CHEMICAL SCIENCES AND TECHNOLOGY

Seed Money Fund

Molecular Modeling and Simulation of the Formation and Decomposition of Methane Hydrates

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The broad goal of this research was to answer two fundamental questions from a molecular-based viewpoint. (1) How can we predict phase stability in multicomponent systems containing methane hydrates, and (2) how does the pore size and surface chemistry of a porous solid matrix affect the phase stability of methane hydrate systems? In order to tackle this broad goal successfully, we proposed to demonstrate our modeling capabilities by performing several groundbreaking calculations. Particularly, our goals for this project were (1) the determination of thermodynamic and structural properties of methane and carbon dioxide hydrates, and (2) the determination of relative stability between methane and carbon dioxide hydrates. Toward the achievement of these goals we studied, by isobaric-isothermal molecular dynamics of realistic host-guest representations, the structure and the thermodynamic properties of methane and carbon dioxide hydrates. The simulation results were then used to interpret the limitations and main implications behind the most popular macroscopic modeling tool, that is, the so-called van der Waals-Platteeuw theory (vdWP) and its modifications. In addition, we developed a simulation methodology to analyze the feasibility of methane replacement by carbon dioxide sequestration as an alternative way to exploit methane by simultaneously mitigating the buildup of carbon dioxide in the atmosphere.

Introduction

ORNL has established the Energy and Environmental Systems of the Future (E²SF) Initiative to develop comprehensive, systematic approaches to meeting growing global energy needs. Energy systems of the future have been identified in E²SF as having the following characteristics: clean (minimal adverse health and environmental effects), efficient (more efficient than today's energy systems), affordable (no more expensive than today's energy systems), available (accessible worldwide), and abundant (drawing on plentiful resources). If it can be recovered safely and in an environmentally sound fashion, gas hydrates fit the definition for an energy system of the future almost perfectly, and so it is not surprising that this is one of the energy systems in which E²SF is making investments. The E²SF initiative, mirroring DOE interests and funding priorities, is also focusing on the fundamental science related to future energy systems and includes the goal of understanding the molecular chemical and physical processes underpinning future energy system exploitation, production, and use. This proposal addresses the goal of understanding the fundamental molecular basis for gas hydrate formation, stability, and decomposition, and so fits naturally within the E²SF initiative. The primary tool to achieve this goal is redeployment and application of more than a decade's experience in molecular simulation of aqueous systems and complex organic systems, implemented on massively parallel supercomputers, to the problem of gas hydrates.

Technical Approach

Gas hydrates are nonstoichiometric inclusion compounds showing that water can form solutions (solid) with hydrophobic molecules. The resulting solid solution (hydrate) consists of a crystalline host lattice (water) that encloses the guest (usually a natural gas component). The gas hydrate structure differs from that of corresponding ice and depends on the nature of the solute guest. The empty hydrate lattice is thermodynamically unstable (i.e., the hydrophobic guest makes it possible for the water to be solid at conditions at which water would otherwise be liquid).[1]

Gas hydrates occur naturally in sediments under pressure and temperature conditions of permafrost (polar) regions and in outer continental margins (deep ocean regions). Even though other gases such as light hydrocarbons and carbon dioxide can form gas hydrates, provided the conditions are appropriate, methane hydrates are the most common naturally occurring type of clathrate. Methane is typically the main constituent of these hydrates, with a ten-fold higher energy density (STP volume of gas per volume of sediment/rock) than that of other nonconventional gas sources, such as coal beds, and two to five times larger energy density than conventional natural gas.[2]

Two important factors make methane hydrates a potentially attractive clean energy resource—the size of the methane fields in the form of hydrates in shallow seafloor sediments and the wide geographical distribution of the gas hydrates. Conservative estimates indicate that the worldwide amount of methane hydrates is of the order of 10 teratons (i.e., about twice the carbon found in all fossil fuels on earth).[3] Moreover, the use of natural gas from hydrates as a source of energy, as opposed to coal or petroleum, can help mitigate greenhouse gas emissions, provided we prevent release of methane into the atmosphere. In fact, the stability of gas hydrates is strongly affected by changes in pressure and/or temperature of the shallow seafloor where they form. Thus any increase in temperature and/or decrease in pressure have the potential to destabilize the hydrates, resulting in decomposition and consequent release of methane into the atmosphere. Considering that methane exhibits a greenhouse effect approximately 20 times stronger than carbon dioxide, any substantial additional methane release from the decomposition of hydrates will contribute a positive feedback in the global warming cycle, which eventually could result in the so-called runaway greenhouse effect.[4] Therefore, although methane hydrates represent a significant untapped hydrocarbon source, their physical chemistry at the seafloor conditions is not yet well understood and becomes an important factor hindering large-scale exploitation of these potential energy sources due to environmental concerns.[5]

Our current understanding of clathrate behavior hinges around the cell theory, proposed about 55 years ago by van der Waals,[6] who assumed that the encaging of the guest molecules into a metastable empty host structure (β-modification) leads subsequently to a stable configuration (the α-modification). This theory (vdWP) encompasses in principle three clear assumptions within the frame of classical statistical mechanics: (1) the host cavities exclude multiple guest occupancy, (2) the host-host interactions can be neglected, and (3) the guest molecules (host occupancy) do not affect the contribution of the β-structure to the hydrate partition function. Based on these assumptions, van der Waals derived a simple expression for the hydrate Helmholtz free energy. Unfortunately, to make practical use of the vdWP theory, it is necessary to introduce two additional assumptions: (4) the guest molecules behave as ideal gas free rotors, and (5) the potential energy of the guest molecule is represented by a spherically symmetric cavity potential centered at the center of the host cage. Under this set of assumptions, the hydrate formation can be (formally) analyzed as an adsorption isotherm through the definition of the corresponding Langmuir constant.

Over the years, much of the molecular-based work on hydrates has been directed toward the testing of the validity of the hypotheses behind the vdWP model. These simulations have typically involved simple nonpolarizable water intermolecular potential models with rigid geometry as well as one-site spherically symmetric solutes usually described as Lennard-Jones atoms. The main thrust behind these simulations has been the explicit description of the three water sites, while little emphasis has been placed into the actual solute geometry and the corresponding molecular asymmetry of the resulting intermolecular potential.

However, a more realistic representation of the host-guest interactions is a crucial factor for the successful molecular-based description of condensed fluids, since the molecular asymmetry (difference between solute-solvent and solvent-solvent intermolecular forces) between species in solution ultimately dictates the phase equilibria of the system. In addition, a stringent test of the hypothesis behind the vdWP hydrate theory is only possible if we are able to realistically describe not only the host-host but also the host-guest and the guest-guest interactions.

In this investigation we analyzed, by molecular dynamics simulation at isobaric-isothermal conditions, the thermophysical and structural properties of methane and carbon dioxide clathrate hydrates, as described by the most accurate and realistic available multisite intermolecular potential models. We compared these results with those corresponding to the solutes described by spherically symmetric Lennard-Jones potentials to test underlying hypotheses behind hydrate modeling. In particular, we analyzed the effect of the strength of the host-guest interactions, the effect of the type of water potential, and the degree of cage occupancy on the resulting properties of the hydrate. We also used the simulation results to interpret the main assumptions behind the original vdWP theory of clathrates, as well as the numerous modifications currently in use for the modeling of hydrate phase equilibria. Finally, we studied the methane replacement in the hydrate by carbon dioxide sequestration via free energy computer simulation.

Results and Accomplishments

In our original research proposal we stated two main goals that have been accomplished and their results are part of two publications (see below).

The first goal was the molecular dynamics study of the thermodynamic and structural properties of methane and carbon dioxide hydrates, where the species were described by the most accurate (available) intermolecular potentials. The predicted properties were then used to interpret the limitations and implications of the main assumptions behind the original vdWP theory of clathrates and its modifications, which are currently used in the modeling of hydrate phase equilibria. On the one

hand, our results suggest that two of the main hypotheses of the vdWP theory are far from being appropriate approximations (i.e., assumptions 2 and 3 in the Technical Approach are usually violated). On the other hand, this theory and its current modifications have become versatile tools for the regression of experimental data.

In order to interpret this dichotomy, we must address a few issues regarding the validity of the assumptions in the vdWP theory at the light of the molecular dynamics results. Much of the modelers' emphasis has been placed on determining the adequacy of the spherical cell approximation invoked in the calculation of the so-called cavity potential and resulting Langmuir constant. In particular, the effort has been directed to the more accurate computation of the Langmuir constants,[7] aimed at improving their estimations over those originally based on the Lennard-Jones Devonshire smooth cell approximation.[8–11] Other alternative modifications to the original vdWP theory were focused on softening the inadequacies of the spherical approximation for the cavity potential and account for any possible lattice distortion. Even though these modifications might have improved the regression capability at the expense of introducing yet more adjustable parameters, the resulting models and corresponding adjustable parameters tend to lose the original and clear physical picture of the clathrate solution.

The success of the vdWP theory in correlating experimental data should not be a surprise,[1,12] if we consider that it involves a rather large number of adjustable parameters used in the regression of the equilibrium data, namely, four thermodynamic properties (the differences of properties between the empty lattice and pure water at the reference state), two parameters per cavity type (the cavity radius and the number of water molecules forming the cavity), and two to three force-field parameters per guest type. Thus, the ten or so adjustable parameters defining the cavity potential and the host's reference state properties are invariably determined ad hoc by regression of phase equilibrium data, and consequently, they are not uniquely defined.[1]

More recently, Sparks[13–15] revisited Tester's[7] original work to analyze the inadequacy of the nearest-neighbor approximation used in the determination of the host-guest partition function and corresponding Langmuir constant for specific cavity types within the vdWP theory of hydrates. While they explored several computational methods to evaluate accurately and efficiently the host-guest partition function, placing emphasis on the contributions farther away from the first host cell, they also, somewhat surprisingly, included the guest-guest interactions in their calculations. The latter is inconsistent with the definition of the Langmuir constant; that is, the host-guest partition function in the vdWP theory was not formulated to include the guest-guest interactions (assumption 2 in the Technical Approach).

The second goal of our proposal was the determination of the relative stability between of methane and carbon dioxide hydrates. The rationale behind this analysis is that a promising approach to the exploitation of gas hydrates is the methane replacement by carbon dioxide at the seafloor in that CO_2 (gas) can provide the hydration energy necessary to decompose the CH_4 hydrates.[16,17] The novelty of this gas replacement approach is the simultaneous CO_2 mitigation, by long-term sequestration as a gas hydrate, and simultaneous extraction of CH_4 gas from the gas hydrates. To test the feasibility of CH_4 displacement by CO_2 in the clathrate matrix (toward its fixation in the deep ocean floor), we need to determine the relative stability between CH_4 and CO_2 hydrates.[18] With that purpose we developed a coupling parameter approach to assess the change of Gibbs free energy in the replacement process by molecular dynamics. This approach was used to determine the free energy isotherm for the replacement of a fully loaded methane hydrate to a fully loaded carbon dioxide. Our results indicate that the molecular description of the water has a negligible effect on the free energy of guest replacement.

Summary and Conclusions

Our molecular dynamics study[19] suggests that much of the effort spent in the modification of the original vdWP theory of hydrates has been misdirected, in that it has wrongly targeted either a more "accurate" or "realistic" calculation of the cavity potential (and consequent Langmuir constant) rather than the most obvious failure of the vdWP theory and its modifications (i.e., the neglect of the small but significant guest-guest interactions). Obviously, this simplifying assumption made it possible to derive a flexible and simple theory for "ideal" gas clathrates that takes the simple form of Langmuir adsorption isotherms. In that sense, the vdWP theory can be considered the zero approximation (ideal lattice model) to a more general formalism. The general theory should explicitly include the guest-guest interactions (nonideal lattice model) in the same spirit as the Henry's Law is in the development a fundamentally based theory of gas solubility.[20]

Unfortunately, most hydrates of industrial interest do involve non-negligible guest-guest interactions, and consequently, their behavior cannot be analyzed within the framework of the vdWP theory. The fundamental reason is that the clathrate partition function in the vdWP theory does not involve guest-guest interactions. The explicit inclusion of the guest-guest partition function alters obviously the mathematical expression for the

clathrate's Helmholtz free energy. In turn, this changes the functional relationships between the species fugacities, the state conditions, the host occupancy, the host-guest and guest-guest interactions, and consequently, the resulting expressions for phase equilibrium calculations. These expressions cannot be obtained from or simply mapped to re-parameterized (more accurate, or re-interpreted) Langmuir constants as is frequently done in engineering calculations.

In summary, most modifications to the vdWP theory designed to deal with real systems are fundamentally flawed. They can be regarded as more flexible data correlators at the expense of losing all molecular-based foundation (i.e., their adjustable parameters carry little physical meaning). It is also rather surprising that little attention has been placed on the developments that invoke explicitly the guest-guest interactions, which can be treated as natural extensions of the vdWP theory; that is,

$$A^H = A^H_{ideal} + A^H_{guest-guest} \; ,$$

where the first term on the right-hand side is the vdWP expression and the second is the explicit contribution of the guest-guest interactions.

The successful completion of this project positions ORNL well for future funding opportunities in hydrate research. Unfortunately, the projected $5–12 million/year national hydrates program recommended by the President's Council of Advisors on Science and Technology has not materialized at anything like this scale. We have kept ORNL Fossil Energy Program Manager Rod Judkins apprised of our work and have forwarded manuscripts to Claudia Rawn of the Metals and Ceramics Division and Bryan Chakoumakos of the Solid State Division, who plan to perform neutron scattering experiments on gas hydrates. Our simulation capability would be a valuable component of a larger project, either fundamental or applied, that involved experimental work on gas hydrates.

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A. A. Chialvo et al., "Molecular Dynamics Study of the Structure and Thermophysical Properties of Model sI Clathrate Hydrates," *Journal of Physical Chemistry B*, accepted for publication (2001).

E. M. Yezdimer et al., "Extraction of Methane from its Clathrate by Carbon Doxide Sequestration: Determination of the Gibbs Free Energy of Gas Replacement by Molecular Simulation," *Journal of Physical Chemistry B*, to be submitted (2001).

A Novel Method to Improve Productivity of Fermentation Processes

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The main objective of this project was to test a new method to improve the productivity of fermentation processes, which are used for the production of industrial chemicals derived from the glycolytic pathway. The method involved addition of 2,5-anhydro-D-mannitol (2,5AM) to a microbial culture. It was expected that this compound would be taken up by the cells and converted to 2,5-anhydro-D-mannitol-1,6-bisphosphate (2,5AMP2), which is a potent activator of two key enzymes of the glycolytic pathway and would increase carbon flow toward the final products of this pathway. The experimental results unfortunately disproved this hypothesis. However, they provided direct evidence of a rate-limiting step of the most important pathway in many biological systems, which included two key industrial microorganisms. This discovery was a very significant contribution to scientific knowledge. It also is very useful in the development of industrial microorganisms, which can synthesize glycolysis-derived products at higher rates than those currently available.

Introduction

In most microorganisms, glycolysis, which is also known as the glycolytic pathway, is the key sequence of biochemical reactions responsible for the dissimilation of glucose and many other sugars. The glycolytic pathway ends with the formation of pyruvate. This pathway leads to the formation of many industrial chemicals, which are produced in fermentation processes. These include ethanol, butanol, lactic acid, succinic acid, acetic acid, propionic acid, butyric acid, citric acid, and others. The total market for these industrially important chemicals is on the order of billions of dollars. Since the glycolytic pathway constitutes the initial steps of the reaction sequence, it is highly likely that productivity of the fermentation processes used for production of glycolysis-derived chemicals can be significantly improved if faster rates of glucose conversion by this pathway are achieved. The main objectives of this project are as follows:

1. Test a novel method to improve the conversion rate of glucose by the glycolytic pathway in industrial microorganisms. The method uses a non-metabolizable chemical activator of key enzymes of the pathway.
2. Test the hypothesis that higher rates of glucose conversion by the glycolytic pathway will result in higher rates of formation of chemicals derived from this biochemical pathway.

Technical Approach

The flux (i.e., the flow rate) of carbon through the glycolytic pathway has been thought to be under the regulation of two key enzymes, phosphofructokinase 1 (PFK1) and pyruvate kinase (PK). Both PFK1 and PK are activated by D-fructose-1,6-bisphosphate (F1,6P2), which is the product of the step catalyzed by PFK1. It is converted to dihydroxyacetonephosphate (DHAP) in the next step of glycolysis. Therefore, the intracellular concentration of F1,6P2 tends to fluctuate, which subsequently causes a fluctuation in the activity of PFK1 and PK. If the intracellular concentration of F1,6P2 were maintained at a high level, high activities of PFK1 and PK also might be maintained. A possible result would be a dramatic increase of carbon flux through the glycolytic pathway, which may cause significant increases in formation rates of chemicals derived from this pathway.

It is possible to maintain high activities of PFK1 and PK by using a nonmetabolizable analog of their natural activator F1,6P2. One such compound is 2,5-anhydro-D-mannitol-1,6-bisphosphate (2,5AMP2). Our approach was to add 2,5-anhydromannitol (2,5AM), which is commercially available, to a microbial culture. This compound will be taken up by the cells, converted first to its monophosphate, 2,5AMP, then to 2,5AMP2.[1] This bisphosphate product should then accumulate inside the cells and act as an activator for both PFK1 and PK. Since 2,5AMP2 cannot be converted further, it was anticipated that high activities of PFK1 and PK would be maintained independent of the metabolic state of the cells.

Results and Accomplishments

Both objectives were accomplished. Unfortunately the experimental results proved that our hypothesis was incorrect. In other words, the addition of 2,5AM to the selected microbial cultures did not improve production

rates of target compounds derived from the glycolytic pathway. However, the results led to a very important discovery, namely, the identification of a rate-limiting step of this pathway. To the best of our knowledge, this step has not been considered as a rate-limiting step before. Our experimental results provided the strongest evidence thus far. This discovery and its impacts are discussed below.

The first microorganism tested was *Escherichia coli* KO11, which is a recombinant strain of *E. coli* genetically engineered for enhanced ethanol production. When 5 mM 2,5AM was added to an aerobic culture with glycerol as the sole carbon source, complete inhibition of glycerol metabolism was observed. Glycerol enters the *E. coli* cells by simple diffusion and subsequently is converted to glycerol-3-phosphate (G3P) by a kinase. This enzyme is strongly inhibited by F1,6P2. The inhibition of glycerol metabolism, therefore, gave the evidence that 2,5AM indeed entered the cells and was converted to 2,5AMP2, which is the structural analog of F1,6P2, and caused the observed inhibition.

When 2,5AM at concentrations above 1 mM was added to cultures of *E. coli* KO11 under either aerobic or anaerobic conditions with either glucose or fructose as the sole carbon source, inhibition of metabolism of both sugars was observed. The degree of inhibition of fructose metabolism was significantly higher than that of glucose. Glucose is converted to glucose-6-phosphate (G6P) on its entrance into the glycolytic pathway. G6P then is converted to F6P by an isomerase before it continues down the pathway. On the other hands, fructose is converted directly to F6P on its entrance into the glycolytic pathway. Since 2,5AMP, the monophosphate product of 2,5AM, is the structural analog of F6P, inhibition of fructose metabolism must have been caused by this monophosphate compound, and the site of inhibition was the kinase, which converted fructose to F6P. This is a typical feedback inhibition in a biochemical pathway. In the case of glucose, the site of inhibition must have been the isomerase, which converted G6P to F6P. Ethanol production also was monitored in these experiments, under both aerobic and anaerobic conditions. Inhibition of ethanol production was observed in parallel with inhibition of sugar metabolism. The degree of ethanol inhibition matched that of inhibition of glucose. Since ethanol is the final product of glycolysis, this information indicated that the site of inhibition of glucose metabolism (i.e., G6P isomerase) was the rate-limiting step of this pathway. We attempted to measured the intracellular pools of G6P and F6P with various amounts of 2,5AM added to obtain further evidence. Unfortunately, we exhausted our funds and time.

The second organism tested was *Saccharomyces cerevisiae* ATCC 24855. This is a wild-type yeast strain, which produces ethanol as the main product. Experiments were performed under conditions similar to those used for *E. coli* KO11. The same results were obtained. In other words, inhibition of glucose (or fructose) metabolism and ethanol production was observed and the degree of ethanol production inhibition matched that of sugar metabolism. These results again indicated the site of inhibition (i.e., G6P isomerase) is the rate-limiting step of glycolysis.

Two other organisms were also tested. These were *E. coli* ATCC 202021, which is the organism used for development of the patented and licensed succinic acid technology at ORNL, and *Bacillus nghiemis*, which is a newly isolated thermophilic organism capable of producing lactic acid at high temperatures. Since the products of fermentation in these two cases were strong organic acids, the pH of the cultures dropped very quickly. We made numerous attempts to maintain the pH by using various buffering systems but failed. Due to the high cost of commercial 2,5AM, we could not perform the experiments in large-volume fermenters, where the pH could be easily controlled. The results of these experiments, therefore, were inconclusive.
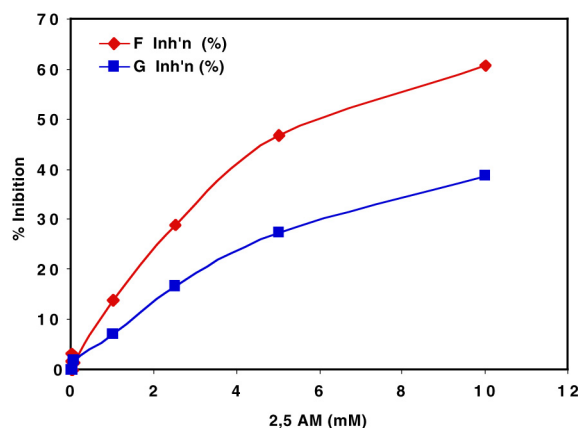

Fig. 1. Effect of 2,5AM on sugar consumption by Saccharomyces cerevisiae *ATCC 24855.*

Summary and Conclusion

Our hypothesis has been proved incorrect. Consequently we did not have a patent to cover many broad areas of industrial fermentation as we had hoped. However, we discovered a rate-limiting step of glycolysis, which is the most important pathway of glucose metabolism. This discovery is a significant contribution to scientific knowledge. We are considering presentation of our finding at a national meeting and also preparation of a manuscript for publication in a scientific journal. The discovery also may lead to many important developments. One such development will be use of genetic engineering techniques to increase the levels of the enzyme responsible for the rate-limiting step. The probable result is significant

increases in the rates of production of compounds of interest, which are derived from glycolysis, such as ethanol, lactic acid, succinic acid, acetic acid, and others. A number of requests for proposals (RFP) will be issued soon. These include the RFP by the Office of Fuel Development on yeast platform ethanol production and the RFP by the Office of Industrial Technology on bio-based products. We are considering partnerships with several industrial concerns to respond to these calls.

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Publication Derived from This Project

N. P. Nghiem and T. M. Cofer, "Experimental Evidence of a Rate-limiting Step of Glycolysis in Microorganisms," in preparation.

Synthesis of High-Performance Polymer Composites by Electron Beam Curing

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Cationic polymerization of epoxy resins can be induced by ultraviolet (UV) or electron beam radiation and proceeds very efficiently in the presence of appropriate photoinitiators. Although good thermal properties have been obtained for some electron beam–cured epoxy resins, other important mechanical properties such as interlaminar shear strength, fracture toughness, and compression are poor and do not meet the manufacturer's standards. We have initiated a comprehensive study to investigate the kinetics and mechanism of UV-cured and electron beam–cured cationic polymerization of the model compound phenyl glycidyl ether (PGE) and Tactix-123 in the presence of the initiator CD-1012. The objectives of this study is to determine whether or not the same reaction conditions and kinetic parameters (e.g., radiation dose, concentration, temperature) control the properties of final polymeric products under both radiation conditions. The ultimate goal of this study is to identify the parameters that give rise to improved thermal and mechanical properties of the final composite prepared by electron beam curing. We have employed fast kinetic spectroscopy to elucidate the mechanism of polymerization and identify the reactive intermediates involved.
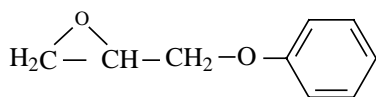
Introduction

Currently, there is considerable interest in developing high-strength, lightweight polymer matrix composite materials for the aerospace and automotive industries. One class of resins that has the proper thermal and mechanical properties for these applications is toughened epoxies. These materials are typically processed by thermal (i.e., autoclave) curing methods, but recently, composites with comparable thermal and mechanical properties have been prepared by radiation curing. UV and electron beam curing of resins and composites has received considerable attention in recent years.[1–6] Radiation curing typically uses high-energy radiation from electron beams to induce polymerization and cross-linking reactions. Electron beam curing is of great interest to industry because it has many advantages over thermal curing methods, which include lower cost, improved polymer performance, reduced energy consumption, lower residual thermal stress, reduced volatile toxic by-products, and simpler less-expensive tooling. Electron beam processing is currently used for curing thin films for can and beverage coatings, printing inks for folding cartons, and anticorrosion coatings for automobile wheels.[7] Recent advances in electron beam curing of polymers has invoked onium salt promoters in cationic polymerization of vinyl ether monomers[8] and epoxy resins.[9] A fundamental understanding of the chemical events that lead to the desired material properties as well as a knowledge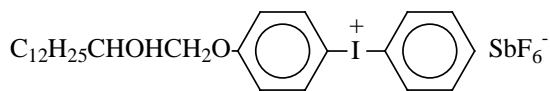 of the materials that undergo these radiation-induced reactions would provide researchers with the insight needed to control properties of the end products and to make advances in the development of novel resin systems for use in composites and adhesive applications. However, there is a lack of understanding concerning the chemical reactions that occur in the radiation curing of polymeric materials and the chemical structures that produce the desired mechanical properties. The goal of this study was to identify and optimize the parameters that control the material properties to facilitate preparation of new composites with advanced mechanical and thermal properties from epoxy resins by radiation curing.
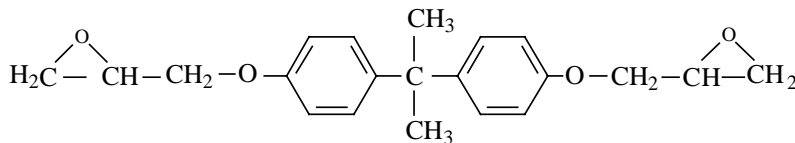
Technical Approach

The structure of all the reagents used in this study are shown in Scheme 1. All the solvents used were high-performance liquid chromatography (HPLC) grade. Bromophenol blue (3',3'',5',5''-tetrabromophenol-sulfonephthalein) was used as received. Gel permeation chromatography (GPC) was performed on a Waters 600E Instrument. HPLC analysis of the samples was carried out under isocratic conditions (75% acetonitrile, 25% water) using a Hewlett Packard Model 1090 equipped with a diode array detector. Absorption spectra of the samples were obtained on a Cary 4 spectrophotometer. Steady-state γ-radiolysis and fast kinetic studies by pulse radiolysis were conducted at the Notre Dame Radiation Facilities.

$$H_2C - CH - CH_2 - O - \text{(phenyl)}$$

Phenyl Glycidyl Ether (PGE)

$$C_{12}H_{25}CHOHCH_2O-\text{(phenyl)}-\overset{+}{I}-\text{(phenyl)}\quad SbF_6^-$$

CD-1012

$$H_2C - CH - CH_2 - O - \text{(phenyl)} - \underset{CH_3}{\overset{CH_3}{C}} - \text{(phenyl)} - O - CH_2 - CH - CH_2$$

Tactix-123

Scheme 1. Regent structures.

Results and Accomplishments

UV Photolysis Studies

Table 1 shows the data for a set of samples irradiated at various times. As can be seen from the data presented in Table 1, the yield of insoluble polymer (cross-linked material) increases with increasing irradiation time and accounts for about 70% of the total polymer formed. Samples irradiated to >90% conversion of Tactix-123 completely solidified and were hard to remove from the glass tube by tetrahydrofuran (THF) as the solvent. As a result, the exact weight of insoluble polymer could not be determined for high conversion samples.

The internal rise in temperature as a result of bond breaking during the polymerization process was not significant. The effect of external temperature on the reaction rate was studied for samples of Tactix-123 + CD-1012 to obtain the activation energy E_a and the Arrhenius A factor for the polymerization process. From the slope and intercept of the plot of rate constant as a function of inverse temperature we obtain an activation energy of $E_a = 61$ kJ/mol and an Arrhenius A factor of 2.4×108 s^{-1} for the polymerization process. The observed values are in good agreement with the reported values for polymerization processes.

Pulse Radiolysis Studies

Steady state γ-radiolysis of CD-1012 in methanol (5E-4 M) containing bromophenol blue (9E-5 M), an acid indicator, using dose rates of 1, 5, and 20 krad/min has revealed that super acid HSbF$_6$ is indeed formed. The formation of HSbF$_6$ by degradation of initiator CD-1012 is evident from the observed changes in the absorption spectra of bromophenol blue. Figure 1 shows the change in the absorption spectra of bromophenol blue as a function of total irradiation (0 to 75 krad) for a 1-krad/min dose rate. Similar spectra were also observed for dose rates of 5 and 20 krad/min. It is clear from such plot that the acidity is increased as total dose absorbed by CD-1012 is increased. Presence of two isosbestic points at around 325 and 475 nm further supports the notion that bromophenol blue (λ_{max} = 595 nm) is converted to its corresponding protonated form, which absorbs at λ_{max} = 425 nm.

Plots of increase in the acidity of media as a function of total dose for 1, 5, and 20 krad/min (not shown) show that the acidity increases sharply as a function of total dose. Such data is consistent with the formation of more superacid HSbF$_6$ at higher total doses. These results have clearly shown the formation of HSbF$_6$, a key intermediate in cationic polymerization of epoxies, by decomposition of CD-1012 upon radiation.

Table 1. HPLC and GPC analysis data for the photolysis (300 nm) of Tactix-123 in the presence of CD-1012 as the photoinitiator

Irradiation time (min)	Moles Tactix at time 0	Moles Tactix after irradiation	% loss Tactix[a]	% Polymeric cross-linked insoluble product[b]	% Polymeric soluble products[c]
3	2.4E-3	2.2E-3	8.8	0.0	100
6	2.6E-3	2.1E-3	20.9	19.0	81.0
9	3.1E-3	1.7E-3	47.7	22.0	78.0
12	3.0E-3	1.7E-3	43.7	56.0	44.0
18	2.8E-3	1.1E-3	60.4	67.0	33.0
24	2.7E-3	9.7E-4	64.1	69.0	31.0

[a]Data obtained by HPLC.

[b]% Cross-linked insoluble polymeric product is based on the weight of material that was insoluble in THF.

[c]% Polymeric soluble product is based on GPC data for products that dissolved in THF and shows three broad peaks eluting at 19, 21, and 24 min (unreacted CD-1012 and Tactix-123 elute at 26 and 29 min, respectively).
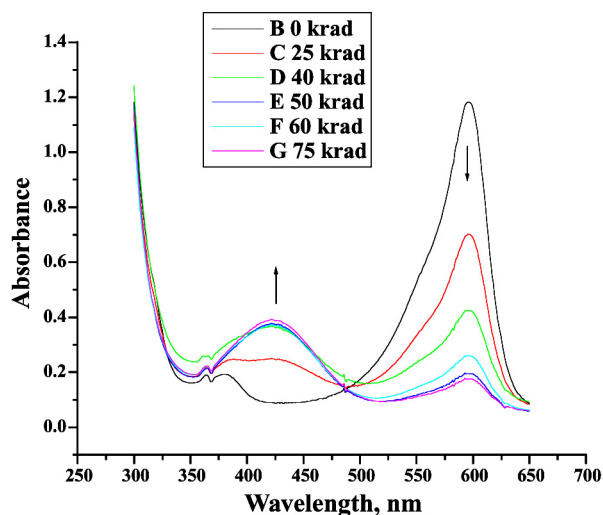
Fig. 1. Changes in the absorption spectra of bromophenol blue in methanol upon radiolysis of CD-1012 using a 1-krad/min source; [CD] = 5E–4 M, [BPB] = 9E–5 M.

Fig. 2. Transient absorption spectrum of degassed PGE in the presence of ~3% of CD-1012 taken at: (A) 0.75, 3.5, 5.75, and 10.75 μs and (B) 20 μs, 40 μs, 60 μs, 110 μs, and 160 μs after the pulse.

Pulse Radiolysis/Optical Detection of Degassed Phenyl Glycidy Ether (PGE) Containing 3% (wt) CD-1012

The evolution and decay of the transient absorption spectrum of degassed PGE in the presence of 3 wt % CD-1012 is shown in Fig. 2. The spectral features of 0.75 μs intermediate with maxima at 360 and 400 nm changes with time. Growth of the 400 nm absorbance maximizes in approximately 11 μs after the pulse and decays on a much longer time scale. Figure 2B shows that at longer times (>20 μs after the pulse) the 435-nm band becomes a major absorption band in the transient spectrum and decays on a much longer time scale. About one-third of the transient spectrum decayed in a time scale of 160 μs (limit of pulsed probe lamp). Similar spectral changes were also observed for the transient in the 500- to 800-nm region. This transient exhibits maximum absorption peak at λ_{max} > 800 nm and two shoulders at 605 and 685 nm, respectively (not shown). Spectral features of these intermediates change with time. At 160 μs after the pulse, only the spectrum of transient with λ_{max} > 800 nm is still present. We observe significant variation in the rise-time of transients (e.g., from 2 μs for 605-nm absorbance to 20 μs for the 435-nm band). Almost all the absorption bands reach their maximum value between 20 and 60 μs after the pulse, suggesting that these transients are formed by secondary processes. Our extensive transient absorption studies have allowed us to assign the observed intermediates to (a) the cation radical of PGE produced by the interaction of $HSbF_6$ with PGE and (b) other PGE radicals produced upon the interaction of PGE with carbon-centered radicals formed by decomposition of CD-1012 (cleavage of carbon-iodine bond). These intermediates are the precursors to the polymerization process.
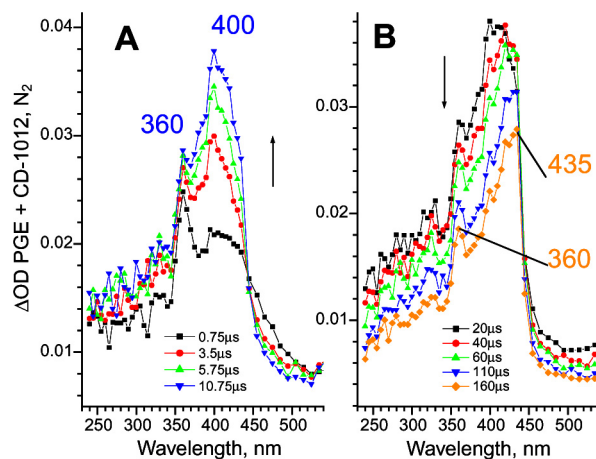
Summary and Conclusions

Our γ-radiolysis studies of the photoinitiator CD-1012 have confirmed the formation of super acid $HSbF_6$, a key intermediate in the cationic polymerization of epoxies. Pulse radiolysis/optical detection studies of model compound PGE in the presence of photoinitiator CD-1012 have shown that the cation radical of PGE (formed by the addition of superacid $HSbF_6$, which is generated upon decomposition of CD-1012) as well as other free radicals (generated by the cleavage of carbon-iodine bond in CD-1012) are responsible for the polymerization process. These new kinetic findings have been well received and recognized by the CRADA partners involved with the Engineering Science and Technology Division in the development of new epoxy resins for toughened composites. Among the benefits that this project has brought to the investigators and ORNL are (a) these kinetic findings have impacted the scientific communities to such a degree that we have been able to attract new partners for the ongoing CRADA project and (b) the success of this project has led to some follow-on fundings from the Army and NASA to investigate the cure kinetics of cycloaliphatic epoxy resins and polyimides that are of interest to both Army and NASA. An oral presentation entitled "Cure kinetics of cationic polymerization of phenyl glycidyl ether and Tactix-123 using CD-1012 as photoinitiator" was given at SAMPE 2001, May 6–10, Long Beach, California. A full paper on this research has also been published in the Proceedings of SAMPE 2001, SAMPE Journal, May 2001.

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R. Dabestani and I. N. Ivanov, "Cure Kinetics of Cationic Polymerization of Phenyl Glycidyl Ether and Tactix-123 Using CD-1012 as Photoinitiator," *Proceedings of SAMPE 2001, SAMPE Journal*, Book 2, 2075 (2001).

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Novel Catalytic Membranes for Alkane Dehydrogenation

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Membrane reactors are of great interest to DOE and the petrochemical and chemical industries because of potential large-scale applications, including energy-efficient petrochemical production and cost-effective upgrading of natural gas. This project focused on a novel catalytic membrane approach that would allow simultaneous reaction and separation with efficient mass and heat transfer, aiming to provide much higher throughput and conversion than that achievable with current technology. Significant progress was made towards achieving this goal. A new method was developed for synthesis of MFI-type zeolite membranes on the inner surfaces of porous α-alumina supports. The method developed in this work has the advantages of improved controllability, minimal waste generation, and reduced chemical consumption that are desirable for large-scale production of zeolite membranes. This accomplishment overcomes the largest technical hurdle in developing the advanced membrane reactors and is the subject of an invention disclosure that has been elected for filing and one research paper. We plan to continue progress in testing the approach on a model reaction characteristic of nonoxidative dehydrogenation of low alkanes and anticipate follow-on collaborative work with universities and industry.

Introduction

Low-alkane (C_1-C_4) dehydrogenation and hydrogen separations are important processes in the petrochemical, chemical, and energy industries. Dehydrogenation is the key step in converting natural gas into useful products, and hydrogen separation is important in many chemical processes as well as in the development and application of fuel cells. Recent DOE funding of over $70 million on processes for oxidative dehydrogenation of natural gas illustrates the importance of one of these areas. Nonoxidative dehydrogenation has several advantages over oxidative processes, including higher selectivity, lower reaction temperature, and reduced production of greenhouse gases. Research for nonoxidative processes has not been funded well in the past because available technologies are limited to very low conversion rates and throughputs.

The aim of this project, initiated in May 2000, was to demonstrate the concept of a new materials production process that would be the basis of a novel reactor for dehydrogenation and gas separations. This novel reactor is, in principle, capable of overcoming the dual obstacles of low conversion and low throughput currently associated with traditional nonoxidative dehydrogenation reactors. The new membranes are designed for simultaneous reaction and separation with efficient mass and heat transfer and could provide much higher throughput and conversion than that achievable with current technology.

Proof of principle of this new type of membrane reactor could have significant impact on the conversion of methane into valuable higher hydrocarbons.

The membrane reactor concept consists of a submicron polycrystalline separative film supported on a porous ceramic substrate, with a nanoscale zeolite catalyst coating on the surfaces of the substrate pores (Fig. 1). The novel membrane reactor can be thought of as a compact assembly of numerous catalytic micromembrane reactors. The nanoscale zeolite film on the substrate pore surface provides a catalytic surface area that could be three to six times larger than that of mesoporous or layered structures. The ultrathin separative film provides a high hydrogen permeance, needed to maintain conversion and throughput by matching the reaction rate. Since the catalyst layer is directly grown on the pore walls of the support, efficient heat transfer can be realized. Thus, a uniform and stable temperature distribution can be maintained in the reaction zone. With these marked improvements, the new membrane reactor is expected to provide much higher throughput and higher conversion rates than those obtained using currently available membrane reactors.

The major technical challenge faced in producing such a membrane reactor is the synthesis of the nanoscale catalyst layer on the surfaces of the micron-scale pores of the substrate. This project focused on developing a synthesis method that would overcome the difficulties in
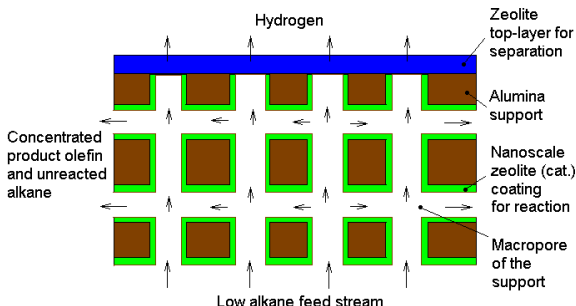
Fig. 1. Schematic of membrane reactor.

Technical Approach

Supported polycrystalline zeolite membranes have been studied for more than a decade. To date, most of the work has focused on MFI-type zeolite membranes, ZSM-5, and silicalite-1 membranes in particular because of their (1) readiness to form polycrystalline films on different substrates, (2) excellent performance in hydrocarbon separations, (3) high thermal and chemical stabilities, and (4) potential applications in membrane reactors. However, industrial applications of zeolite membranes have been hindered due to some major disadvantages associated with current synthesis techniques that make large-scale production difficult and expensive. In this study, a new type of vapor-phase transport (VPT) method using a template-containing wet gel layer was developed for synthesizing MFI-type zeolite membranes on porous α-alumina supports. Such a modified vapor-phase method uses tetrapropyl ammonium hydroxide (TPAOH) as the structure-directing agent and, thus, can combine the advantages while avoiding the major disadvantages of conventional hydrothermal and VPT methods.

Synthesis sols were prepared in aqueous solutions of fumed silica, sodium hydroxide, and TPAOH. After the synthesis sol was aged at room temperature, the polished side of an α-alumina disc was dipped in the sol to coat it with a uniform precursor layer. The disc was then placed with coated side upward on a Teflon stand in an autoclave. The liquid volume in the autoclave was large enough to avoid complete vaporization and to maintain a vapor-liquid coexisting state in the autoclave under the synthesis temperature and autogenous pressure. The autoclave was sealed and was preheated to 190°C for vapor-phase treatment. The vapor phase was provided by a 1 M TPAOH aqueous solution, which had never been used before in conventional VPT synthesis. We used TPAOH as the structure-directing agent because it is (1) the most effective template for formation of MFI-type zeolites and (2) less toxic than the commonly used amines. Other

liquids, including pure water and a mixture of ethylene diamine (EDA), triethylamine (TEA), and water, were also used to investigate their effects on zeolite crystallization. The duration of vapor-phase treatment was several days. After synthesis, the discs were washed with deionized water and dried/stored in an oven at 50°C.

Results and Accomplishments

X-ray diffraction (XRD) patterns of the discs after vapor-phase treatment are shown in Fig. 2. For the gel layers coated from the parental synthesis sol containing template TPAOH, the XRD patterns indicate that MFI-type zeolite crystallites are formed on the α-alumina supports after being treated in the vapor phases of the TPAOH solution and the EDA/TEA/water mixture. However, the gel layer remained in an amorphous phase after treatment in the vapor of pure water.
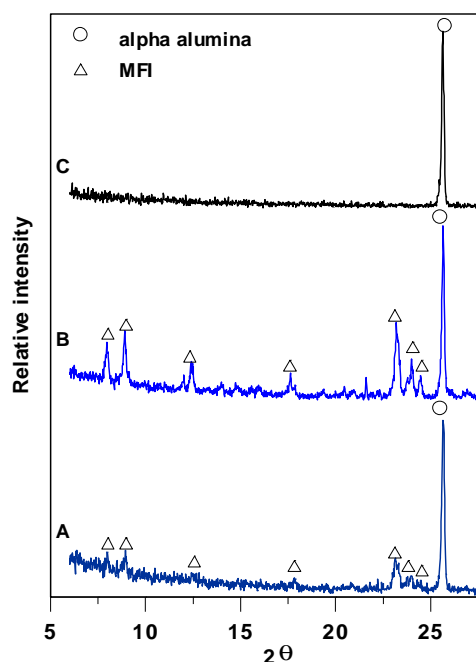

Fig. 2. XRD patterns of membranes after treatment in different vapor phases: (a) treated in vapor of 1 M TPAOH aqueous solution; (b) treated in vapor of EDA/TEA/water mixture; and (c) treated in water vapor.

Interesting results were obtained when the precursor layers were coated from TPA$^+$-free colloidal silica suspension. In these experiments, MFI zeolite was not formed in either one of the vapor phases of the TPAOH solution or the EDA/TEA/water mixture. A cubic Na-P1 zeolite phase was formed on the support after treatment in the vapor phase of the TPAOH solution, and a cubic analcime phase was formed in the vapor phase of the EDA/TEA/water mixture. These results indicate that the

that step to allow the further development of novel membrane reactors.

structure-directing organic molecules play a key role in determining the crystallization of the precursor layer and utilization of template TPAOH in the parent synthesis sol is critical to the formation of the MFI zeolite films.

Figure 3 shows scanning electron microscopy (SEM) images zeolite synthesized in the vapor of TPAOH solution in the form of a membrane. These images clearly show a compact zeolite film, composed of intergrown crystals, which is estimated to be ~3 μm thick. This observed morphology of aggregates consisting of numerous smaller intergrown crystallites is characteristic of VPT synthesis wherein a well-defined single crystal shape is unable to develop through gel particle crystallization in a bulk liquid phase. The helium permeance of the membrane before calcination was 3.8×10^{-9} mols$^{-1} \cdot$ m$^{-2} \cdot$ Pa^{-1}, which indicates that the membrane is of good quality, with minimized macrodefects.
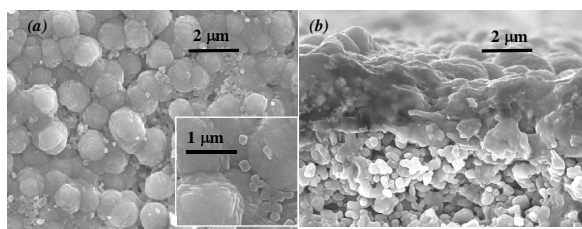

Fig. 3. MFI zeolite membrane synthesized in vapor of TPAOH solution: (a) surface, (b) cross section.

A modified VPT technique was developed in which the zeolite was formed on the inner pores of the α-alumina supports. Figure 4 presents cross-section images showing zeolite crystal coating on the inner surface of α-alumina substrates. The zeolite crystals are approximately 0.5 microns in diameter and are evenly distributed throughout the pores of the substrate. The specific surface area of the coated alumina, as measured by nitrogen adsorption, was 0.3 m$^2 \cdot$ g^{-1} before calcination and 5.0 m$^2 \cdot$ g^{-1} after calcination. Although the zeolite comprises a small fraction of the total mass of the membrane, it contributes the majority of the surface area. The α-alumina membranes internally coated with zeolite crystals are suitable for further testing for reactive membrane applications.

The accomplishment described above opens the way for further development and testing of the novel catalytic membrane reactor. Procedures have been developed for the remaining steps of the membrane synthesis—formation of the separative top layer and incorporation of catalyst metals into the zeolite in the support pores. A remaining task is the testing of the product membranes. A membrane test rig, which includes a high-temperature membrane reactor test cell, has been set up for performance testing using the model reaction of dehydrogenation of
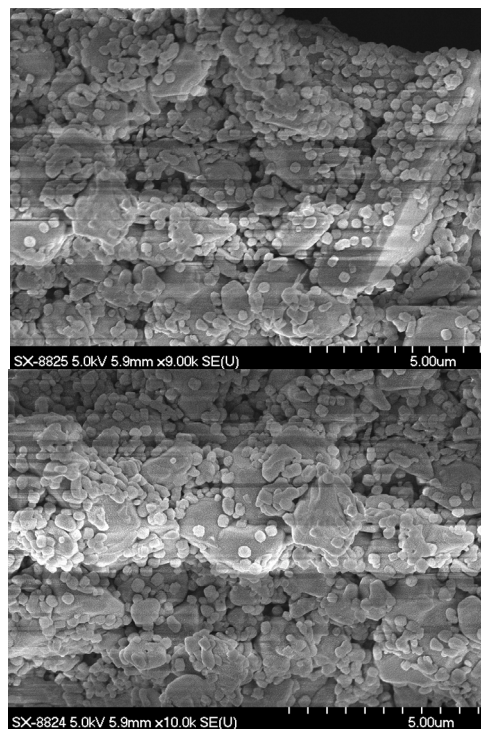

Fig. 4. Cross-sectional images of porous α-alumina coated with zeolite crystals: (top) at surface, (bottom) near center of alumina support.

isobutane. The testing task has been slowed due to a safety shutdown and to the departure of the postdoctoral associate participating in this research. We aim to complete the membrane testing experiments in FY 2002.

Summary and Conclusions

This project has made significant progress in developing a novel catalytic membrane approach for simultaneous reaction and separation. A new method that takes advantages of both in situ crystallization and VPT processes has been developed for the synthesis of MFI-type zeolite within the pores of an α-alumina substrate. The method developed in this work is a major step forward in the production of membranes that can be operated in chemical processes with efficient heat and mass transfer. The method also has advantages of improved controllability, minimal waste generation, and reduced chemical consumption that are desirable for large-scale production of zeolite membranes. This accomplishment overcomes the largest technical hurdle in developing the envisioned advanced membrane reactors.

To date, this work has produced one invention disclosure that has been elected for filing and one research paper submitted to a peer-reviewed journal. We plan to continue progress in testing the approach on a model reaction characteristic of nonoxidative dehydrogenation

of low alkanes and anticipate follow-on collaborative work with universities and industry.

Publications and Intellectual Property Derived from This Project

J. Dong, E. A. Payzant, M. Z. Hu, D. W. DePaoli, and Y.S. Lin, "Synthesis of MFI-Type Zeolite Membranes on Porous α-Alumina Supports by Wet Gel Crystallization in the Vapor Phase," submitted to *J. Mater. Sci.* (2002).

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Bacterial Cellulose as a Matrix for Construction of Fuel Cells and Biosensors

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During studies on the inhibition of enzymatic cellulose hydrolysis by platinum group metals, a novel phenomenon, the deposition of palladium black from hexachloropalladate solutions by the action of bacterial cellulose, was discovered. Since this hydrated form of cellulose can be easily dried to a thin membrane, it was purposed that bacterial cellulose doped with metals, enzymes, and polyelectrolytes could be used for the assembly of cellulose-based devices for construction of fuel cells and biosensors. The original concept was demonstrated by the assembly of working fuel cells from bacterial cellulose containing deposited palladium. Immobilization of enzymes for biosensor assembly in purified bacterial cellulose was carried out. Bacterial cellulose with defined properties was produced by cultivation of *Gluconoacetobacter hansenii* in the laboratory. This bacterium produces enzymes of interest for hydrogen production and biosensor applications, and these were immobilized in the cellulose pellicules by chemical cross-linking. These enzymatic activities were maintained after subsequent deposition of palladium, indicating that such membranes can be used for direct production of electricity from glucose and other sugars. The attachment and growth of mammalian kidney cells on purified bacterial cellulose pellicules was demonstrated and opens new possibilities for the design of biosensors and artificial organs.

Introduction

As part of a continuing study on the inhibition of cellulases,[1,2] various samples of cellulose such as cotton linters and filter paper were permeated with solutions of ammonium hexachloropalladate before enzymatic hydrolysis with cellulase. When this procedure was carried out with cubes of bacterial cellulose obtained from the Philippine food product nata de coco, a black precipitate formed on and within the cubes that appeared to palladium black. No deposition was observed with samples of plant-derived cellulose, although both types of cellulose are composed of chains of glucose molecules linked by β–1,4-glycosidic bonds. There are, however, obvious differences in the physical properties and microstructure of plant cellulose, which is a fibrous crystalline solid, while bacterial cellulose contains 200-fold its weight in water and resembles a gel when hydrated but can be dried to a thin membrane. Cellulose from cotton (*Gossypium* spp.) has a structure consisting of many small microfibrils that are assembled into larger macrofibrils that are then twisted together to form the fiber. Imaging of cotton linter fibers by atomic force microscopy found an average macrofibril diameter of approximately 100 nm.[3,4] The bacterial cellulose used commercially is produced by the bacteria *Gluconoacetobacter xylinus* and *hansenii* (formerly classified as strains of *Acetobacter xylinum*). The bacteria secrete cellulose macrofibrils 40 to 60 nm wide that assemble into sheets outside the cell to form a layer or pellicule on the surface of the culture medium.[5]

The palladium-cellulose cubes were dried to thin membranes that conducted electricity and produced hydrogen from sodium dithionite. The ease of preparing the palladium-cellulose membranes indicated that they might be used in fuel cells and small electronic devices.

The hydrogen-powered fuel cell is constructed from a cathode and an anode separated by an ion-selective membrane, usually Nafion117, a perfluorosulfonic acid polymer film. Hydrogen gas is infused at the anode side. Platinum black is often used as the catalyst layer at the anode side that splits hydrogen gas into protons, that flow through the membrane to the catalyst at the cathode, where they combine with oxygen to form water, and electrons, that are routed through the anode as electric current. Both platinum and palladium are used for catalysis of hydrogen reactions.

The objective of the project was the development and demonstration of the potential of bacterial cellulose for the design and synthesis of novel membrane materials that can be used for fuel cells and biosensors. The ultimate goal is design of a device that combines immobilized enzymes and fuel cells to generate electrical power directly from glucose or other substrates derived from renewable bioproducts.

Technical Approach

The methods that were demonstrated in the course of the project included infusion of metal salts, electrolytes, and enzymes into the cellulose, chemical modification of

the cellulose, growth of mammalian cells in the cellulose matrix, and immobilization of the cellulose-synthesizing bacteria and their enzymes in the cellulose. The approach can be separated into three main categories: (1) infusion of target molecules into hydrated cellulose and subsequent drying of the cellulose to a membrane for testing; (2) infusion of target molecules followed by a chemical modification step such as cross-linking, precipitation, or oxidation-reduction catalyzed by addition of appropriate reagents; (3) direct chemical modification of cellulose by sulfonation and other reactions.

The first task was the assembly and demonstration of a working fuel cell from layers of bacterial cellulose. The second task was the immobilization of active enzymes in the bacterial cellulose matrix and the demonstration of possible detection systems for biosensor assembly. For this part of the project, the initial plan called for the use of commercially available purified enzymes. An important subtask for the entire project was the growth and preparation of bacterial cellulose with defined characteristics in the laboratory. It was discovered that another technique, immobilization of whole bacteria containing enzymes of interest, could be easily used to obtain enzymatically active membranes. The intrinsic enzymes of the cellulose-synthesizing bacteria include several of interest for the ultimate goal of utilization of glucose and other sugars for production of hydrogen and electricity. Another technique for design of biosensors as well as biocapsules for medical implantation was demonstrated, the attachment and growth of mammalian cells on the bacterial cellulose matrix.

Results and Accomplishments

Preliminary experiments had been carried out using the bacterial cellulose cubes obtained from the commercial food product nata de coco. The cellulose-synthesizing strain *G. hansenii* was purchased from the American Type Culture Collection and cultivated under defined conditions to obtain cellulose pellicules of desired size and properties for the different project tasks (Fig. 1). Production of

cellulose with medium components derived from agricultural products such as maize and soybeans was demonstrated, an important factor in eventual cost-effective large-scale production of the cellulose. The laboratory-grown bacterial cellulose catalyzed deposition of palladium, confirming the results observed with the commercial nata de coco.

Fuel cell membrane electrode assemblies (MEAs) were assembled from layers of palladium-cellulose, untreated cellulose, and cellulose infused with electrolytes such as potassium chloride (Fig. 2).
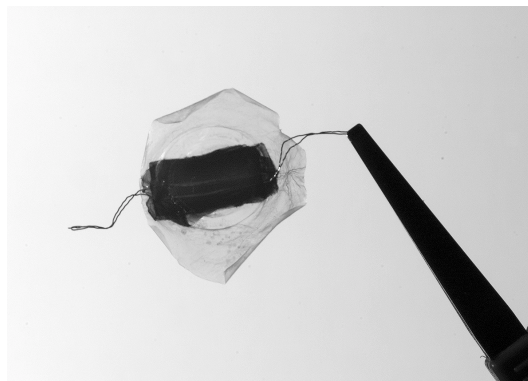

Fig. 2. Bacterial cellulose MEA.

The MEAs were tested for fuel cell behavior, with the resistance increased in 2-kΩ increments from 1 kΩ to 11 kΩ and reaction conditions of 26°C and 1 atm, 4% H_2 at 40 mL/min (Fig. 3). Membrane characterization was carried out, including quantification of palladium by atomic absorption (Galbraith Laboratories, Inc., Knoxville, TN), hydrogen crossover, and thermogravimetric stability of cellulose membranes (Fig. 4). A mechanism for the deposition reaction was proposed in which oxidation of the cellulose reducing ends was followed by decarboxylation. Supporting evidence was obtained when the evolution of carbon dioxide during palladium deposition by bacterial cellulose was experimentally
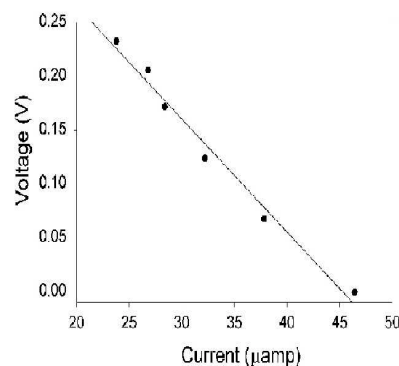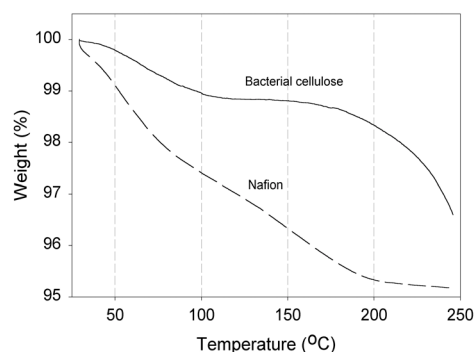

Fig. 1. Laboratory-grown bacterial cellulose pellicule.

Fig. 3. Voltage-vs-current curve for MEA.

Fig. 4. Comparison of the thermogravimetric analysis profiles of bacterial cellulose and Nafion 117®.

Fig. 6. AFM image of bacterial cellulose after palladium deposition.

verified and deposition of palladium from hexachloropalladate solution by glucose and other reducing sugars was demonstrated.

Imaging of bacterial cellulose before and after palladium deposition by atomic force microscopy was carried out by Dr. Ida Lee, Department of Electrical Engineering, University of Tennessee, Knoxville. Comparison of the bacterial cellulose images with those of cotton linters[3,4] show that, after drying, bacterial cellulose fibers form a dense net-like structure very different from the large, parallel macrofibrils of cotton cellulose (Fig. 5). The palladium particles appeared to be deposited in clusters approximately 60 nm in diameter (Fig. 6).

The second task of the project objective was the immobilization of enzymes in bacterial cellulose for the design of biosensors and biohydrogen generation.[6] Target enzymes included glucose oxidase, β-glucosidase, β-fructofuranosidase (invertase), glucose dehydrogenase, and hydrogenase. In preliminary experiments, *Aspergillus* and *Pyrococcus* β-glucosidases were infused in cubes of nata de coco cellulose. After infusion, the *Aspergillus* enzyme maintained activity better than the *Pyrococcus* enzyme but exhibited a gradual loss of activity over time.
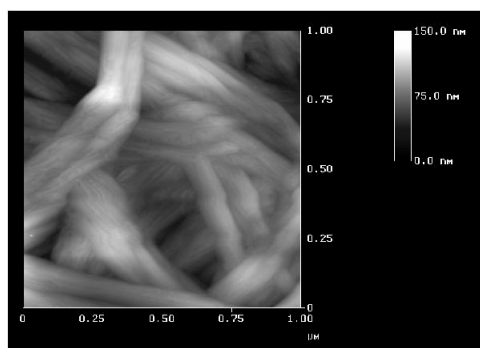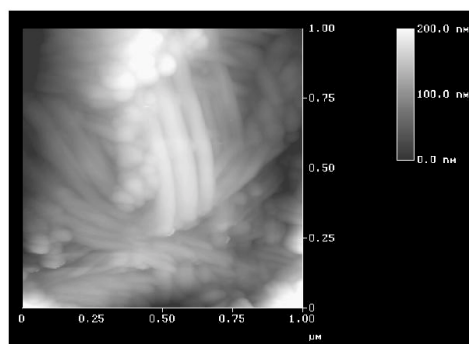
The best performance was exhibited by glucose oxidase from *Aspergillus niger*, which, after simple infusion into the cellulose matrix, maintained activity in repeated assays, as well as after drying, after deposition of palladium in the cellulose, and after formation of sol gel within the cellulose. A glucose sensor was constructed from dried bacterial cellulose containing glucose oxidase and a pH electrode.

Co-immobilization of the *Aspergillus* enzymes glucose oxidase and β-glucosidase in laboratory-grown cellulose pellicules was carried out by glutaraldehyde cross-linking. Once again, the glucose oxidase maintained high levels of activity, while the β-glucosidase exhibited lower stability.

In addition to cellulose, the *G. hansenii* bacteria themselves synthesize many enzymes of interest for biosensor construction. By simply immobilizing whole cells in the cellulose pellicule that they produced during growth, membranes containing β-glucosidase (BG), glucose dehydrogenase (GDH), alcohol dehydrogenase, and electron transport activities were obtained. Electron transfer to the redox dyes 2,6-dichloroindophenol and Neutral Red was demonstrated (Fig. 7). The redox dyes can be employed as biosensor indicators or can be utilized to couple sugar oxidation to hydrogen production by
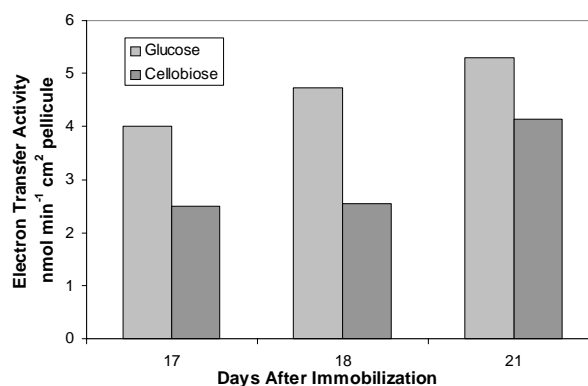

Fig. 5. AFM image of bacterial cellulose.

Fig. 7. Stability of instrinsic GDH (substrate glucose) and GDH + BG (substrate cellobiose) in cross-linked pellicules.

hydrogenase without the utilization of the labile cofactor nicotinamide adenine dinucleotide phosphate. Since enzymatic activity was maintained in such membranes after the subsequent deposition of palladium, electron transfer directly to palladium can be employed in the design of bioelectronic devices.

Purified, sterilized cellulose pellicules were incubated with mammalian kidney cells by Dr. Minoo Askari, Life Sciences Division, ORNL. The kidney cells attached to the cellulose were metabolically active and formed colonies. These results provided the basis of a new concept for the design of biodevices.

Summary and Conclusions

The results obtained in the course of this project have not only proved that bacterial cellulose can be utlilized for fuel cells and biosensors but have led to the conception of additional applications for this novel material in the fields of biomedical engineering and bioremediation as we have consulted with colleagues and program managers.

This project has started three new research areas (fuel cells, enzyme-based biomembranes, biomedical engineering) for our research group that have the potential for full programmatic funding and that fit well with ORNL's current program goals. Follow-on funding was obtained from the Fuel Cell Initiative of the Office of Transportation Technology (OTT), DOE, for the specific task of development of a proton–conductive membrane from bacterial cellulose (March–September, 2001; FY 2002). Further funding for development and characterization of metal deposition and electron-transfer mechanisms has been obtained from the Electrochemistry Initiative of the Office of Chemical Science, DOE (FY 2002). A small academic-linked business partner was located for development of the biomedical implant concept, and funding is being sought from the Biomedical Engineering Joint Program, NIH/DOE, and other sources.

Preliminary results from the project and the resultant subtask that has received follow-on funding from OTT were presented in a poster at the Annual Laboratory R&D Meeting of the DOE Fuel Cells for Transportation Program, Oak Ridge, TN, June 6–8, 2001.

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Publications and Intellectual Property Derived from This Project

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Novel Nanocoatings for Silicon-Based Microelectromechanical Systems Devices

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This research focuses on developing a fast, reliable, and practical method of fabricating performance-enhancing polymer coatings for microelectromechanical system (MEMS) sensors. The goal of the project is to develop a versatile state-of-the-art method for preparation of nanometer-thick (ca less than 50 nm) polymer coatings ("nanocoatings") on silicon substrates. Control over the chemical composition of the nanocoating is required if molecular interactions between analytes and the coating are to be used to enhance the sensor chemical selectivity and sensitivity. Surface coating thicknesses of a few nanometers are required to achieve the theoretical sensitivity limits of modern MEMS sensors. Current coating technologies cannot meet the challenge of preparing nanocoatings with total control over the chemical composition of the film. This research builds on recent breakthroughs in surface chemistry and polymer synthesis which hold promise for the controlled preparation of uniformly distributed nanoscale polymer array coatings on MEMS sensors. Surface-bound growth of low-molecular-weight polymers by living free radical polymerization (LFRP), atom transfer polymerization (ATRP), and photochemically initiated polymer binding are utilized for nanocoating preparation.

The untreated surfaces of miniaturized MEMS devices (sensors, microfluidics, and other silicon/silica-based devices) can be considered as passive, relying on physics to define their performance characteristics. Engineered chemical coatings are required to introduce chemical selectivity to these devices, to extend their functionality, and to optimize their performance characteristics. However, the small size of a modern MEMS device introduces a fundamental technological barrier in generating surface coatings. Conventional coating techniques often apply large masses of unequally distributed high-molecular-weight polymers to a surface. Large masses of polymer severely diminish the mass-sensitive properties of the device. A film of organic polymer that was "thin" by the standards of even 5 years ago can render today's MEMS sensors virtually useless. To overcome these barriers to modern sensor development, we are examining the controlled construction of low-molecular-weight polymer arrays (nanocoatings) on small silicon devices through the use of surface-initiated polymer growth and photochemically initiated surface modification.

Surface-initiated polymer growth eliminates problems of grossly uneven coverage while providing an elegant technique for the preparation of nanoscale polymer arrays with controlled chemical functionality. Photochemical surface attachment of polymer chains offers an additional method of generating polymer nanocoatings of well-defined thickness. The polymer's radius of gyration, which is related to polymer molecular weight, is linearly related to the coating thickness achievable through single-point polymer attachment. The objective of this research is to demonstrate the preparation of nanocoatings with controlled chemical functionality on a miniaturized MEMS sensor platform and to test the performance of the sensor in a laboratory setting.

In FY 2001, we focused our efforts on the preparation of surface-linking molecular units for polymer nanocoating preparation. Surface-linking units have been synthesized for ATRP polymer growth and photochemically initiated polymer binding. Organo-silanes produced through multistep organic synthetic procedures have been attached to hydroxylated silicon wafers to produce nanometer-thick surface-attached layers. Polymer photochemical attachment was investigated as a method of producing nanocoating of polystyrene on a hydroxylated silicon substrate. ATRP polymer growth studies and additional photochemical polymer attachment studies are under way. Polymer attachment methodologies are also being applied for surface modification of a silicon-based MEMS sensor platform.

Data gathered from this research will form the basis of external research proposals targeted at sensor needs in the areas of long-term stewardship, homeland defense/counter-terrorism, and biomedical applications. This research will also form the groundwork for proposals directed toward understanding the fundamental physical and chemical properties of nanophases in soft materials. The project has also assisted in forging new collaborations within ORNL which derive advantages from a multidisciplinary approach to sensor development.

Ammonia-Scrubbing Technology for Removal of Industrial CO_2 Emissions

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The objective of this project is to determine if the ammonium bicarbonate (NH_4HCO_3)-production reaction can be used as an effective scrubbing technology to remove CO_2 emissions for many industrial combustion facilities (such as steelmaking or coal-fired power plants). New technology for efficient gas-liquid contacting recently developed at ORNL will be employed. The success of this project should enable us to attract outside funding to fully develop this technology.

The goal of this project is to perform proof-of-principle experiments to answer a fundamental question: Is it feasible to use an NH_4HCO_3-production reaction to remove CO_2 emissions from industrial flue gas? According to our assessment, an innovative application of this ammonia carbonation reaction could lead to an effective scrubbing technology to remove CO_2 emissions for many industrial combustion facilities and produce a valuable fertilizer product (NH_4HCO_3) that can be sold and placed into soil and subsoil terrains through proper agricultural application. Although the ammonia carbonation reaction has been used in manufacturing NH_4HCO_3 for decades, the existing fertilizer manufacturing technology, which typically uses CO_2 streams of high purity, is not designed to treat large volumes of industrial flue gas for carbon management. In most of the fossil energy industries, CO_2 is emitted in large volumes of flue gas (commonly containing 15% CO_2 by volume) that are continuously generated from combustion processes. A successful carbon management technology must be highly efficient and capable of cleaning large volumes of flue gas at a low cost. Studies are needed to develop this ammonia-scrubbing concept into a practical and cost-effective technology for carbon management. In this project, we plan to demonstrate this CO_2-removal concept by creating "snows" of solid NH_4HCO_3 in flue gas at laboratory-bench scale.

We started this project in June 2001. Ammonium hydroxide solution and synthetic flue gas (containing 15% by volume CO_2) were purchased, and a bench-scale reactor system was constructed and set up in our laboratory. We conducted a preliminary experiment with technical assistance of a summer student (Robert Townsend).

Formation of NH_4HCO_3 crystals has been now demonstrated for the first time in our laboratory. In the experiment, flue gas was passed through a reaction chamber while a fine spray (<10-μm droplet diameter) of ammonium hydroxide (NH_4OH) solution was concurrently introduced to the resulting flue gas cloud using an electric field atomizer. Theoretically, a reaction then occurs by which NH_4HCO_3 is formed from NH_4OH and CO_2. This product then precipitates out as tiny white particles or "snow." Upon spraying, we found that particles did not form instantly but instead only formed along the walls of the reactor where the NH_4OH solution coalesced as it was sprayed. The photograph in Fig. 1 shows the crystals of NH_4HCO_3 formed on the wall of the reaction chamber. This product, which was harvested and analyzed using nuclear magnetic resonance analysis techniques, was entirely pure NH_4HCO_3, proving the effectiveness of this method. According to our analysis, the reason that the preliminary experiment yielded "rains" instead of snows of NH_4HCO_3 was likely due to the fact that the ratio of H_2O to NH_3 in the NH_4OH solution was somewhat too high. We can overcome this problem by adding some anhydrous NH_3 in our further experiments.

Our postdoctoral research associate, Dr. Xiaonian Li, who is specialized in NH_3 fertilizer production, has now arrived at ORNL to join this project effort. Dr. Li has extensive experience in handling anhydrous NH_3 and will contribute significantly to this project. Success of this project will enable us to attract outside funding to fully develop this technology, which can potentially enable the fossil energy industries to operate their facilities in a manner that is in harmony with efforts to protect the Earth's environment for many years to come.

Fig. 1. Photograph of the NH_4HCO_3 crystals formed on the wall of the reaction chamber.

Mass Spectrometry and Bioinformatics for Determination of Protein Structure

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The three-dimensional (3-D) structure of a protein provides valuable clues as to its function. Current methods for determining 3-D protein structure are not capable of keeping pace with the huge numbers of proteins predicted from the Human Genome Project. We propose to combine chemical crosslinking and mass spectrometry to generate low-resolution spatial constraints on protein structure. These constraints will then be used as inputs for rapid computational prediction of protein structure. This approach offers advantages of speed and economy over current protein structure determination methods.

As large-scale DNA sequencing of the human and other organisms progresses toward completion, the focus of biology research is shifting to the structure and function of proteins. For example, the Protein Structure Initiative, also known as "structural genomics," is a large National Institutes of Health (NIH) program aimed at predicting a protein's tertiary structure from its gene sequence. In large part, this prediction is based on comparison with known protein structures ("folds"). To expand and improve the database of known protein structures, and to complement structure predictions for new or unknown proteins, experimental methods for tertiary protein structure elucidation must be used. Currently, X-ray diffraction and nuclear magnetic resonance (NMR) are the accepted experimental approaches. However, not all proteins are amenable to these techniques. In order to increase the flow of experimental data available for developing computational methods of predicting protein structure, other experimental techniques are sorely needed to complement X-ray diffraction and NMR.

In this project, we are combining mass spectrometry with intramolecular chemical crosslinking for tertiary structure interrogation. The crosslink produces a change in molecular weight, thus offering a way to exploit the strengths of mass spectrometry for studying protein structure. By determining the regions of the protein that are crosslinked by this approach, one in essence inserts "rulers" into the protein, thus providing information on distances between pairs of crosslinked amino acids in the 3-D structure of the protein. This information will be used as constraints in protein fold prediction programs such as PROSPECT, developed by the Computational Protein Structure Group of the ORNL Life Sciences Division.

To date, preliminary crosslinking and mass spectrometry results have been obtained for the model protein, bovine ribonuclease. A. Matthew Sega, a graduate student in The University of Tennessee(UT)/ORNL Genome Science and Technology Graduate School, identified favorable crosslinking conditions for this protein. On subsequent tryptic digestion of the crosslinked protein, a crosslinked pair of peptides was identified in the mass spectrum. This crosslinked peptide pair was subjected to tandem mass spectrometry to generate a series of fragments that contain information about the sequences of the two peptides, as well as the specific amino acid residues through which the peptides are crosslinked. This experiment demonstrated the feasibility of a key component of our overall approach—namely, the ability to interpret tandem mass spectrometry results from crosslinked peptides.

This project is benefiting the investigators and ORNL by providing preliminary data for a novel combination of experimental and computational protein structure determination methods that should be of interest to the Protein Structure Initiative at NIH. Furthermore, this project strengthens collaborations between ORNL and Prof. Cynthia Peterson, UT Department of Biochemistry and Cellular and Molecular Biology. Once mass spectrometric and computational methods are in place for model proteins, we will apply these techniques to the study of the 3-D structure of vitronectin, an important circulatory protein that is a focus of Prof. Peterson's research.

Nanocatalysts

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Composite nanocrystals (i.e., metallic cores surrounded by organic ligands) will be synthesized and separated according to specific metallic core sizes. We will investigate the use of nickel and gold nanocrystals to direct the growth of carbon nanotubes and silicon nanorods, respectively. Size-selected nanocrystals will provide unprecedented control over the size and structure of carbon nanotubes and silicon nanowires while also providing metallic nanocrystals that can be used in related studies throughout ORNL.

The goal of this project is to develop a series of catalytic nanocrystals that can be used to direct the growth of carbon nanotubes and silicon nanowires. The nanocrystals will be separated by their respective metallic-core size by fractional crystallization or electrophoresis. We will address the fundamental question: Can the structure of carbon nanotubes and silicon nanowires be controlled by the choice of appropriate metal nanocrystal?

Synthesis and separation of gold and nickel nanocrystals was the primary goal for the FY 2001 portion of this project. Since the synthesis and separations of the gold nanocrystals have been well documented and performed previously, this aspect of the project was addressed first. Three sets of gold-nanocrystal compounds were synthesized using various *n*-alkanethiols: butanethiol, hexanethiol, and dodecanethiol. Both butanethiol and hexanethiol were subjected to size separations, and as a result, tens of milligrams of purified (~90% size-selected) nanocrystals are ready for silicon nanorod growth studies and for other ancillary investigations (see below). Unlike gold nanocrystals, synthesis of nickel nanocrystals over the desired nickel-core-size range (~1–3 nm diameter) has not been previously reported. Early indications are that the growth of the nickel-nanocrystals likely follows the same synthetic route as the gold nanocrystal [i.e., reduction of smaller Ni(II)SR oligomers to form the larger ligand-capped nanocrystal products]. However, characterization of these extremely large and complex compounds is not as straightforward as for the gold nanocrystals. High-resolution electron microscopy (HREM) and laser desorption ionization mass spectrometry (LDI-MS) have proven mainstays of purifying and characterizing the gold nanocrystals but have thus far not provided the same wealth of information about nickel nanocrystals. The failure of these methods,

thus far, is likely due to the low atomic number of nickel (detrimental to HREM) and the lack of size purity (detrimental to LDI-MS). Optical absorption spectroscopy can provide information on the presence of large metallic nanocrystal/colloidal compounds but is by no means a definitive characterization technique and cannot be used to monitor the separation of nanocrystal compounds into distinct sizes. Further characterization of this new class of nickel-nanocrystal compounds will be addressed more fully in FY 2002.

Growth of carbon nanotubes has been cursorily investigated using the unpurified nickel nanocrystals (not separated by core size). One of the initial concerns revolved around the presence of sulfur, which could possibly "poison" the metallic nanocrystal catalyst particles. Initial experiments have provided essential information for determining the effect of sulfur present in the form of thiol capping ligands in the nanocrystal compounds. In chemical vapor deposition (CVD) experiments, a drop of nickel nanocrystals was placed on two different substrates (titanium on tungsten and silicon oxide on silicon) and the treated substrates were subjected to typical CVD growth conditions (partial pressure of ethylene at elevated temperatures). In both cases, carbon nanotubes were formed and the size of the multiwalled tubes (or fibers) was found to depend on the apparent coverage of the nickel nanocrystals, indicating the aggregation of individual nanocrystals to form larger nanocrystals. In the condensed phase growth, the nickel nanocrystals were dispersed with C_{60} in a toluene solution and placed onto a molybdenum grid and annealed at 900°C. A web-like material, which is similar to previous carbon nanotube products, formed between extremely large nickel colloids. Large colloids were not apparent in the secondary electron micrographs taken before annealing, also indicating

aggregation of the smaller nickel nanocrystals. The initial experiments have confirmed that the presence of sulfur does not seem to hinder the growth of the carbon nanotubes. Further *growth* studies this year will concentrate on (1) the appropriate "loading" of the nickel-nanocrystals to avoid aggregation, (2) further characterization of the carbon nanotube products, and (3) the investigation of silicon-nanorod growth from the gold nananocrystals.

Ancillary studies have revolved around nanocrystal films formed by condensation of gold nanocrystals into well-defined three-dimensional "superlattices." The coupling between nanocrystals separated by a sufficiently small gap, which can be controlled by the capping ligand, can yield films with interesting and potentially useful nonlinear optical properties. Mike Sigman and I are investigating the optical properties (absorption, reflectance) of these films. Currently, a collaborator for determining non-linear components in optical properties is being sought. FY 2002 investigations will focus on the remaining goals for the project and the publication of these results: (1) further characterization of the Ni-nanocrystals, (2) "fine-tuning" the production of carbon nanotubes by CVD and condensed phase growth, (3) pursuing the silicon-nanorod growth from the separated gold nanocrystals, and (4) identifying future collaborators for projects using the unique size-separated nanocrystal compounds.

Condensed-Phase Synthesis of Fullerenes and Nanotubes

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We propose to develop a novel method for synthesizing high-purity carbon nanotubes (CNTs) that would significantly reduce the cost of production. Our method is unique because it uses the liquid phase (precursor soot) as a synthesis medium. We have evidence that this medium can produce fullerenes without producing other forms of carbonaceous material. Incorporation of transition metal catalysts into this medium should also readily produce single- or multi-walled CNTs (depending on catalysts) without producing amorphous carbon or mature soot. The CNTs can readily be separated from this medium by solvent extraction with very high efficiency. Moreover, the extracted medium can be reincorporated into the synthetic process to yield a highly efficient synthesis. The other product of the synthetic process is molecular hydrogen. In an industrial process, the hydrogen product could be burned separately to help maintain the temperature of the pyrolysis to help lower of the cost of production or it could be harvested and sold separately. The kinetics of this synthetic process will be defined with unique diagnostics that define both the liquid- and gas-phase processes. The developed synthetic process should provide the reduction in cost necessary to bring the utilization of CNTs into the industrial sector and make production of CNT-based materials economically more feasible.
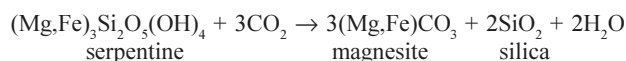

Carbonation of Serpentine for Long-Term CO_2 Sequestration

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Carbonation of serpentine by the reaction

$$(Mg,Fe)_3Si_2O_5(OH)_4 + 3CO_2 \rightarrow 3(Mg,Fe)CO_3 + 2SiO_2 + 2H_2O$$

serpentine   magnesite   silica

is a promising technology for sequestering CO_2 emitted from fossil-fuel-fired power plants. The solids produced in the process, crystalline magnesite and amorphous silica, are highly stable and environmentally benign at ambient temperature and pressure. The serpentine feedstock required to implement the technology can be obtained from magnesium-rich rocks that occur worldwide.

To accelerate R&D on the technical feasibility and cost-effectiveness of serpentine carbonation, the U.S. DOE recently launched the Mineral Carbonation Study Program (MCSP). After nearly 3 years of work, experimentalists in the program have achieved 78% conversion of serpentine to magnesite in 30 min at 155°C and 185 bars. This represents a major step forward in the effort to demonstrate the technical viability of the process. However, to achieve this rapid and extensive carbonation, serpentine must first be heat-treated at 600–650°C to drive off structurally bound water. In an industrial-scale carbonation technology, such heating would consume ~20% of total plant electrical output. Recognizing that this large energy penalty threatens the economic viability of serpentine carbonation, MCSP researchers are actively investigating alternative reaction pathways that do not require an initial heat-treating step.

Thus, it is significant that a promising new approach—carbonating *untreated* serpentine at an elevated fugacity of hydrogen to prevent extensive oxidation of iron—is not being researched currently in the MCSP program. Several lines of reasoning suggest that maintaining iron in the ferrous state will be essential in eliminating the serpentine heat-treating step. Therefore, we propose a series of experiments to determine whether a high hydrogen fugacity (i.e., a low redox state), along with one or more chemical "additives," will accelerate serpentine → magnesite conversion. If these experiments are successful, the foundation would be laid for a sustained, externally funded program involving not only process optimization but also comprehensive studies of potential product end use.

Online Characterization of Individual Airborne Bacteria

W. B. Whitten, P. T. A. Reilly, and J. M. Ramsey

Chemical Sciences Division

The purpose of this project is to explore methods for generating representative biomolecular ions from individual airborne bacteria by laser ablation/atmospheric pressure chemical ionization. The ions would then be introduced into an ion trap mass spectrometer for chemical analysis. If successful, the research could lead to a method for real-time identification of airborne bacteria and spores on a single-particle basis. The methodology could be used in fixed monitors or in portable survey instruments for detecting the presence in the environment of harmful species.

Stable-Isotope Forensics of Biological and Chemical Agents

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Our hypothesis is that all candidate materials for bioterrorism possess distinct stable-isotope ratios of essential light elements (C, H, O, N, etc.) depending on the raw materials, production methods, and geographic locations. If this hypothesis is proven, it would be possible to use such "stable-isotope fingerprints" as a forensic tool for identifying the source of the biological and chemical agents. To test our hypothesis, we will culture bacteria similar to real biological agents, namely, *Bacillus globigii* and *Erwinia herbicola*, and measure the isotopic ratios of carbon and hydrogen in the bacteria and culture media. Successful demonstration of this idea along with ORNL's expertise in chemical and biological mass spectrometry will place ORNL in a central position for the detection and characterization of biological and chemical warfare agents.