obtained and support validation of the methodology. Calculations have been initiated on ceramics relevant to nuclear waste, such as zircon, which have limited data bases. In the case of zircon, preliminary calculations indicate a displacement energy of 23 eV for oxygen.

Damage Cascades from α -decay

Displacement damage in zircon (ZrSiO_4) caused by α -decay of substitutional actinides on the Zr sites is being studied at the atomic scale using the binary collision approximation computer code MARLOWE. With MARLOWE, the collisional phase of the displacement cascade is modeled, following collisions of individual atoms in the zircon crystal structure until they no longer have enough energy to permanently displace another atom. From this method, information is obtained on cascade morphology and energy deposition as a function of material and irradiation conditions, such as the projectile mass and energy and the displaced atom type, as well as producing cascades at much higher energies and greater numbers than possible with MD.

The decay of ^{238}Pu , which results in a 5.5 MeV α -particle and a 94 keV recoiling ^{234}U nucleus, is being used as a test case because of the availability of experimental data. The α -particle has an extremely long path (relative to the "cascade" concept) and loses most of its energy to electronic excitation rather than displacement damage. The average recoiling ^{234}U creates a cascade about 25 nm in diameter, initially displacing about 650 atoms (120 Zr, 60 Si and 470 O). The fractions of the initially displaced atoms that remain after the cascade energy dissipates and after subsequent local annealing occurs by diffusion will be determined from further modeling using MD and stochastic annealing simulations. Cascade morphology was studied as a function of Zr recoil energy, revealing that the threshold energy for subcascade formation (more than one distinct damage region per cascade) is about 4 keV, which is consistent with lower-Z metals such as aluminum (2 keV). Because of its high atomic mass, ^{234}U recoils produce cascades that are significantly more compact than those from Zr recoils. Thermal spike

effects on defect survivability may be significantly different for ^{234}U recoils as compared to those of lighter ions, although the effect of recoil mass may not be as large in zircon as those observed in metals because of the light elements involved.

Models of α -decay-induced Amorphization

Zircon and apatite form as actinide host phases in several high-level waste forms and have been proposed as host phases for the disposition of Pu residues, excess weapons-grade Pu, and other high-actinide waste streams (e.g., highly separated commercial nuclear waste in France). Additionally, closely related structure types appear as actinide-bearing phases among the corrosion products of spent nuclear fuel and high-level waste glasses. Self-radiation damage from α -decay of the incorporated Pu or other actinides can affect the durability and performance of these actinide-bearing phases. For both zircon and apatite, these effects can be modeled as functions of storage time and repository temperature and validated by comparison with data from natural occurrences. Natural zircons and apatites, with ages up to 4 billion years, provide abundant evidence for their long-term durability because of their widespread use in geochronology and fission-track dating. Detailed studies of natural zircons and apatites, ^{238}Pu -containing zircon, a ^{244}Cm -containing silicate apatite, and ion-irradiated zircon, natural apatite, and synthetic silicate apatites provide a unique basis for the analysis of α -decay effects over broad time scales. Models for α -decay effects in zircon and apatite have been developed that show α -decay of Pu and other actinides will lead to a crystalline-to-amorphous transformation in zircon, but not in apatite, under conditions typical of a repository, such as the Yucca Mountain site. (Details of these models are provided in a manuscript that was submitted for publication.)

Based on these models, the critical temperatures above which amorphization cannot occur are 15°C and 29°C for silicate apatite containing 1 wt. % and 10 wt. % ^{239}Pu , respectively, and are 196°C and 218°C , respectively, for zircon containing 1 wt.% and 10 wt. % ^{239}Pu . These results suggest that apatite phases containing excess weapons Pu or other actinides will remain crystalline, due to high thermal recovery rates, under conditions expected for a near-surface repository, such as the Yucca Mountain site. In the case of zircon, α -decay of Pu and other actinides will lead to amorphization at conditions expected in a near-surface repository; however, the initial elevated temperatures in a geologic repository may retard amorphization during early storage times. The predicted amorphization behavior for zircon containing 1 wt. % ^{239}Pu is shown in Figure 1 as a function of time for several temperatures. These results show that the amorphization rate for zircon decreases rapidly above 190°C . At the higher temperatures, the amorphization rate is decreased even further due to both an increase in the recovery rate and a decrease in the actual α -activity, $A(t)$, due to the longer time scales (approaching one half-life).

Figure 1. Predicted amorphization behavior in zircon.

The actual behavior for apatites and zircons containing actinides may differ slightly from that discussed above due to compositional variations and impurities, which have a significant effect on kinetic processes. These models will be refined as more experimental and computational data become available.

Experimental Studies

Pu-Containing Glasses and Ceramics

Density, XANES/EXAFS, diffuse x-ray scattering, and stored energy measurements have been performed on a suite of compositionally identical Pu-doped (1 wt.%) waste glasses prepared with different α -activities by varying the $^{239}\text{Pu}/^{238}\text{Pu}$ isotopic ratio. The resulting α -activities in these glasses, which were prepared in July 1982, range from 1.9×10^7 to 4.2×10^9 Bq/g, and the accumulated dose ranges from 8.7×10^{15} to 1.8×10^{18} α -decays/g. Similar measurements have been carried out on a Pu-zircon (8.1 mole% ^{238}Pu was substituted for Zr) prepared in August 1981; the α -activity and accumulated dose in the zircon are 5.6×10^{10} Bq/g and 2.6×10^{19} α -decays/g, respectively. The macroscopic volume expansion of these glasses is shown in Figure 2 as a function of cumulative dose. The results suggest that for this range of α -activities there is no significant effect of the α -activity (i.e., dose rate) on the macroscopic density change in waste form glasses. Self-radiation from α -decay in the Pu-zircon results in the simultaneous accumulation of point defects and amorphous domains that eventually lead to a completely amorphous state.² The macroscopic volume expansion of the zircon is much larger than in the glasses as a result of a radiation-induced crystalline-to-amorphous transformation, reaching a saturation value of almost 17%.²

Figure 2. Macroscopic volume expansion as a function of cumulative dose.

Initial results from the XANES measurements at the Pu and U L_{III} -edges indicate that Pu and U occur in more highly oxidized valence states in the Pu-doped glasses than in the Pu-containing zircon. XANES measurements at the Fe and Zr K-edges reveal that the Fe and Zr oxidation states are unaffected by accumulated dose. Analyses of the Zr K-edge and Pu and U L_{III} -edges in the EXAFS show that the trend of local structural modification as a result of self-radiation damage is highly element-specific. Analysis of the Zr K-edge in the EXAFS of the Pu-doped glass samples indicates that the first shell atoms, consisting of O atoms, are increasingly disordered with increasing accumulated α -decay dose. However, the second and third shells, consisting of Si and O atoms, respectively, are much less affected. In contrast, analysis of the U L_{III} -edge EXAFS of the glass samples reveals a significant change in the first shell O atoms that is consistent with the reduction of U(VI) to U(IV) concomitant with the breakup of U(VI) oligomers that are present in the low-dose glass. Interestingly, analysis of the Pu L_{III} -edge EXAFS shows very little change in local structure with accumulated α -decay dose, suggesting that the initial Pu environment in the glass is a stable configuration for this α -activity level.

Because there is only one sample of zircon at a high dose (i.e., amorphized), it is not possible to draw conclusions about the systematic effects of self-radiation damage. The zircon cations reside in two distributions, one that is similar to a highly disordered zircon and a second that has Zr--Zr distances that are similar to baddeleyite, the monoclinic form of ZrO_2 . However, in the fully amorphous state that was analyzed, the U daughter product is present as U(IV), and it resides in a site unlike that of the Pu parent, suggesting that it is not in thermal equilibrium with the host. The Pu is present as Pu(III) and resides in a site that is very similar to $PuSiO_4$, the plutonium analog of zircon. Whether these effects are the result of self-radiation or artifacts of synthesis conditions will be tested in upcoming experiments.

Stored energy measurements have recently been completed on these glasses. The protocols for the preparation of specimens for transmission electron microscopy have been developed, and specimens will be prepared once the protocols are approved.

Gamma-Irradiation Studies

Temperature-controlled capsules have been designed and constructed for irradiations studies using the PNNL gamma irradiation facility. Six different glass compositions have been prepared, cut into wafers, and polished. Samples of each composition have been loaded into four different temperature-controlled capsules for long-term gamma irradiation (3 months to 1 year). Each capsule will be held at a different temperature (50°C, 100°C, 150°C, and 200°C). The glass samples are currently under irradiation in the PNNL gamma (Co-60) irradiation facility.

Electron and Ion Irradiation Studies

Several reference glasses are being used in initial electron and ion irradiation studies at Argonne National Laboratory and at Los Alamos National Laboratory to determine the conditions under which beam-charging effects can be minimized. Approaches that are currently being investigated include: 1) using electron flood guns, 2) using carbon coatings, and 3) using metal (aluminum, silver, or gold) coatings. Several series of irradiations on glasses are planned for the summer 1997. In addition, several ceramic specimens have been prepared for irradiation at the HVEM-Tandem facility (Argonne National Laboratory); the first of these irradiation studies on ceramics is scheduled for June 1997.

Reference Samples

Standard reference samples have been provided to Argonne National Laboratory and Los Alamos National Laboratory as part of informal collaborations. Single crystals of UO_2 have been provided to Los Alamos National Laboratory and the University of New Mexico under similar informal collaborations. Samples of reference phosphate glasses have been received from the University of Missouri - Rolla for radiation effects testing as part of a collaboration with their EMSP project on glass.

References

1. Hall, N.A., and K.B. Whaley. 1996. "Two-Particle Calculation of Excitonic Effects in Semiconductor Nanocrystals." *Chem. Phys.* **210**:117-133.
2. Weber, W.J. 1990. "Radiation-Induced Defects and Amorphization in Zircon." *J. Mater. Res.* **5**:2687-2697.

Soil and Groundwater Cleanup

Dynamics of Coupled Contaminant and Microbial Transport in Heterogeneous Porous Media

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

T.R. Ginn
(916) 752-1707 (Phone)
email: trginn@ucdavis.edu
172 Everson Hall
Department of Civil and Environmental Engineering
University of California, Davis
Davis, CA 95616-5294

Contributors and Collaborators

D.R. Boone
J.H. Cushman

Co-Investigators

M.M. Fletcher
(803) 777-3916 (Phone)
email: fletcher@biol.sc.edu
Belle W. Baruch Institute for Marine Biology and Coastal Research
University of South Carolina
Columbia, SC 29208

D.M. Friedrich
(509) 376-9637 (Phone)
email: dm_friedrich@pnl.gov
Environmental Molecular Sciences Laboratory
Pacific Northwest National Laboratory
P.O. Box 999, MSIN K8-96
Richland, WA 99352

E.M. Murphy
(509) 375-5914 (Phone)
email: em_murphy@pnl.gov
Environmental and Health Sciences Division
Pacific Northwest National Laboratory
P.O. Box 999, MSIN K3-61
Richland, WA 99352

Research Objective

Dynamic microbial attachment/detachment occurs in subsurface systems in response to changing environmental conditions caused by contaminant movement and degradation. Understanding the environmental conditions and mechanisms by which anaerobic bacteria partition between aqueous and solid phases is a critical requirement for designing and evaluating *in situ* bioremediation efforts. This interdisciplinary research project will provide fundamental information on the attachment/detachment dynamics of anaerobic bacteria in heterogeneous porous media under growth and growth-limiting conditions. Experiments will provide information on passive and active attachment/detachment mechanisms used by growing anaerobes capable of reductive dechlorination. Theoretical representations of these attachment/detachment mechanisms will be incorporated into existing groundwater flow and contaminant transport models that incorporate heterogeneity effects and can be used to predict behavior at field scales. These mechanistic-based models will be tested against experimental data provided through controlled laboratory experiments in heterogeneous porous media in large (meter-scale) 2-D flow cells. In addition to a mechanistic-based predictive model, this research will lead to new theories for the transient spatial distribution of microbial populations and contaminant plumes in heterogeneous porous media, improving our capability for designing staged remediation strategies for dealing with mixed contaminants.

Research Statement

The central challenge of *in situ* remediation strategies is the control of the transient spatial distribution of contaminants and remediation reagents (e.g., nutrients or microorganisms) in the presence of natural physical and chemical heterogeneities. Currently available predictive tools rely almost exclusively on the representation of passive attachment/detachment processes (e.g., filtration, sedimentation) with the bacterium treated as an inert biocolloid. One of the most intractable aspects of bioreactive transport, however, is active microbial partitioning (e.g., attachment/detachment) under growth and transport in physically and chemically heterogeneous systems. Active attachment/detachment is associated with microbial physiologic response to changes in local aqueous concentrations of nutrients and electron-acceptors: this process is treated rarely in field-scale bacterial transport theories, and not at all in available predictive tools.

Understanding coupled contaminant and bacterial transport in real subsurface media is critical to the success of potential future bioremediation/biobarrier strategies at DOE sites. Disposal of chlorinated hydrocarbons has generated extensive contaminant plumes in sand/gravel aquifers at DOE's Savannah River Site (SRS) in South Carolina and Paducah Gaseous Diffusion Plant (PGDP) in Kentucky, and such organic solutes interact with natural environments in coupled complex ways that have serious ramifications for remediation. For example, during the SRS *in situ* bioremediation demonstration, 90% of the increase in methanotroph biomass arising from degradation of trichloroethylene (TCE) partitioned into the aqueous phase. Initially, this methanotroph population was primarily attached to solid surfaces. If this partitioning behavior during bioremediation were understood and could be predicted, the knowledge could be exploited to effectively distribute the biomass across a contaminated area and to plan an injection

network so as to minimize costly well construction and dampen or eliminate microbially induced reductions in hydraulic conductivity. This basic knowledge on the transport response of microorganisms is required for any subsurface remediation involving microbes, including staged approaches that sequentially degrade and immobilize multiple contaminants. Staged approaches (temporal or spatial) can exploit the different transport properties of distinct microbial populations and are especially well suited to DOE mixed contaminant problems.

The purpose of the proposed research is to address the limitations in understanding reactive contaminant transport and dynamic bacterial attachment/detachment under growth and growth-limiting conditions in natural porous media. This research will assess the coupled degradation and transport processes involving biological agents, with focus on the active and passive attachment/detachment and transport processes of anaerobic microorganisms involved in TCE degradation, including their persistence under transient variations in substrate and electron-acceptor availability. Experiments will be performed in natural geologic materials that display general physical and chemical heterogeneity patterns representative of TCE-contaminated sandy media at SRS and at PGDP. The goals of the research are to develop basic knowledge of the fundamental relationship between contaminant transport and active bacterial transport and attachment/detachment under transient local chemical conditions. This knowledge will afford a quantitative understanding of the transient spatial distribution of contaminants and microbial populations during contaminant degradation and microbial growth in physically and chemically heterogeneous media. To accomplish this end, the proposed research includes an integrated effort toward the development of predictive models of contaminant transport and degradation in heterogeneous media, through a combined multi-scale experimental and theoretical approach.

Research Progress

The work and associated spending on this project are purposely accelerated during the second half of fiscal year 1997. The reason for this is threefold: 1) the DOE research contracts with key collaborators (Fletcher and Cushman) and the subcontract with Dr. Boone were not initiated until December 1996, 2) the establishment of contracts and subcontracts delayed the search and hiring of post-doctoral associates, and 3) one of the principal investigators, Timothy Ginn, has moved to the University of California, Davis and the subcontracting to this PI has only recently been completed. This multidisciplinary research project is highly integrated. As such, the progress and results are also highly integrated among the different institutions. The collaborators met in October 1996 to discuss the selection of microbial strains and initial growth kinetics. An additional meeting is planned for July 1997 to enhance collaboration and interpretation of results.

Selection of Strains

Four strains were selected for study in this project. *Desulfomonile tiedjei* was selected because it is capable of dechlorination. The kinetics of this organism can be studied as a pure culture, both in suspension and in porous media, by providing it with a reduced substrate (e.g., formate) from which it can acquire the reducing equivalents it needs to dechlorinate. As well, this organism can be combined with another bacterial strain that can produce organic compounds

such as formate. The propionate-oxidizing bacterium, strain LYP, that can oxidize propionate in co-culture with hydrogen- or formate-using strains, was selected. Thus, co-cultures of strain LYP and *D. tiedjei* can be grown with propionate as the electron donor and one of several chlorinated compounds as electron acceptor. Two other bacteria that grow by using hydrogen and formate as electron donors were also selected. Co-cultures of strain LYP (which produces formate) with *D. tiedjei* will be studied for kinetics of growth and interspecies formate transfer, but we can also add another formate-using bacterium as a third member to determine whether it can out-compete *D. tiedjei* for formate. The two other formate-using bacteria selected are *Desulfovibrio* strain G-11 and *Methanospirillum hungatei*. These two species have very different thresholds of formate concentration that are required for formate uptake. In addition, the natural fluorescent properties of *M. hungatei* will be used to directly measure real-time, *in situ* biomass concentrations during the transport experiments (see In Situ Spectroscopy progress below). These methanogens contain the unique electron carrier, F420, that autofluoresces a blue green color with a peak at 420 nm when excited by UV light.

Determination of Growth Kinetic Constants

Experiments have begun at the Oregon Graduate Center to determine Monod constants for the growth of the four selected microbes. A 14-liter New Brunswick fermentor has been modified to serve as a continuous culture fermentor, with culture medium added by an high-performance liquid chromatograph (HPLC) pump and passing through a bacteriological filter in-line to the fermentor. The first "shake-down" experiment in the fermentor is complete. In this experiment, *Desulfovibrio* strain G-11 with formate and sulfate were grown as substrates. The culture grew in the fermentor, demonstrating steady-state growth. After several modifications to the fermentor to enhance our ability to acquire samples, these experiments will be repeated in an attempt to determine the growth constants (Monod) in a dilute buffer solution that will be used for the adhesion experiments and kinetic studies of growth in saturated media. At the same time as the continuous culture studies, batch culture studies will be carried out to determine the maximum growth rate and the growth yield of this culture.

Surface Characteristics of Anaerobes

A post-doctoral associate was recently recruited to carry out the work on adhesion properties and kinetics of attachment/detachment of anaerobic microorganisms under the guidance of Dr. Fletcher at the University of South Carolina. Paula van Schie has just obtained her Ph.D. from Rutgers University, and was trained in anaerobic microbiology in Dr. Lily Young's laboratory. When she begins work at USC at the end of June, Dr. Schie will initially assess the adhesive properties of the test organisms and determine the influence of physiological conditions on degree and stability of adhesiveness. These experiments are detailed as follows.

Previous studies with aerobic organisms have demonstrated that cell adhesiveness can be affected by different nutrient conditions, such as types and concentration of available nutrients, carbon: nitrogen ratios, and degree of starvation of the organisms. The variability in adhesion with nutrient conditions varies with the organism; some show little change while others

demonstrate significant changes in adhesiveness. Thus, adhesion properties of anaerobic organisms might be expected to alter with their physiological state. It is important to assess the adhesive properties of *D. tiedjei* and *M. hungatei* and the variability in these properties with growth and starvation conditions, because of their important roles in subsurface bioremediation. Adhesion is first assessed by batch kinetic assays, using flat coupons of glass and siliconized or iron-coated glass as test substrata. Test conditions include cells freshly grown in a nutrient-rich medium and cells starved in MSNS-PIPES buffer (a dilute buffer solution more representative of nutrient levels found in groundwater) for periods of 48 to 96 hours. After establishing the degree of variability in adhesiveness with the test strains, specific growth and maintenance conditions are established for batch kinetic adhesion experiments. Relative hydrophobicity and surface charge of the test organisms under different growth and maintenance conditions will also be determined by retention of cells on octyl- or phenyl-Sepharose beads and ion exchange resins. In this way the precise culture and maintenance conditions that must be used for adhesion assessment and kinetic experiments for these organisms can be assessed, so that these data are relevant to future transport experiments.

Preliminary measurements of surface charge and morphology have been made on the selected anaerobes in this project at Pacific Northwest National Laboratory (PNNL). The electrophoretic mobility of the different strains was measured using a Brookhaven Instruments-ZetaPlus analyzer. The charge of the particles is measured by applying an external electrical field and measuring the direction and rate of movement of the bacteria. The anaerobic strains were suspended in a dilute media that was designed to provide the necessary growth nutrients for the anaerobes, but at levels that are more realistic for groundwater. As expected for most bacteria, the membranes carried a net negative charge expressed as mobility (e.g., ratio of particle velocity in Fm s⁻¹ to field strength expressed as Vcm⁻¹). The mobilities measured were -0.92, -2.72, and -2.32 for *M. hungatei*, *Desulfovibrio* strain G-11, and *D. tiedjei*, respectively. These values are preliminary, but are similar to other reported values for bacteria (Bayer and Sloyer 1990). *M. hungatei* have flagella and are chemotactic, which presents an additional challenge for describing their transport in porous media.

The cell morphology affects transport in porous media and is also being measured for these selected strains. Preliminary analyses using a Macrotrac Ultrafine Particle Analyzer were inconsistent with cell size information found in the literature. This is likely because this analytical procedure is based on Stokes law for spherical particles. The anaerobic cells used in this project are not spherical. *D. tiedjei* is an elongated rod, 4-5 Fm long by ~0.5 Fm wide, while *M. hungatei* cells are curved, 0.5 by 7 Fm in diameter. Cell size is currently being assessed using phase-contrast microscopy.

In Situ Spectroscopy

As mentioned above, the natural fluorescent properties of methanogens, *M. hungatei* in particular, are being used to develop the capability to directly measure real-time, *in situ* biomass concentrations during the transport experiments. Fluorescence emission and excitation spectra of *M. hungatei* in dilute aqueous suspensions were measured at PNNL to determine optimum

wavelengths for excitation and detection of the microbes in column and flow cell experiments. Original samples ($\sim 5 \times 10^7$ cells/ml) were diluted 10 times to $\sim 6 \times 10^6$ cells/ml and placed in a 1 cm x 1 cm fused quartz cuvette in the fluorometer. Excitation was by continuous xenon lamp through a double monochromator, which provided monochromatic light at selected wavelengths and protected the sample from scattered UV and visible light. Fluorescence was detected at right angles to the excitation direction through a double monochromator with a cooled, photon-counting photomultiplier detection system. Excitation spectra were measured by recording emission intensity near one of the fluorescence wavelength peaks (426 nm) as a function of the excitation wavelength, which was scanned. Emission spectra were measured by setting the excitation wavelength near one of the two excitation maxima (292 nm or 340-350 nm) followed by recording emission intensity as a function of the scanned wavelength of the emission monochromator.

Under UV excitation (290-350 nm) in the fluorometer, bright fluorescence was observed visually from the original, undiluted samples. The 10x diluted samples produced a fluorescence intensity of $\sim 18,000$ counts/second. The intensity was approximately proportional to the concentration. Repeated scans indicate that the samples were relatively stable under continuous monochromatic UV radiation. Excitation maxima were observed at 292 nm (narrow band) and 340 nm (broad band) (Fig. 1a). The emission maxima depended on the excitation wavelength. Near UV excitation (340 nm) produced broad, somewhat structured blue emission peaking at 426 nm (Fig. 1b). Excitation in the quartz-UV (292 nm) produced a broad, unstructured near-UV emission band peaking around 365 nm (Fig. 1c). The buffer solution showed no fluorescence in the absence of microbes. (Fig. 1b).

These two excitation and emission band systems correspond to two different fluorophore centers in the microbes. The longer wavelength system (340 nm excitation, 426 nm emission) is suited for direct excitation and observation through pyrex (borosilicate) glass columns. Initial experiments will monitor transport of *M. hungatei* through 1" dia x 8" long glass columns filled with Accusand. Excitation will be from an argon ion laser (351 nm). Fluorescence will be detected through wavelength selective filters using a photon-counting photomultiplier.

Modeling

Significant advances have been made on two fronts in the modeling on this project at Purdue University. First, we have developed a multidimensional mathematical model representing sticky brownian motion with drift, as a general representation of microbial transport with attachment/detachment. This model has been implemented on the computer, and is being tested using data from Madilyn Fletcher's laboratory. A sticky brownian motion with drift allows a particle (e.g., microbe) to diffuse like a classical brownian motion in a pressure-induced convective field, except when it hits a wall. When it hits the wall, it sticks for a random period of time, which is dictated by a probabilistic stickiness condition. For comparison to Dr. Fletcher's data, the model is being applied within the experimental setting of the microflow

chamber that is being used in her laboratory (i.e., the particles move in a similar dimensioned cell under similar pressure gradients). We are currently computing exit time probability densities as a function of pecelet number and the flow cell length ($Pe=d/vl$ where v is the flux, d is the diffusion coefficient, and l is the flow cell length).

Figure 1. Excitation (a) and Emission (b) Spectra of *M. Hungateii* in Buffer Solution. The emission spectra (c) of the bacteria diluted 10% with excitation at 292 nm.

Second, a revolutionary result on upscaling stochastic transport of chemicals and bacteria has been obtained. Full characterization information of physical and chemical heterogeneity is rarely available for field sites, hence stochastic representations are often used to describe transport. Stochastic representations of transport arise in the presence of uncertain subsurface properties, which are treated as random (e.g., stochastic) variables. The resulting expressions for stochastic concentrations relate the statistical properties of concentration to the statistical properties of the porous media. These expressions involve an infinite series of statistical moments and thus have been impossible to solve explicitly (e.g., the expressions are not closed). This is known as the closure problem, and is inherent in upscaling any transport process in natural porous media. The closure problem has been solved and explicit upscaled solutions for the stochastic solute concentration, its moments, the macroscale dispersive flux, and the variance of fluctuating concentration for several standard problems have been produced. This solution is currently being implemented on the computer at the University of California, Davis so that actual transport problems can be tested. Because of the significance of this closure solution to the field of transport in porous media, a manuscript is in preparation and will be submitted this summer.

Reference

Bayer, M.E., and J.L. Sloyer, Jr. 1990. "The Electrophoretic Mobility of Gram-Negative and Gram-Positive Bacteria: An Electrokinetic Analysis." *J. Gen. Microbial.* **136**:867-874.

Genetic Analysis of Stress Responses in Soil Bacteria for Enhanced Bioremediation of Mixed Contaminants

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

K.K. Wong, Ph.D.

Senior Research Scientist

(509) 375-5097 (Phone)

(509) 376-9449 (Fax)

k_wong@pnl.gov

Pacific Northwest National Laboratory

PO Box 999, MSIN P7-56

Richland, WA 99352

Collaborators

Professor R. Olsen

(313) 764-4380 (Phone)

ronolsen@umich.edu

Department of Microbiology and Immunology

5605 Medical Science Building II

University of Michigan Medical School

Ann Arbor, MI 48109-0620

Research Objective

To provide increased knowledge of stress responses of indigenous microbes at contaminated sites as well as using microbes as molecular probes for monitoring the performance and effectiveness of bioremediation, we propose to identify the stress-inducible genes and promoters from two soil bacteria, *Deinococcus radiodurans* and *Sphingomonas* F199. These organisms represent two phylogenetically distinct groups of soil bacteria, each of which has specific features of interest for bioremediation. *D. radiodurans* exhibits high resistance to external stress; F199 is a deep subsurface (Savannah River Site) organism with unique degradative capabilities.

Research Statement

To realize the full potential of bioremediation, an understanding of microbial community and individual bacterial responses to the stresses encountered at contaminated sites is needed. Knowledge about genetic responses of soil and subsurface bacteria to environmental stresses, which include low nutrients, low oxygen, and mixed pollutants, will allow extrapolation of basic principles to field applications either using indigenous bacteria or genetically engineered microorganisms. Defining bacterial responses to those stresses presents an opportunity for improving bioremediation strategies, and should contribute to environmental management and restoration actions that would reduce the cost and time required to achieve DOE's cleanup goals.

Stress-inducible genes identified in this project can also be used as molecular probes for monitoring performance of indigenous bacteria as well as the effectiveness of bioremediation strategies being employed. Knowledge of survival and catabolic plasmid stability of indigenous bacteria will be needed for devising the most effective bioremediation strategies. In addition, stress-inducible regulatory elements identified in this project will be useful for creating genetically engineered microorganisms able to degrade hazardous wastes under stress conditions at contaminated sites.

Research Progress

Research Performed

Analysis of Starvation Stress on Gene Expression

Our initial effort has been directed toward identifying starvation-induced genes and their promoters using ribonucleic acid (RNA) arbitrarily primed polymerase chain reaction (RAP-PCR) methodology. The first task in this approach was to identify appropriate starvation conditions for *D. radiodurans*. Cultures of *D. radiodurans* were grown to saturation in a minimal media (Raj. et al. 1960), harvested, washed, and suspended to a density of 1×10^{10} cells per liter in the minimal media minus glucose. The starvation cultures were incubated at room temperature without shaking for 0, 4, 14, or 21 days. Direct cell viability counts showed that

D. radiodurans was maintained in this media without growth for at least 21 days. The RNA was isolated from cells at each time point and RAP-PCR performed. Five different arbitrary primers were screened and six tentative differentially expressed genes identified. An example of the RNA fingerprint is shown in Figure 1. Putative differentially identified bands were cut out of the gels, cloned into the pCR-Blunt vector (InVitrogen), and sequenced using an Applied Biosystems 373 automated ribosomal RNA (rRNA) sequencer. Three of the putative clones were identified as either 16S or 23S rRNA. Two clones share sequence homology with open reading frames (ORFs) of unknown function. Northern analysis, a standard method used to analyze levels of RNA, is currently underway to verify the differential expression of these two clones. Sequencing of the sixth identified clone is being pursued.

Figure 1. Identification of Starvation-Inducible Genes From *Deinococcus Radiodurans* Using RAP-PCR.

Starvation culturing condition has also been worked out for *Sphingomonas aromaticivorans* F199. RNAs were isolated from cells that have been starved for 1 day, 4 days, and 21 days. Six different arbitrary primers have been screened and seven putative differentially expressed genes have been identified. Three of them are novel but the rest are either 16S or 23S artifacts. An example of the RNA fingerprint is shown in Figure 2.

Analysis of Other Stresses on Gene Expression

The effect of toluene and peroxide on the viability of both *D. radiodurans* and *S. aromaticivorans* F199 were studied. Preliminary studies indicated that starved or stationary cells of *D. radiodurans* can tolerate toluene at a level of up to 50% (V/V) for a period of 1 hour. However, after 12 hours incubation with 12% toluene, the cell titer decreased by 4 orders of magnitude. On the other hand, exponentially growing cells are highly sensitive to toluene and were killed completely within 1.5 hours. Genetic response to toluene stress at sublethal levels

is being pursued. The physiological response to peroxide stress has been studied; findings indicate that the level of resistance was growth phase-dependent (Figure 3). Efforts are currently underway to identify genes whose expressions are growth regulated or induced during late log phase (Figure 4).

Figure 2. Identification of Starvation-Inducible Genes From *Sphingomonas Aromaticivorans* Using RAP-PCR.

Figure 3. Peroxide Resistance of *D. Radiodurans* at Different Growth Phase.

Figure 4. Identification of Genes Whose Expression is Growth-Phase-Dependent by RAP-PCR. Arrows indicate genes that are growth-phase regulated.

Methodology Development

To further improve the RNA fingerprinting methodology, a 16S and 23S subtraction step is being developed that will eliminate the cloning of 16S and 23S from background amplification. Besides the RAP-PCR technology, several new approaches are being pursued to identify stress-induced promoters. One of these approaches uses a promoter probe plasmid for promoter fusion experiments. A promoter probe plasmid has been constructed based on a *Deinococcus* cloning vector that contains a promoter-less chloramphenicol reporter gene. A *Deinococcus radiodurans* promoter library has been constructed with this plasmid and promoter clones will be screened for the expression of chloramphenicol resistance under different stress-induced environments. A promoter library has been constructed from *Sphingomonas* using a broadhost range promoter probe plasmid pRKZ1-um (supplied by Ronald Olsen) using *lacZ* reporter gene. Current efforts are aimed at establishing an effective transformation system in both *D. radiodurans* and *S. aromaticivorans* F199. Dr. Ronald Olsen, University of Michigan, will provide several plasmid vectors that include the clone containing toluene 3-monooxygenase for establishing a genetic system in these bacteria. Furthermore, a set of 96 arbitrary primers, which are available from another unrelated sequencing project, are being screened for generating more informative fingerprints. DNA fragments and probes are currently being designed to define stress responses of genes involved in contaminant metabolism, plasmid replication, and plasmid transfer in *Sphingomonas aromaticivorans* F199.

Summary of Findings

- Two putative novel genes that expressed at starvation condition from *Deinococcus radiodurans* were cloned and sequenced.
- Three putative novel genes that expressed at starvation condition from *Sphingomonas aromaticivorans* F199 were cloned and sequenced.
- Findings indicate that starved or stationary cells of *Deinococcus radiodurans* are more resistant to toluene stress than exponentially growing cells.
- It has been shown that the high resistance to hydrogen peroxide (up to 100mM) of *Deinococcus radiodurans* is growth-phase-dependent.

The isolated starvation genes from both *S. aromaticivorans* and *D. radiodurans* will be used to develop gene expression systems to express toluene 3-monooxygenase in the corresponding bacteria. Based on our findings, stationary cells of the to-be-engineered bacteria will be evaluated for their degradative ability to trichloroethylene (TCE) and toluene in pure culture study. In addition, we will test whether hydrogen peroxide will create a niche for *D. radiodurans* when the bacteria is inoculated to soil samples with an established microbial community. Because of its high level of hydrogen peroxide resistance, it is expected that *D. radiodurans* could be established in any new environment when applied in environmental soil samples with an established microbial community.

Reference

Raj, H.D., F.L. Duryee, A.M. Deeney, C.H. Wang, A.W. Anderson, and P.R. Elliker. 1960. "Utilization of Carbohydrates and Amino Acids by Micrococcus Radiodurans." *Canadian Journal of Microbiology* 6:289-298.

Molecular-Level Processes Governing the Interaction of Contaminants with Iron and Manganese Oxides

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigators

S.A. Chambers

(509) 376-1766 (Phone)

sa_chambers@pnl.gov

Pacific Northwest National Laboratory

PO Box 999, MSIN K8-95

Richland, WA 99352

G. Brown

(415) 723-9168 (Phone)

gordon@pangea.stanford.edu

Stanford University

Department of Geological and Environmental Sciences

Stanford, CA 94305-2115

Contributors and Collaborators

J.E. Amonette (PNNL)

D.A. Dixon (PNNL)

S.A. Joyce (PNNL)

J.R. Rustad (PNNL)

Research Objective

The central tenet of this proposal is that *a fundamental understanding of specific mineral surface-site reactivities will substantially improve reactive transport models of contaminants in geologic systems, and will allow more effective remediation schemes to be devised.* Most large-scale, macroscopic models employ global chemical reaction kinetics and thermochemistry. However, such models do not incorporate molecular-level input critical to the detailed prediction of how contaminants interact with minerals in the subsurface. A first step leading to the incorporation of molecular-level processes in large-scale macroscopic models is the ability to understand which molecular-level processes will dominate the chemistry at the microscopic grain level of minerals. To this end, our research focuses on the fundamental mechanisms of redox chemistry at mineral surfaces. As much of this chemistry in sediments involves the Fe(III)/Fe(II) and Mn(IV)/Mn(II) couples, we focus on mineral phases containing these species. Of particular interest is the effect of the local coordination environment of Fe and Mn atoms on their reactivity toward contaminant species. Studies of the impact of local atomic structure on reactivity in combination with knowledge about the types and amounts of various surfaces on natural grain-size minerals provide the data for statistical models. These models in turn form the basis of the larger-scale macroscopic descriptions of reactivity that are needed for reactive transport models. A molecular-level understanding of these mechanisms will enhance the ability to design much greater performance efficiency, cost effectiveness, and remediation strategies that have minimal negative impact on the local environment. For instance, a comprehensive understanding of how minerals that contain Fe(II) reduce oxyanions and chlorinated organics should enable the design of other Fe(II)-containing remediation materials in a way that is synergistic with existing minerals in the subsurface environment.

Research Statement

Contamination of subsurface sediments with radionuclides, organic solvents, and Resource Conservation and Recovery Act (RCRA)-listed metals is one of the many challenging environmental problems at U.S. Department of Energy (DOE) weapons sites. Addressing this problem requires: (i) an accurate assessment of the health and environmental risks associated with these subsurface contaminants (in particular, a prediction of transport rates and degradation products in the subsurface), and (ii) the development of reliable and economical remediation schemes. These two activities, in turn, critically depend on an adequate understanding of the chemistry that occurs when contaminants encounter the solid surfaces (largely minerals) present in the subsurface. In addition to adsorption and precipitation at these surfaces, many of the contaminants (e.g., Pu, U, Cr(VI), CCl₄, TCE, and perchloroethylene) can undergo electron-transfer reactions that can substantially alter the species and, thereby, either help or hinder the remediation process. Predicting the movement of redox-sensitive contaminants through subsurface materials poses a difficult challenge, made more difficult by a lack of fundamental knowledge about the mechanisms and rates of redox reactions with exposed mineral phases. Manipulation of the redox status of aquifers, and the contaminants they contain, by the *in-situ* reduction of iron-bearing minerals, or the addition of elemental iron, are two examples of

proposed remediation techniques that take advantage of redox chemistry at solid surfaces to remove contaminants from groundwater. Although these remediation techniques have shown some success in laboratory-, intermediate-, and field-scale demonstrations, this success has been somewhat fortuitous in that little is actually known about the manner in which they work, and the manner by which they might fail. Clearly, both risk-assessment and remediation activities can benefit from a better understanding of the mechanisms of redox reactions at mineral surfaces.

Research Progress

Growth of Model Fe-Oxide Surfaces by Molecular Beam Epitaxy

Successfully developed, for the first time, is a comprehensive methodology for growing very well defined surfaces of the oxides of iron by molecular beam epitaxy (MBE). Such surfaces are essential for gaining a detailed, molecular-level understanding of sorption and redox chemistry of contaminants on iron-bearing minerals; naturally occurring iron oxides are, in general, too heterogeneous and ill-defined to be useful for this kind of research. Our approach allows us to span the full range of stoichiometry and surface orientation. In particular, we can now routinely prepare Fe_{1-x}O (wustite) (001) and (011), where $x = 0$ to ~ 0.2 , Fe_3O_4 (magnetite) (001) and (011), $\gamma\text{-Fe}_2\text{O}_3$ (maghemite) (001) and (011), and $\alpha\text{-Fe}_2\text{O}_3$ (hematite) (0001), (1120) and (1012) surfaces. All surfaces are phase-pure and possess negligible densities of point defects. With the exception of (011)-oriented FeO , Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$, these surfaces are quite flat, with mean roughnesses of a few Å, as measured by x-ray reflectivity. The (011)-oriented FeO , Fe_3O_4 , and $\gamma\text{-Fe}_2\text{O}_3$ are rough only because the $\text{MgO}(011)$ substrates upon which they are grown facet while being thermally cleaned prior to MBE growth. The key parameters that determine the characteristics of the as-grown surfaces are substrate temperature, iron flux, oxygen flux, and growth rate. We have developed a phase diagram for the formation of MBE-grown films of the four iron oxides mentioned above, and can now produce these surfaces with very high yields for the other investigators in this program.

Scanning Probe Microscopy Images of Model Iron Oxide Surfaces

Two epitaxially grown $\text{Fe}_3\text{O}_4(100)$ surfaces have been examined with scanning tunneling microscopy. One sample was 50 nm thick, the other 250 nm. Both were cleaned in ultrahigh vacuum by repeated annealing in oxygen. The thinner sample was heated to 300°C, while the thicker film was generally annealed at 380°C. A small amount of magnesium out-diffusion from the MgO substrate was observed for the thinner sample after ~ 30 hours of cumulative annealing. No such out-diffusion was observed for the thicker sample, even though it was heated to higher temperatures, consistent with standard diffusion models, which predict a time-squared rate dependence on thickness. In general, both surfaces are found to be relatively flat. Over length scales of a few hundred nanometers, roughly 4 to 5 exposed layers are typically observed. For the 50 nm film, the uppermost layer comprises dispersed, oriented square islands a few nanometers in size. These islands are on successively larger islands, which ultimately coalesce. A preferred orientation of the edges of the islands is observed. Based on preliminary low-energy electron diffraction experiments, the orientation is tentatively assigned to the $\langle 100 \rangle$ direction.

For the 250 nm film, the islands are larger, the edges more irregular, and they show a pronounced anisotropy (i.e., the islands are longer in one direction than the other). Low-energy electron diffraction experiments are planned to determine the relevant crystallographic directions of this anisotropy. Based on these observations, it appears that the films grew via an island-nucleation mechanism, as opposed to either step flow or ballistic deposition. Step-flow growth is characterized by large, flat terraces with little or no island density. Ballistic or random growth is characterized by statistically rough surfaces. For example, the 50-nm-thick film would have, on average, 16 exposed layers if ballistic growth occurred, as opposed to 5 layers observed. The island edges represent surface sites that are under-coordinated with respect to the ideal terrace sites and as such may have different chemistries. A detailed analysis of the edge densities is currently underway. Initial estimates suggest that the densities are only of the order of 10% for the 50 nm film and lower yet at 5% for the 250 nm film. For comparison, single crystals of "high quality" samples, such as commercially available semiconductors or metals, still have defect densities of 2-3%. Comparison of the heterogeneous chemistry on samples with differing defect densities will allow for an assignment of the importance of defects in influencing sorption and redox of contaminants.

Synchrotron Radiation Studies of Aqueous/Fe-Oxide Interfaces

Grazing-incidence x-ray absorption fine structure spectroscopy (GI-XAFS) studies of aqueous Pb(II) sorbed on single-crystal MBE-grown films of $\alpha\text{-Fe}_2\text{O}_3(0001)$, $(11\bar{2}0)$ and $(10\bar{1}2)$ were carried out at Stanford Synchrotron Radiation Laboratory (SSRL). The XAFS results show that Pb(II) is chemi-sorbed on $\alpha\text{-Fe}_2\text{O}_3(10\bar{1}2)$ in an inner-sphere fashion, which is the sorption mode of aqueous Pb(II) on $\alpha\text{-Al}_2\text{O}_3(10\bar{1}2)$. Pb(II) also appears to chemi-sorb on $\alpha\text{-Fe}_2\text{O}_3(0001)$ in an inner-sphere fashion, initially, but apparently undergoes some type of coupled photocatalytic oxidation-reduction reaction in which a gas is liberated in the sample cell; the Pb-XAFS spectrum changes significantly after an hour of exposure of the sample to the synchrotron x-ray beam. We plan to repeat this experiment to more fully document and understand these changes.

Soft x-ray XAFS studies of the interaction of aqueous PO_4 with natural hematite surfaces were also carried out. A natural $\alpha\text{-Fe}_2\text{O}_3(0001)$ surface was prepared by cleaving and polishing for these experiments. The sample was placed in our main vacuum chamber on SSRL beam line 3-3 for LEED and surface EXAFS studies. The clean surface was found to give a good LEED pattern characteristic of the (0001) surface. Photoemission experiments also showed the surface to be relatively free of contaminants, with only minor amounts of C, Si, and Al detected. This sample was then reacted with an aqueous solution containing 30 μg of KH_2PO_4 for ten minutes in a nitrogen-filled glove bag that was connected to the preparation chamber of the UHV system. P K-XAFS spectra were collected out to an energy of 250 eV above the P K-edge. A similar experiment also was carried out on PO_4 deposited on a reduced hematite surface, which is similar to magnetite. The EXAFS spectra and Fourier transforms of these two samples show significant differences that suggest a different sorption mode for PO_4 on the two surfaces. The data are currently being fit to derive detailed models of the sorption geometry of PO_4 surface complexes.

A study was initiated of the interaction of water with MBE-grown hematite and magnetite as a necessary step before reacting them with aqueous solutions containing chromium and chlorocarbons. These experiments were carried out on SSRL beamline 10-1. Photoemission (O 1s) and Fe L-edge and O K-edge XAFS data were collected on $\alpha\text{-Fe}_2\text{O}_3(0001)$ and $(10\bar{1}2)$, and on $\text{Fe}_3\text{O}_4(110)$ magnetite model surfaces. These measurements were also made on natural hematite (0001) and magnetite (110) surfaces, and on natural polycrystalline samples of goethite and limonite. The goethite and limonite data show clear signatures of OH groups coordinating iron, which serve as fingerprints for the dissociative chemisorption of water on water-exposed hematite and magnetite surfaces. The photoemission data show that the threshold water pressure required for extensive reaction on $\text{Fe}_2\text{O}_3(0001)$ is a 2×10^{-4} torr exposure for three minutes. In contrast, the same orientation of $\alpha\text{-Fe}_2\text{O}_3(0001)$ required a threshold water pressure of 2-4 torr for three minutes before significant interaction occurred.

Plans are underway to continue studies of the interaction of water on other orientations of MBE-grown hematite and magnetite surfaces. This work should lead to an estimate of defect levels on these surfaces, which is critical for understanding reactivity on iron oxide surfaces in general. This work will have direct bearing on planned studies of the reaction of chromium on these surfaces, which will be a major focus of the work during the remainder of year 1. In addition, GI-XAFS studies of Cr deposited from aqueous solutions onto MBE-grown hematite and magnetite surfaces will be initiated. These studies will reveal the geometric details of the sorption products, which are necessary for defining reaction mechanisms. The uptake of Cr(VI) and Cr(III) on high-surface-area hematite and magnetite powders using powder XAFS methods will also be investigated.

Chromate Adsorption Kinetics Using Photoacoustic Spectroscopy

The ability to detect very low concentrations of chromate in solution in real-time using pulsed-laser photoacoustic spectroscopy has been developed. This technique relies on absorption of light at a specific wavelength by the analyte and the generation of an acoustic wave from heat released as the atom or molecule returns to the ground state. The amplitude of the acoustic wave is proportional to the concentration of the analyte and is detected using a pressure transducer in acoustic contact with the solution. Because 90-100% of the light energy absorbed by molecules is typically released as heat, rather than as fluorescence, photoacoustic detection has an inherent sensitivity up to two orders of magnitude greater than fluorescence spectroscopy and several orders of magnitude greater than conventional absorption spectroscopy.

Initially, we used light at 355 nm from a Nd-YAG laser to excite chromate in a pH-3 HCl solution in a standard 1-cm path-length quartz cuvette. The maximum absorbance of chromate is at about 372 nm, and the relative absorbance at 355 nm is ~50% of the maximum. Thus, the detection limits we are able to obtain thus far are at least twice as large as those achievable with light of the appropriate wavelength. The appropriate laser capability will become available in June 1997 with the final acceptance of an Alexandrite tunable laser. Detection was accomplished using a Pb-Zr-titanate (PZT) pressure transducer attached to the outside of

the cuvette. The transducer signal was amplified 500 times and the area under the initial photoacoustic signal was integrated using a boxcar integrator.

Preliminary experiments consisted of measurements of the photoacoustic signal with 1.5 ml of stock solution in the cuvette in the presence and absence of chromate and of an 8-mm diameter $\alpha\text{-Al}_2\text{O}_3(0001)$ disk of the type used as substrates for MBE synthesis of hematite surfaces. The solution was spiked with 30 ng of Cr(VI); it was then possible to establish a detection limit of 2-4 ng Cr(VI). The sapphire disk, which had been equilibrated with pH-3 HCl for a minimum of 30 minutes, was then immersed in the solution and rested at the bottom of the cuvette below the light path. The decrease in concentration of Cr(VI) to 0 ng could then be followed over a period that ranged from 90 minutes to 210 minutes, depending on whether chromate adsorbed to the disk had been cleaned off prior to a specific run. Adsorption of Cr(VI) to the quartz cuvette was negligible in tests conducted without sapphire present.

It is believed that this technique represents a major step forward in real-time analysis of Cr(VI) in solution in that no other technique can nondestructively analyze Cr(VI) at these low levels during an actual kinetics run. The performance of a series of experiments are planned at different pHs with sapphire, after which we will move to the hematite and magnetite surfaces prepared by MBE to measure adsorption and reduction kinetics of Cr(VI) by these materials. A special flow-through cell will also be designed to allow adsorption testing on the MBE surfaces without contributions from the substrates.

Theoretical Modeling of Electron Transfer Rates

Theoretical modeling efforts thus far have been directed at producing an empirical potential for Fe(II)-O-OH-H₂O interactions, consistent with the current model for Fe(III)-O-OH-H₂O interactions. The goal is to predict relative electron affinities of surface species within a framework that allows both surface solvation and variable proton stoichiometry. The latter has been shown to be crucial in homogeneous electron transfer reactions in solution where the rates are strongly dependent on pH. Benchmarks for success were the successful prediction of: (i) the Fe(II)-O $\ddot{\text{O}}$ distance in hexaaqua Fe(II) (213 pm), (ii) the a_{1g} octahedral stretch (380 wavenumbers) and, (iii) the heat of solution of Fe(II). Moreover, to simulate electron transfer reactions, the model must also reproduce analogous quantities involving Fe(III) without a change in any parameter other than the change in charge (+2e to +3e). Experimental and calculated quantities differ systematically because the former are measured in condensed phases and calculations are performed on gas-phase quantities. The extent of this variation was assessed with quantum mechanical DFT and *ab initio* calculations. It was found that Fe(II)-O and Fe(III)-O bond lengths are overestimated by approximately 3% and a_{1g} stretching frequencies were underestimated by approximately 50 wavenumbers. Taking into account this systematic variation, this final model predicts bond lengths and energies to better than 2% for all Fe(II) and Fe(III) species. Because of constraints imposed by the model for water, however, a consistent overestimate was made of the a_{1g} stretching frequencies by 100 wavenumbers. The heats of solution are accurate to within 5%. Hydrolysis constants for both Fe(II) and Fe(III) correlate

well with gas-phase acidities, except for the third hydrolysis constant for Fe(II). A paper is in progress that describes these findings and applies them to the calculation of electron affinities of octahedral sites on hematite (012).

well with the present evidence. For the time being, however, it is not possible to say whether the results are due to the action of the solvent or to the action of the reagent. A further investigation is required.

Health Effects

Mechanisms Involved in Trichloroethylene- Induced Liver Cancer: Importance to Environmental Cleanup

Pacific Northwest National Laboratory

June 1, 1997

Progress Report

Principal Investigator

R.J. Bull, Ph.D.

(509) 373-6218 (Phone)

(509) 376-6767 (Fax)

rj_bull@pnl.gov

Pacific Northwest National Laboratory

PO Box 999, MSIN P7-56

Richland, WA 99352

Co-Investigator

B.D. Thrall

(509) 376-3809 (Phone)

(509) 376-0302 (Fax)

bd_thrall@pnl.gov

Pacific Northwest National Laboratory

PO Box 999, MSIN P7-56

Richland, WA 99352

Contributors and Collaborators

L.B. Sasser (PNNL)

J.H. Miller (PNNL)

I.R. Schultz (PNNL)

Research Objective

The objective of this project is to develop critical data for changing risk-based cleanup standards for trichloroethylene (TCE).

Importance to DOE

Cleanup costs for chlorinated solvents found on DOE sites are most frequently driven by TCE because it is both a widespread contaminant and is generally present at the highest concentrations. Data that would permit changes in risk-based standards for TCE would reduce complex-wide cleanup costs by several billion dollars while still protecting humans and the environment.

Current Regulatory Actions Research Will Impact

EPA is currently reviewing its risk assessment for TCE. One of this project's principal investigators (Dr. Bull) is working with EPA on this review. The scheduling of tasks in the project is carefully keyed to the final rulemaking process to ensure that the data generated have the greatest potential benefit to DOE.

Problem Statement

The main impetus for this research is that recent data indicates that TCE induces a shift in the mutation spectra observed in the H-ras codon 61 in mouse liver tumors. Such effects have been generally interpreted as indicating that the chemical is acting as a mutagen. However, preliminary data argue that selection (or promotion) is most likely involved.

The present project is organized around two interrelated tasks:

- Task 1 addresses the pharmacokinetic questions in the TCE-pretreated mouse, seeks to establish whether the selection hypothesis is reasonable, and then will seek to prove that this mode of action is responsible for TCE-induced tumors. This project will supply the basic dose-response data from which extrapolations would be made.
- Task 2 seeks specific evidence for the dichloroacetate (DCA)/selection mechanism by studying the biological consequences of this H-Ras mutant-dependent effect *in vitro*. Mechanisms can be most directly demonstrated *in vitro*. These data are necessary to select between a linear (i.e., no threshold) and non-linear model.

Research Progress

Task 1. Tumorigenesis Studies

Biologically based models for assessing health risk from TCE exposure will only be useful if it can be firmly established which metabolite is responsible for the carcinogenic effects of TCE and the likely mode of action by which this metabolite(s) operates. A key argument for the role of DCA as the metabolite primarily responsible for TCE-induced liver tumors is that the incidence and spectra of H-ras mutations is similar to tumors induced by DCA (62%) or TCE (51%) (Anna et al. 1994). However, data describing the mutation incidence and spectra for trichloroacetate (TCA)-induced tumors is either limited or inferred from animals treated with tetrachloroethylene, of which TCA is an active metabolite. Ferreira-Gonzalez et al. (1995) reported a H-ras mutation incidence of 45% in mice treated directly with TCA; however, the low number of total tumors (11) in this study causes concern over the accuracy of this estimate and does not necessarily suggest or exclude a role for TCA in TCE-induced tumors. To establish a more accurate estimate of the H-ras mutation incidence, we have now sequenced over 40 tumors induced by TCA in B6C3F1 mice in prior experiments. Our analysis has shown that 29% (12/41) of these tumors bear mutations in H-ras. This incidence agrees with the incidence of mutations in tetrachloroethylene-induced tumors (26%; Anna et al. 1994) and clearly demonstrates that the mutation incidence in TCE-induced tumors is more closely reflected by that of DCA than TCA.

A pilot initiation/promotion study was designed to determine if DCA- or TCA-induced liver cancer is caused by mechanisms that select a particular H-ras genotype. Three chemical initiators were selected that give rise to liver tumors when injected into B6C3F1 neonatal mice. Vinyl carbamate (VC) induces tumors with the H-Ras-61-leu mutation, N-Hydroxy-2-acetylaminofluorene (N-OH-AAF) produces the H-Ras-61-lys mutation, and diethylnitrosamine (DENA) produces tumors without H-ras mutations.

The study includes 72 mice, which were divided into 12 treatment groups of 6 mice each as follows: (1) untreated, (2) VC, (3) VC + DCA, (4) VC + TCA, (5) N-OH-AAF, (6) N-OH-AAF + DCA (7) N-OH-AAF + TCA (8) DENA, (9) DENA + DCA, (10) DENA + TCA (11) DCA, and (12) TCA. A single dose of the specific initiator (VC, N OH-AAF, or DENA) was administered at 15 days of age as appropriate. The mice were weaned at 3 weeks of age and given DCA (2 g/L) or TCA (2 g/L) in the drinking water or no further treatment, as described above.

The study has been ongoing for about 19 weeks and no treatment-related problems have developed. A selected number of animals were imaged for liver tumors by *in vivo* MRI spectrometry, but no indication of liver tumors are evident at this point. It is anticipated that a high number of nodules and tumors can be generated from these studies in 30 to 36 weeks. Administration of VC alone (3 mg/kg) to neonatal mice has been shown to produce about 8 nodules and tumors per liver in 8 months and similar numbers are expected with N-OH-AAF and DENA treatment.

Pharmacokinetic Results

Initial experiments have concentrated on determining DCA levels in blood of male B6C3F1 mice given intravenous injections of TCE via a jugular vein cannula. Pilot experiments established that mice could tolerate a bolus, 100 mg/Kg i.v. dose of TCE. This high dose of TCE was then used in later experiments to increase the potential for measuring DCA in blood. After i.v. injection of TCE, blood was repetitively sampled from individual mice ($n=8$) via the cannula for eight hours. Immediately after injection, high levels of chloral hydrate (CH) were observed, which rapidly declined as blood levels of TCE and TCA gradually increased and peaked between 0.5 and 1 hour. Trichloroacetic acid blood levels gradually decreased and were still present eight hours after injection of TCE. No detectable quantities of DCA were observed at any time points after TCE injection. The effective limits of detection for DCA in blood were between 10 and 20 ng/ml.

Later experiments characterized the pharmacokinetics TCE metabolites CH and TCE after 100 mg/Kg doses were administered via the jugular vein cannula. These experiments were performed to obtain blood clearance parameters for these metabolites and to confirm recent reports of high DCA blood levels formed after i.v. (tail vein) injection of CH. Initial results were negative and consistent with the previous experiment, which did not measure detectable levels of DCA in blood. The results from these experiments, along with previous studies using TCA and DCA, were used to construct a compartmental pharmacokinetic model to describe the appearance and decline of CH, TCE, and TCA in blood after an i.v. bolus injection of TCE. Initially, the observed data for CH, TCE, and TCA were simultaneously fit to the model-based equations to obtain estimates of the formation rates of these metabolites. Next, a series of computer simulations was performed to predict the blood concentrations of DCA that should be observed if i) all DCA formation occurred via TCA metabolism and ii) additional DCA is formed via an unknown pathway(s) from CH, which circumvents TCA as an intermediate. These simulations were made using blood clearance parameters for DCA obtained after a high (100 mg/Kg) dose administered via the tail vein.

The model simulations indicated that DCA blood levels should have risen above our detection limits and produced measurable levels of DCA in blood if at least a small portion (i.e., 5 %) of the excretion of TCA or CH occurs through a metabolic pathway with DCA as an intermediate. Thus, the lack of quantifiable levels of DCA in blood suggests that DCA may be formed at a lower rate than previously thought. However, the model predictions were very sensitive to DCA blood clearance parameters and changes in these parameters directly affect model predictions of DCA blood levels. Therefore, current experiments are characterizing DCA clearance from blood after low i.v. doses (5 and 20 mg/Kg). These results will be used to assess whether DCA blood clearance is increased at lower doses and whether predictions of DCA blood levels after TCE injection were overestimated by relying on DCA parameters obtained from unrealistically high doses.

Task 2. Studies of Selectivity

Our molecular analysis of tumor genotypes strongly argues that DCA plays a greater role than TCA in the carcinogenicity of TCE. The major question we are currently evaluating is whether the carcinogenic mechanism of DCA involves promoting the survival and clonal expansion of pre-existing initiated cells in mouse liver. Initial studies have focused on determining whether *in vitro* exposure of hepatocytes to DCA or TCA promotes clonal expansion of cells with initiated characteristics, in particular cells that have lost anchorage-dependence. Hepatocytes isolated from untreated mice and grown over soft agar for 10 days form anchorage-independent colonies at an incidence of approximately 5 colonies per 25,000 hepatocytes. However, colony formation is increased in a dose-dependent manner when either DCA or TCA are included in the medium. A significant increase in the number of colonies (8 or more cells) was observed at concentrations of DCA ranging from 0.5 - 2 mM, whereas no increase in colonies was produced by 0.2 mM DCA after 10 days. The effects of TCA on colony formation were similar to that of DCA, although TCA was slightly less effective at stimulating colony formation at the highest concentration used (2 mM). These results are, to our knowledge, the first demonstration that haloacetates can stimulate clonal expansion of cells with initiated characteristics *in vitro*. In addition, immunocytochemical analysis of the reactivity of these cells to anti-c-Jun antibody demonstrated that the phenotype of the colonies promoted *in vitro* reflects phenotypic differences observed between TCA- and DCA-induced tumors. Tumors induced by DCA react positively with antibodies against c-Jun (c-Jun⁺), whereas TCA-induced tumors are of the c-Jun⁻ phenotype (Stauber and Bull 1997). *In vitro*, a high fraction of both spontaneous colonies (10/13; 76.9%) and DCA-promoted colonies (28/34; 82.3%) react positively with c-Jun antibody. In contrast, the c-Jun⁺ phenotype was less frequent (5/22; 22.7%) in colonies promoted by TCA. These results indicate either that TCA and DCA promote clonal expansion of different hepatocyte populations, or that the mechanisms of clonal expansion by these two chemicals are distinct, or both. Studies are underway to determine if prior exposure to these metabolites *in vivo* stimulates the clonal expansion of colonies isolated *in vitro*, and whether the growth of these colonies are dependent on the continued presence of the chemical. In addition, we are pursuing molecular approaches, including amplification and sequencing of the H-ras gene from isolated colonies, to determine if these metabolites indeed promote the clonal expansion of pre-existing initiated hepatocyte populations. This latter approach will provide a powerful model for assessing the mode of action by which metabolites of TCE induce liver cancer in mice, and will also provide a vehicle for examining interspecies differences in sensitivity to tumor promotion.

Structural Bases of Selectivity

A modeling of the protein products of various H-ras-61 mutations' interaction with Raf is an initial effort that will be followed up experimentally if appropriate hypotheses can be developed. The resulting structural studies are to be separately funded if the modeling results are promising.

All of the common codon-61 mutations of Ras found in tumors prevent hydrolysis of Ras-GTP to Ras-GDP, thereby locking this membrane-bound G-protein into a state with affinity for the serine/threonine kinase Raf. A strong correlation has been observed between Ras-Raf

binding affinity and expression of reporter genes linked to transcription factors in the MAPK pathway (Block et al. 1996). Consequently, differential Ras-Raf affinity could be the basis for conferring a growth advantage to a subset of spontaneous codon-61 Ras mutants. The working hypothesis is that interference with down-regulation of a mutation that maintains some sensitivity to down regulation could explain the shift in the spectrum of Ras mutations seen in tumors induced by DCA relative to tumors from control animals.

Exposure to DCA produces an initial mitogenic response in mouse hepatocytes that is suppressed by continued treatment (Stauber and Bull 1997). The interaction between Ras and Raf is a focal point for cross-talk between growth factor- and cAMP-dependent signaling pathways. Phosphorylation of Raf by protein kinase A (PKA) reduces Ras-Raf binding affinity and inhibits activation of Raf (Hafner et al. 1994) as well as subsequent downstream kinase activity (VanRenterghem et al. 1994).

Molecular modeling techniques are being used to investigate the impact of various codon-61 Ras mutations on the structure of the effector-binding region, residues 32-40. Recent data from x-ray crystallography (Nassar et al. 1996) provide atomic-level detail on the interaction between the wild-type Ras effector region and the Ras binding domain (RBD) of Raf, residues 51-131. We are also constructing a model of the long-form of the Raf RBD, residues 1-131, that includes the PKA phosphorylation site at serine 43. The goal of this modeling activity is to understand how specific Ras mutations affect Ras-Raf interactions with and without phosphorylation at serine 43. We have obtained a sample of Raf 1-131 from Dr. Jonathan Cooper at the Fred Hutchinson Cancer Research Center in Seattle. Experimental binding and structural studies with this fragment will be used to test results obtained by molecular modeling.

References

- Anna, C.H., R.R. Maronpot, M.A. Pereira, J.F. Foley, D.E. Malarkey, and M.W. Anderson. 1994. "Ras Proto-Oncogene Activation in Dichloroacetic Acid-, Trichloroethylene- and Tetrachloroethylene-Induced Liver Tumors in B6C3F1 Mice." *Carcinogenesis* 15:2255-2261.
- Block, C., R. Janknecht, C. Herrmann, N. Nassar, and A. Wittinhofer. 1996. "Quantitative Structure-Activity Analysis Correlating Ras/Raf Interaction in Vitro with Raf Activation in Vivo." *Nature Struct. Biol.* 3:244-251.
- Ferreira-Gonzalez, A., A.B. DeAngelo, S. Nasim, and C.T. Garrett. 1995. "Ras Oncogene Activation During Hepatocarcinogenesis in B6C3F1 Male Mice by Dichloroacetic and Trichloroacetic Acids." *Carcinogenesis* 16:495-500.
- Hafner, S., H.S. Adler, H. Mischak, P. Janosch, G. Heidecker, A. Wolfman, S. Pippig, M. Lohse, M. Ueffing, and W. Kolch. 1994. "Mechanism of Inhibition of Raf-1 by Protein Kinase A." *Mol. Cell. Biol.* 14:6696-6703.

Maronpot, R.R., T. Fox, D.E. Malarkey, and T.L. Goldsworthy. 1995. "Mutations in the *Ras* Proto-Oncogene: Clues to Etiology and Molecular Pathogenesis of Mouse Liver Tumors." *Toxicology* **101**:125-156.

Nassar, N., H. Gudrun, C. Herrmann, C. Block, R. Janknecht, and A. Wittinhofer. 1996. "Ras/Raf Effector Specificity Determined by Charge Reversal." *Nature Struct. Biol.* **3**:723-724.

Stauber, A.J., and R.J. Bull. 1997. "Differences in Phenotype and Cell Replicative Behavior of Hepatic Tumors Inducted by Dichloroacetate (DCA) and Trichloroacetate (TCA)." *Toxicol. Appl. Pharmacol.* **144**: In press.

VanRenterghem, B., M.D. Brownin, and J.L. Maller. 1994. "Regulation of Mitogen-Activated Protein Kinase Activation by Protein Kinases A and C in a Cell-Free System." *J. Biol. Chem.* **269**:24666-24672.

Distribution

**No. of
Copies****No. of
Copies****OFFSITE**

Professor Mansoor Alam
Department of Materials
New Mexico Tech
Socorro, NM 87801

A.L. Alm
Office of Environmental Management
U.S. Department of Energy
19901 Germantown Road (5A-014)
Germantown, MD 20874-1290

G. Ballew
Environmental Technology Partnership
Department of Community, Trade, and
Economic Development
State of Washington
2001 Sixth Avenue, Suite 2700
Seattle, WA 98121

J.R. Beall
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road (ER-72)
Germantown, MD 20874-1290

Jimmy Bell
Bell Consultants, Inc.
137 Bowsprit Lane
Kingston, TN 37763

David R. Boone
Department of Environmental Science
and Engineering
Oregon Graduate Institute of Science
and Technology
2000 Northwest Walker Road
Beaverton, OR 97006

Michelle Broido
U.S. Department of Energy
19901 Germantown Road, MSIN F-240
Germantown, MD 20874-1290

G. Brown
Stanford University
Department of Geological and
Environmental Sciences
Stanford, CA 94305-2115

Barry Burks
Oak Ridge National Laboratory
PO Box 2008, Bldg. 7601, MSIN-6304
Oak Ridge, TN 37831-6304

B. Calloway
Westinghouse Savannah River Company
Savannah River Technology Center
Building 773-A/Rm A-229
Mail Stop 28
Aiken, SC 29802

John Carberry
Dupont
Experimental Station
Building 249/119
PO Box 80249
Wilmington, DE 19880-0249

P.M. Castle
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 5205
Idaho Falls, ID 83415

**No. of
Copies****No. of
Copies**

Gregory Choppin
Chemistry Department
Florida State University
600 W College Ave.
Tallahassee, FL 32306-3006

Paul Clayton
Vice President for Academic Affairs
and Provost
Oregon Graduate Institute
PO Box 91000
Portland, OR 97291-1000

J.M. Clemmensen
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 3750
Idaho Falls, ID 83415

Jim Cochran
Washington State Univ. at Tri-Cities
100 Sprout Road
Richland, WA 99352-1643

Roger G. Collis
Environmental Technology Partnership
Department of Community, Trade, and
Economic Development
State of Washington
2001 Sixth Avenue, Suite 2700
Seattle, WA 98121

L. Coleman
Washington State Department of Ecology
1315 W. 4th
Kennewick, WA 99336

Julie Conner
U.S. Department of Energy
Idaho Operations Office
785 DOE Place
Idaho Falls, ID 83402

Bob Cook
Yakama Indian Nation
1933 Jadwin, Suite 110
Richland, WA 99352

Allen Croff
Martin Marietta Energy Systems, Inc.
Oak Ridge National Laboratory
PO Box 2008
Oak Ridge, TN 37831-6178

Rico Cruz
Nez Perce Indian Nation
PO Box 365
Main Street and Beaver Grade
Lapwai, ID 83450

John H. Cushman
Department of Mathematics and
Department of Agronomy
Purdue University
West Lafayette, IN 47907

Paul Danielson
Nez Perce Indian Nation
PO Box 365
Main Street and Beaver Grade
Lapwai, ID 83450

P.M. Davidson
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road (ER-15)
Germantown, MD 20874-1290

S.L. Domoter
Office of Environmental Management
U.S. Department of Energy
19901 Germantown Road (EH-412)
Germantown, MD 20874-1290

No. of
Copies

Gary Eller
Los Alamos National Laboratory
NMT-6 Advanced Technology Group
MSIN E5-10
Nuclear Material Technology Division
Los Alamos, NM 87544

Tom Engel
Hanford Advisory Board
University of Washington
Department of Chemistry
Bagley Hall Room 109
Seattle, WA 98195-1700

Robert Erdmann
PO Box 922
Grass Valley, CA 95945

H.H. Farrell
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road
Germantown, MD 20874-1290

Dennis Faulk
U.S. Environmental Protection Agency
MAF Plaza
712 Swift, Suite 5 (B5-01)
Richland, WA 99352

M.M. Fletcher
Belle W. Baruch Institute for Marine
Biology and Coastal Research
University of South Carolina
Columbia, SC 29208

C.W. Frank
Office of Environmental Management
U.S. Department of Energy
19901 Germantown Road (5B-014)
Germantown, MD 20874-1290

No. of
Copies

David Geiser
U.S. Department of Energy EM-52.1
19901 Germantown Road
1183/Cloverleaf Bldg.
Germantown, MD 20874-1290

Joe Gentilucci
JAG Technical Services, Inc.
127 Savannah Drive
Aiken, SC 29803

Kurt D. Gerdes
Office of Science & Technology
U.S. Department of Energy (EM-541)
19901 Germantown Road
1186 Cloverleaf Bldg.
Germantown, MD 20874-1290

P.W. Gibbons
PO Box 1970, MSIN H5-61
Richland, WA 99352-1300

Mark Gilbertson
U.S. Department of Energy (EM-52)
19901 Germantown Road
5A-031/FORS
Germantown, MD 20874-1290

T.R. Ginn
172 Everson Hall
Department of Civil and Environmental
Engineering
University of California, Davis
Davis, CA 95616-5294

Dib Goswami
Washington State Department of Ecology
1315 W. 4th
Kennewick, WA 99336

**No. of
Copies**

D.J. Grimes
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road
Germantown, MD 20874-1290

Stuart Harris
Confederated Tribes of the
Umatilla Indian Reservation
Old Mission Hwy 30
Pendleton, OR 97801

Paul Hart
Morgantown Energy Technology Center
3610 Collins Ferry Road
Morgantown, WV 26507-0880

Carol Henry
U.S. Department of Energy (EM-52)
1000 Independence Ave. S.W. RM: 5A-031
Washington, DC 20585

Thomas Hiron
Los Alamos National Laboratory
PO Box 1663, MSIN J591
Los Alamos, NM 87545

Roland Hirsch
U.S. Department of Energy
Medical Applications and
Biophysical Research Division
19001 Germantown Road (ER-73)
Germantown, MD 20874-1290

Earl Holtzscheiter
Westinghouse Savannah River Company
Savannah River Technology Center
Building 773-A/Rm A-229
Mail Stop 28
Aiken, SC 29802

**No. of
Copies**

Ken Hubbard
Assistant to Associate Provost for Research
and Economic Development
Dean of Graduate School
University of Montana
University Hall 118
Missoula, MT 59812-1329

Professor Art Janata
School of Chemistry and Biochemistry
Georgia Institute of Technology
Atlanta, GA 30332-0400

Moses Jaraysi
Washington State Department of Ecology
1315 W. 4th
Kennewick, WA 99336

Arvid Jensen
Lockheed Martin Idaho Technologies
Company
PO Box 1625
Idaho Falls, ID 83415-3204

Dawn Kaback
Colorado Center for Environmental
Management
999 18th St, Ste 2750
Denver, CO 80202

Roy Koch
Vice Provost for Research
Dean of Graduate Studies
Portland State University
PO Box 751
Portland, OR 97207-0751

Bruce Kowalski
Chemistry Department (BG-10)
University of Washington
Seattle, WA 98195

**No. of
Copies**

Alvin Kwiram
Vice Provost for Research
University of Washington
312 Gerberding Hall
Box 351237
Seattle, WA 98195-1237

Jim Lee
Sandia National Laboratories
PO Box 5800, MSIN 0734
Albuquerque, NM 87185-5800

Brenda Lewis
Westinghouse Savannah River Company
PO Box 616
Aiken, SC 29802

Paul Lurk
U.S. Department of Energy (EM-542)
19901 Germantown Road
1168/Cloverleaf Bldg.
Germantown, MD 20874-1290

R.S. Marianelli
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road (ER-14)
Germantown, MD 20874-1290

M.R. Martin
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 2424
Idaho Falls, ID 83415

Todd Martin
Hanford Advisory Board
West 1408 Broadway
Spokane, WA 99201

**No. of
Copies**

R.N. Massey
Office of Environmental Management
U.S. Department of Energy
19901 Germantown Road (EM-64)
Germantown, MD 20874-1290

L.W. McClure
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 3710
Idaho Falls, ID 83415

C. Phil McGinnis
Martin Marietta Energy Systems, Inc.
Oak Ridge National Laboratory
PO Box 2008
Oak Ridge, TN 37821-6273

W.D. St. Michel
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 1061
Idaho Falls, ID 83415

D.L. Miller
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 2208
Idaho Falls, ID 83415

W.S. Millman
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road (ER-14)
Germantown, MD 20874-1290

Jerry Morin
Westinghouse Savannah River Company
Savannah River Technology Center
PO Box 616
Aiken, SC 29802

**No. of
Copies**

Professor Ronald Olsen
Department of Microbiology and
Immunology
5605 Medical Science Building II
University of Michigan Medical School
Ann Arbor, MI 48109-0620

Robert T. Paine
Department of Chemistry
University of New Mexico
Albuquerque, NM 87131

A.A. Patrinos
Office of Energy Research
U.S. Department of Energy
19901 Germantown Road (ER-70)
Germantown, MD 20874-1290

David Pepson
U.S. Department of Energy (EM-38)
19901 Germantown Road
472/TREV
Germantown, MD 20874-1290

Ken Picha
U.S. Department of Energy (EM-32)
Office of Eastern Operations
19901 Germantown Road 343/TREV
Germantown, MD 20874-1290

Kevin Price
DOE-ID/OPE
850 Energy Drive
Idaho Falls, ID 83402

Bill Prindle
1556 Crestline Drive
Santa Barbara, CA 93105

**No. of
Copies**

Caroline Purdy
U.S. Department of Energy
Office of Technology Development
Cloverleaf Bldg.
19901 Germantown Road (EM-53)
Germantown, MD 20874

Professor Kenneth N. Raymond
Department of Chemistry
University of California at Berkeley
Berkeley, CA 94720

James Rice
Sandia National Laboratories
PO Box 5800
Albuquerque, NM 87185-0726

G.M. Roesler
Office of Environmental Management
U.S. Department of Energy
19901 Germantown Road (EM-52)
Germantown, M.D. 20874-1290

Professor D. Max Roundhill
Department of Chemistry
Texas Tech University
Lubbock, TX 79409-1061

Richard Scanlan
Dean of Research
Oregon State University
Administration Services Bldg A312
Corvallis, OR 97331

Wally Schulz
5314 Arbustos Court, NE
Albuquerque, NM 87111

D.R. Sherwood
U.S. Environmental Protection Agency
MAF Plaza
712 Swift, Suite 5 (B5-01)
Richland, WA 99352

**No. of
Copies**

Jean'ne Shreeve
Vice President for Research and
Graduate Studies
Professor of Chemistry
University of Idaho
Moscow, ID 83843-4199

Robert Smith
Vice Provost for Research
Dean of Graduate School
Washington State University
Pullman, WA 99164-1030

R.N. Snelling
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 2213
Idaho Falls, ID 83415

Stan Sobczyk
Nez Perce Indian Nation
PO Box 365
Main Street and Beaver Grade
Lapwai, ID 83450

Peter Spencer, Director
Center for Research on Occupational and
Environmental Toxicology L606
Oregon Health Sciences University
3181 SW Sam Jackson Park Road
Portland, OR 97202-3098

Harold Sullivan
Los Alamos National Laboratory
PO Box 1663
Los Alamos, NM 87545

John Swanson
1318 Cottonwood
Richland, WA 99352

**No. of
Copies**

Robert Swenson
Vice President for Research
Montana State University
Montana Hall 207
Bozeman, MT 59717

Larry Tavlarides
Syracuse University
334 Hinds Hall
Syracuse, NY 13244

Tom Tebb
Washington State Department of Ecology
1315 W. 4th
Kennewick, WA 99336

Tom Thomas
Lockheed Martin Idaho Technologies
Company
PO Box 1625, MSIN 3458
Idaho Falls, ID 83415-3423

Major Thompson
Westinghouse Savannah River Company
Building 773-A, C140
PO Box 616
Aiken, SC 29802

Steadman Upham
Vice Provost for Research and
Graduate Education
Dean of Graduate School
University of Oregon
112 Johnson Hall
Eugene, OR 97403-1226

Nancy Uziemblo
Washington State Department of Ecology
1315 W. 4th
Kennewick, WA 99336

**No. of
Copies**

George Vandegrift
Argonne National Laboratory
Building 205
9700 South Cass Avenue
Argonne, IL 60439

Paul Wang
Special Technologist Laboratory
5520 Ekwill St., Suite B
Santa Barbara, CA 93111-2335

Jack Watson
Oak Ridge National Laboratory
PO Box 2008
Bldg 4500N, MS-6178
Oak Ridge, TN 37831-6178

Tom Weber
6622 West Victoria
Kennewick, WA 99336

Jerry White
Tri-Cities Commercialization Partnership
201 High Meadows
Richland, WA 99352

JR Wilkinson
Confederated Tribes of the
Umatilla Indian Reservation
Old Mission Hwy 30
Pendleton, OR 97801

Tom Woods
Yakama Indian Nation
1933 Jadwin, Suite 110
Richland, WA 99352

Jim Wright
U.S. Department of Energy
Savannah River Operations Office
PO Box A
Aiken, SC 29802

**No. of
Copies**

Lin Yarborough
U.S. Department of Energy
Albuquerque Operations Office
PO Box 5400
Albuquerque, NM 87185-5400

ONSITE

3 Bechtel Hanford, Inc.

Steven C. Foelber	H0-09
Kim Koegler	H0-05
Stephen K. Pulsford	X3-41

Duke Engineering and Services

Robert J. Cash	S7-14
----------------	-------

2 Fluor Daniel Hanford

Tom L. Anderson	K9-46
Mike Yates	H5-20

3 Lockheed Martin Hanford Corporation

John N. Appel	H6-37
Ken A. Gasper	H6-37
Jim O. Honeyman	G3-21

**25 U.S. Department of Energy
Richland Operations Office**

Jay Austenborg	S7-41
Linda K. Bauer	H0-12
Dennis A. Brown	K8-50
Jeff A. Frey	K8-50
Marcus J. Glasper	K8-50
Rick Gonzalez	R3-79
Charles A. Hansen	S7-41
James P. Hanson	K8-50
June M. Hennig	K8-50
Maureen A. Hunemuller	S7-50
Jackson E. Kinzer	S7-50

**No. of
Copies**

**No. of
Copies**

Pete M. Knollmeyer	A5-11
Cathie S. Louie	B4-55
Lance S. Mamiya	K8-50
Billie M. Mauss	K8-50
John L. Murphy	H0-12
John P. Neath	K8-50
Lloyd L. Piper	A7-50
Robert M. Rosselli	K8-50
Shannon W. Saget	K8-50
Fred R. Serier	H0-12
K. Mike Thompson	H0-12
Deborah E. Trader	K8-50
Joseph J. Waring	S7-55
Don D. Wodrich	S7-50

150 Pacific Northwest National Laboratory

Richard C. Adams	K1-43
Robert W. Allen	K9-69
Jack Bagley	K1-74
Sharon A. Bailey	K5-08
Eddie G. Baker	P8-38
William R. Barchet	K9-30
William F. Bonner	K9-14
Donald M. Boyd	K5-02
Joseph W. Brothers	K9-20
Tom M. Brouns	K9-91
Merwin L. Brown	K5-02
James L. Buelt	P7-41
Richard J. Bull	P7-56
Bruce C. Bunker	K2-25
Don M. Camaioni	K2-44
Scott A. Chambers	K8-93
Steven D. Colson	K2-14
Louis R. Corrales	K1-96
Joseph L. Devary	K6-96
Terrence J. Doherty	K6-49
Dennis D. Doneen	K6-48
Thomas H. Dunning	K2-20
Richard M. Ecker	K6-91
Robert E. Einziger	K2-45
Paul D. Ellis	K8-98
Gregory J. Exarhos	K2-44

Linda L. Fassbender	K8-11
Andrew R. Felmy	K8-96
Mark D. Freshley	K9-36
Donald M. Friedrich	K8-96
Teresa A. Fryberger (5)	K2-20
James L. Fuller	K6-48
Bobbi A. Garrett	K8-09
Suzanne M.K. Garrett	K8-18
Roy E. Gephart (5)	K9-76
Bob L. Gruel	K8-60
Mark S. Hanson	K9-02
Paul D. Hart	K9-25
John S. Hartman	K5-25
Benjamin P. Hay	K9-77
Scott W. Heaberlin	K8-31
Michael A. Henderson	K8-93
Langdon K. Holton	K6-51
Landis D. Kannberg	K5-20
Paul K. Kearns	K6-51
Karen Knutsen	K2-14
David W. Koppenaar	P7-07
W.L. (Bill) Kuhn	K2-21
John P. LaFemina (30)	K9-91
Carlton D. Lee	K7-22
Janice R. Lewis	K1-51
Philip E. Long	K6-91
William J. Madia	K1-46
Judith A. Mahaffey	K8-38
Wayne J. Martin	K9-14
Louis Martucci	K7-10
Gary W. McNair	P7-75
Gary L. McVay	K2-50
Barry J. Merrill	K6-49
Ellyn M. Murphy	K3-61
Jon L. Olson	BSRC/S128
Norman J. Olson	K9-46
Thomas M. Orlando	K2-14
Thomas L. Page	K9-18
Terri Paluszkievicz Sequim/MS	L5-304
Larry R. Pederson	K2-44
Linda R. Pond	K8-23
Brent A. Pulsipher	K5-12
Marilyn J. Quadrel	K9-70

No. of
CopiesNo. of
Copies

Rod K. Quinn (10)	K9-69
Shirley A. Rawson	K2-20
Bernard F. Saffell, Jr.	K5-22
Jeffrey D. Saffer	P7-58
Steve N. Schlahta	K9-69
Michael H. Schlender	K1-73
Paul A. Scott	K9-46
L John Sealock	K2-10
Alireza Shekarriz	K7-15
Billy D. Shipp	K9-01
Steve C. Slate	K9-14
Gary L. Smith	P7-41
Kelvin L. Soldat	K3-53
Steven L. Stein	BSRC/S171

Terri L. Stewart	K9-91
Gerry M. Stokes	K9-95
B. Ray Stults	K9-76
Jim J. Thomas	K7-10
Brian D. Thrall	P7-56
Jud W. Virden	K2-44
Terry L. Walton	K1-46
William J. Weber	K2-44
Walter C. Weimer	P7-27
Raymond E. Wildung	P7-54
Kwong K. Wong	P7-56
TFA Library (2)	K9-69
Information Release (7)	K1-06