
Chemical Science and Technology Laboratory

Annual Report – FY2002



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Annual Report - FY2002**

**Hratch G. Semerjian, Director
Chemical Science and Technology Laboratory**

February 2003



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Donald L. Evans, Secretary**

**TECHNOLOGY ADMINISTRATION
Philip J. Bond, Under Secretary for Technology**

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February 2003

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Chemical Science and Technology Laboratory



The year 2002 marked the tenth year of my tenure as the Director of the Chemical Science and Technology Laboratory at NIST. It has been a very fulfilling period of my career; I have had the pleasure and honor to lead our outstanding research staff in transforming CSTL into a high-impact, customer-focused organization. CSTL is recognized as the world's leading chemical metrology laboratory; our prestige in the international measurements and standards community has created increased demands for our staff to provide leadership for many international comparisons, symposia, and training activities. During the last few years of flat budgets, it has become ever more important to articulate the economic impact of our work, and to focus our efforts on projects and programs that will create the largest return for the nation. Our continuing interactions with industry to help us prioritize our work have been critical, and we all appreciate the ideas and input our industrial colleagues have shared with us so generously.

The last two years have also represented a time of great changes in technology and national priorities, in the aftermath of September 11th. NIST's (and NBS's) illustrious hundred-year history, its technical excellence, broad range of capabilities and flexibility has served us well towards responding to the changing needs of the nation. While maintaining our focus on core mission responsibilities of measurement science and standards, NIST and CSTL are expanding our programs on Nanotechnology, Health and Medical Technologies, Data and Informatics, and Homeland Security. The main body of this report reflects these changes in our programs.

Today's Chemical Science and Technology Laboratory has the most comprehensive array of chemical, physical, and engineering measurement capabilities of any group working in chemical science and technology. We are well positioned to achieve our Vision to be a world-class research laboratory that is recognized by the nation as the primary source for the chemical, biochemical, and chemical engineering measurements, data, models, and reference standards that are required to enhance U.S. competitiveness in the world market.

Finally, I write these remarks with a heavy heart, thinking about the recent and tragic loss of the crew of the Space Shuttle Columbia. NIST has had several experiments on board space shuttles over the past years, and had one on the ill-fated Columbia. Experiments in the microgravity of space remove limitations experienced here on earth. Ironically, the crew of the Columbia had transmitted previously unattainable data back to earth during its mission, completing a highly successful experiment on our behalf. We hope that our use and dissemination of the data they provided will be a tribute to their lives, and a fitting contribution to their legacy.

Hratch G. Semerjian, Director
Chemical Science and Technology Laboratory



<http://www.nist.gov>

Arden L. Bement, Jr., Director
Karen H. Brown, Deputy Director

The National Institute of Standards and Technology is a non-regulatory federal agency within the U.S. Commerce Department's Technology Administration. Founded in 1901, NIST's mission is to develop and promote measurements, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life. NIST has an operating budget of about \$810 million and employs about 3,000 scientists, engineers, technicians, and support and administrative personnel. About 1,600 guest researchers complement the staff. In addition, NIST partners with 2,000 manufacturing specialists and staff at affiliated centers around the country. NIST carries out its mission in four cooperative programs:



- **The Measurement and Standards Laboratories** provide technical leadership for vital components of the nation's technology infrastructure needed by U.S. industry to continually improve its products and services. NIST's seven discipline-based Measurement and Standards Laboratories work at all stages of the pipeline from advancing basic science and pioneering new measurement methods to the development of standard test methods, materials, and data to ensure the quality of commercial products. The seven NIST Laboratories are: Electronics and Electrical Engineering Laboratory (EEEL), Manufacturing Engineering Laboratory (MEL), Chemical Science and Technology Laboratory (CSTL), Physics Laboratory (PL), Materials Science and Engineering Laboratory (MSEL), Building and Fire Research Laboratory (BFRL), and Information Technology Laboratory (ITL).
- **The Advanced Technology Program (ATP)** bridges the gap between the research lab and the market place, stimulating prosperity through innovation. Through partnerships with the private sector, ATP's early stage investment is accelerating the development of innovative technologies that promise significant commercial payoffs and widespread benefits for the nation.
- **The Manufacturing Extension Partnership (MEP)** was established in 1989 as the first federally funded extension centers to help small manufacturers improve their capabilities and performance, which is a necessity for survival in the global marketplace. Today, the Manufacturing Extension Partnership is a nationwide network of more than 400 not-for-profit centers and field offices. MEP makes it possible for even the smallest firms to tap into the expertise of knowledgeable manufacturing and business specialists all over the U.S.
- **The Baldrige Quality Program** is an outreach program associated with the Malcolm Baldrige National Quality Award that recognizes business performance excellence and quality achievement by U.S. manufacturers, service companies, educational organizations, and health care providers. The Malcolm Baldrige National Quality Award, created in 1987, is widely credited with making quality a national priority.



A. Overview

The Chemical Science and Technology Laboratory (CSTL) of the National Institute of Standards and Technology (NIST) is the United States' reference laboratory for chemical measurements, entrusted with developing, maintaining, advancing, and enabling the chemical measurement system for the U.S, thereby enhancing U.S. industry's productivity and competitiveness, assuring equity in trade, and improving public health, safety, and environmental quality. With current world events, the need for accurate and timely chemical and biological measurements is more imperative than ever. CSTL is ready to meet these new challenges.

Today as part of the Commerce Department's Technology Administration, NIST's vision is to be the global leader in measurement and enabling technology, and delivering outstanding value to the nation. NIST provides scientific leadership for the Nation's measurement and standards infrastructure and ensures the availability of essential reference data and measurement capabilities. To discharge these responsibilities, NIST maintains expertise in a broad range of science and technology areas. The Chemical Science and Technology Laboratory is responsible for measurements, data, and standards in chemical, biochemical, and chemical engineering sciences. Building on a one hundred year history of technical excellence, today's CSTL has the most comprehensive array of chemical, physical, and engineering measurement capabilities of any group worldwide working in chemical science and technology.

NIST's vision

Preeminent Performance: *Future economic competitiveness, national security, and public well-being will be shaped by revolutionary developments in the biosciences, nanoscience, and information and knowledge management – a transformation enabled by NIST's unique measurements, standards, and enabling technologies and services.*

NIST's mission

... is to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.

CSTL's vision *is to be a world-class research laboratory that is recognized by the nation as the primary source for the chemical, biochemical, and chemical engineering measurements, data, models, and reference standards that are required to enhance US industrial competitiveness in the world market.*

CSTL's Mission *is to serve as the nation's Reference Laboratory providing the chemical measurement infrastructure to:*

- *enhance U.S. industry's productivity and competitiveness;*
- *assure equity in trade; and*
- *improve public health, safety, and environmental quality.*

CSTL has operated under a published Strategic Plan for more than a decade. In the context of this plan CSTL developed its mission, vision and goals. These are clearly aligned with the current NIST 2010 Strategic Plan.

Periodically, CSTL's Strategic Plan is reassessed and updated to respond to National priorities, changing customer needs, and the rapid evolution of science and technology. Although the details of the strategic planning process have varied over the years, its essential characteristics — **customer-focused** and **open to all stakeholders** — have been preserved. CSTL's Strategic Plan focuses on achievement of three **strategic goals**:

Measurement Standards: *Establish CSTL as the pinnacle of the national traceability and international comparability structure for measurements in chemistry, chemical engineering, and biotechnology, and provide the fundamental basis of the nation's measurement system.* This objective is achieved by:

- Developing and demonstrating international comparability for chemical and physical measurements,
- Supporting and strengthening the vertical traceability structure in the U.S., and
- Supporting and strengthening voluntary standards organizations.

Chemical and Process Information: *Assure that U.S. industry has access to accurate and reliable data and predictive models to determine the chemical and physical properties of materials and processes.* This objective is realized by:

- Developing benchmark data for the properties of important substances, classes of substances, and systems;
- Developing data collections, data prediction methods, and models to meet high-priority industrial and national needs;
- Contributing to the development of consensus standards for key properties, substances, and processes; and
- Developing procedures or protocols for data access, facilitating data exchange, and by disseminating properly formatted data.

Measurement Science: *Anticipate and address next-generation measurement needs of the Nation.* This final goal is achieved by:

- Maintaining a strong and cutting-edge research program to support the Nation's measurement and standards infrastructure, and
- Establishing new measurement capabilities to support new or advanced technology development and dissemination.



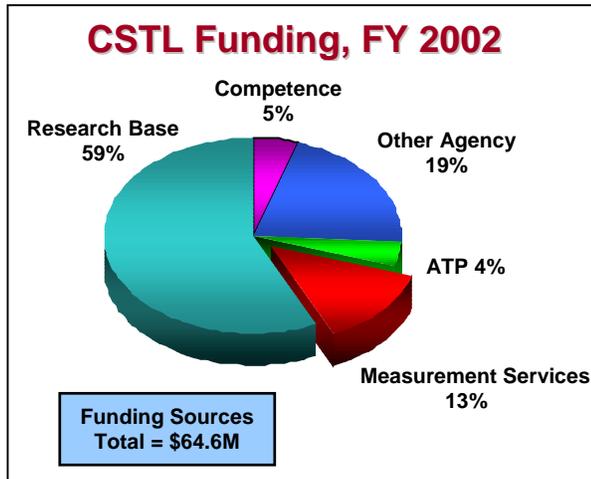
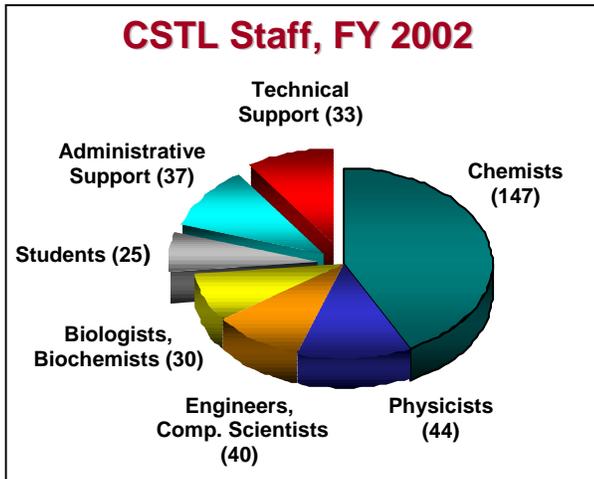
Organizational Structure and Resources

CSTL is organized to reflect the technical expertise that is the foundation of our technical programs and allows us to accomplish our mission. The Laboratory consists of five Divisions: Biotechnology Division, Process Measurements Division, Surface and Microanalysis Science Division, Physical and Chemical Properties Division, and Analytical Chemistry Division. Each Division employs a group structure organized to achieve synergy and critical mass in its technical program areas.



In FY2002, Dr. Gary Gilliland stepped down as Chief of the Biotechnology Division and has assumed the role of Associate Director of CARB. Dr. Vincent Vilker took on the job of Chief of the Biotechnology Division. Dr. W. Mickey Haynes retired as Chief of the Physical and Chemical Properties Division on January 3, 2003. Dr. Gregory Rosasco, retired Chief of the Process Measurements Division, returned to active duty to replace Mickey. The other significant change in CSTL's organization is reorganization in the Surface and Microanalysis Science Division, resulting in the reassignment of all staff in the Atmospheric Chemistry Group to other appropriate groups in the Division, more in keeping with the disciplinary nature of our groups and divisions.

To achieve its goals, CSTL maintains an experienced, well-educated professional staff. The full-time permanent staff numbered 273 in FY02, in addition to 83 temporary and part-time employees. Also, there were 237 guest researchers working closely with CSTL staff in various aspects of the research program. The technical capabilities of CSTL staff are extensive; they hold degrees in chemistry, physics, engineering, biology, and computer science. A capable technical support staff augments the professional staff. Approximately 75% of the technical staff have PhD degrees.



CSTL's physical facilities are located at the major NIST sites in Gaithersburg, Maryland and Boulder, Colorado, as well as at the Center for Advanced Research in Biotechnology (CARB) in Rockville, Maryland and the Hollings Marine Laboratory (HML) in Charleston, South Carolina.



The Physical and Chemical Properties Division has operations located at the NIST sites in Gaithersburg and Boulder. Biotechnology Division staff work closely with that of the University of Maryland Biotechnology Institute located at CARB. The Analytical Chemistry Division is the primary liaison with HML, having staff located in Charleston. HML is a cooperative research facility involving

NIST, the National Oceanic and Atmospheric Administration, the South Carolina Department of Natural Resources, the University of Charleston, and the Medical University of South Carolina.

The Analytical Chemistry and Biotechnology Divisions have now been located in the Advanced Chemical Sciences Laboratory (ACSL) for over three years, the first new research facility on the NIST Gaithersburg Campus in over thirty years. Although ACSL adds significantly to CSTL's research capabilities, it does not meet all facility needs of our program.



Hollings Marine Laboratory



NIST is proceeding with implementing its facilities master plan, the current focus of which is the Advanced Measurements Laboratory (AML). Construction of the Advanced Measurements Laboratory (AML) on the Gaithersburg campus is well underway with completion planned for 2004. This new facility will accommodate most of the Surface and Micro-analysis Science Division, as well as the Pressure and Temperature activities in the Process Measure-

ments Division. Attention is now being directed to improving the facilities for those units not located in the ACSL and AML, including Boulder.

CSTL Strategic Directions

As part of its most recent update of the Strategic Plan, CSTL has established five Strategic Directions:

- Biotechnology
- Nanotechnology
- Healthcare
- Data and Informatics
- International Measurement Standards

NIST Programmatic SFAs

- **Nanotechnology**
- **Healthcare**
- **Information /Knowledge Management**
- **Homeland Security**

The last one reflects an increased emphasis on issues related to global trade. New projects started over the last five years reflect these Strategic Directions. Investment decisions are made jointly by the Laboratory Director, Deputy Director and Division Chiefs. CSTL's decisions are in clear alignment with the NIST 2010 Programmatic Strategic Focus Areas (SFAs) shown above (in box). Some of the projects selected contribute to more than one Strategic Direction, and some contribute to Homeland Security.

It is critically important to ensure that CSTL programs and projects are dynamic and flexible enough to respond to national and international technology trends, and the changing needs of our customers and stakeholders. CSTL has several mechanisms in place that facilitate the development of new projects at the Division, Laboratory and NIST levels. Each year, CSTL Management reviews its portfolio of programs and projects, and considers proposals for high priority projects. Priority is given to new projects that closely align with NIST and CSTL strategic goals.

Selection of new projects for reprogramming and identification of projects to be phased out require specific criteria. CSTL has used the following criteria for its decision-making:

- Industrial Need - Magnitude and immediacy of industrial need
- Match to Mission – CSTL provides the Nation's chemical measurement infrastructure
- Making a Difference – Is the CSTL contribution critical for success?
- Nature and Size of Impact – Measure of anticipated impact relative to investment (rate of return)
- Timely Quality Output - The ability to respond in a timely fashion with high-quality output
- Science/Technology Opportunity - Recent scientific and technological advances present new opportunities

NIST Program Selection Criteria Include the Following Elements

- **Fit to Mission**
- **Unique NIST value-add**
- **Potential external impact**
 - **Customer needs**
 - **Impact path**
 - **Strategic significance of impact arena**
- **Potential impact on NIST**
- **Probability of success**

To provide our staff with stimulus for innovative ideas and new areas of research, CSTL continues its tradition of one-year funding for small seed projects that evaluate the feasibility of new technologies and measurement methods. In FY2002, CSTL funded 12 such projects, following a rigorous internal peer review process. Progress reports for these projects are included in Section C of this Annual Report.

CSTL Programs

CSTL is a multifaceted, synergistic organization with a unique customer base. Perhaps the most remarkable aspect of the CSTL customer-base is its breadth. The primary customers we serve are: industry; federal, state, and local government agencies; standards and industrial trade organizations; and the academic and scientific communities.

U.S. industry is the largest consumer of our products and services. These customers come from established industrial sectors, such as the chemical manufacturers, and emerging industries, such as biotechnology. The products and services they rely on are as varied as Standard Reference Materials (SRMs), Standard Reference Data (SRDs), calibration services, and novel measurement methods. Reference materials and calibrations provide traceability to the International System of Units (SI), which is essential to fair trade, improved reliability of measurements, and regulatory compliance. Data compilations facilitate modeling of chemical processes and rational product design. New measurement techniques ensure improved product quality and process efficiency, and in turn enhance competitiveness.

Federal, state, and local governments are another important consumer of our products and services. These agencies use our products and services in ways similar to the commercial sector, but in different arenas. For example, calibrations, evaluated data, and reference materials are used to ensure the reliability of environmental monitoring programs. Carefully characterized physiological samples lend credibility to forensics tests. Measurement technologies developed and perfected in CSTL verify compliance with international treaties. Other national laboratories exploit the expertise and techniques developed here in discharging their mandates.

A final group that uses our expertise includes trade organizations, standards committees, and the academic and scientific community. These groups rely on CSTL's expertise for advice and guidance in establishing practical standards and uniform protocols. The academic and scientific communities rely on the data produced and compiled by our staff as touchstones for their own research.

CSTL's programs are customer-based and serve to focus our activities. The agility of our technical staff permits CSTL to respond in a timely manner to changes in customer needs within each industry segment, changes in priorities among industries as well as National priorities. CSTL has identified 11 programmatic areas aligned with industrial segments and National priorities, and these are highlighted in Section B of this report. It is clear that those projects listed contribute to strengthening the metrological infrastructure of each industry sector.

- 1. Automotive and Aerospace**
- 2. Biomaterials**
- 3. Pharmaceuticals and Biomanufacturing**
- 4. Chemical and Allied Products**
- 5. Energy Systems**
- 6. Environmental Technologies**
- 7. Food and Nutrition**
- 8. Forensics and Homeland Security**
- 9. Health and Medical Technologies**
- 10. Industrial and Analytical Instruments and Services**
- 11. Microelectronics**

It is also beneficial to articulate CSTL projects by activities that cross-cut industry sectors. Therefore, CSTL has three cross-cutting programs, that are clearly aligned with CSTL's three strategic goals.

- 12. Measurement Standards**
- 13. Data and Informatics**
- 14. Technologies for Future Measurements and Standards**

Measures of Success

CSTL employs many mechanisms to capture feedback on the quality, relevance and impact of our programs and activities. In its mandated review of NIST programs, the National Research Council (NRC) Board on Assessment (BoA) Panel for CSTL provides a rigorous peer review of our programs, to assess the quality and relevance of our technical activities. The Panel is comprised of technical experts from industry, academia, and national laboratories covering the broad range of disciplines corresponding to CSTL program areas. Direct customer feedback on the quality of our work, external recognition of our technical staff, the leadership roles of our staff in standards activities, and the performance of CSTL in Key Comparisons conducted among other countries' National Metrology Institutes, under the auspices of CIPM Consultative Committees, provide direct evidence for the quality of our technical programs. Our performance in CIPM Key Comparisons in a broad range of measurement fields, where our performance is compared to the best laboratories of the world, is an excellent measure of our capabilities and an opportunity to demonstrate our global leadership.

CSTL also organizes many workshops and meetings to assess the needs of specific industry segments, and to prioritize these needs to address the highest impact areas. Workshops, which bring together experts from NIST, industry, academia, and other national agencies, are a particularly useful and cost-effective means of developing competent technical strategies for meeting the NIST mission. CSTL interactions with other federal agencies also provide us with invaluable knowledge on measurements, standards and data needs that may result from promulgation of new regulations, or new more sensitive and selective measurement methods that may be needed to improve the science base needed for decision making. Direct customer and stakeholder feedback on the relevance of our current work and anticipated measurement needs of industry is also sought out through ongoing interactions. This information is used to help develop programs in appropriate directions and assure that CSTL programs are effectively meeting the needs of our customers.

Other direct indicators of the relevance of our work are CSTL outputs, such as new calibration services, SRMs, SRDs, publications, invited talks, patents, licenses, the number of companies using our calibration services, the number of institutions who purchase our Standard Reference Materials (SRMs) in the U.S. and abroad, and the number of institutions who purchase or license our Standard Reference Data (SRD) products, or download our web-based databases. The quality of Measurement Service delivery is another important performance measure for our customers. We constantly monitor and strive to improve the turnaround time of our calibration services. We have been successful in reducing the certification times of our SRMs (to less than two years), and improving our prioritization process to reduce out-of-stock items. CSTL is also making special efforts to expand our web-based data dissemination efforts, and to provide more standardized formatting and single-portal access to all CSTL databases.



Some of the CSTL outputs and interactions in FY2002 are summarized in the following table.

Selected CSTL Outputs – FY 2002										
Div.	Pubs.¹	Talks	Committees²	Seminars	Conferences	CRADAs	Patents Issued	SRMs /RMs³	SRDs	Cals.⁴
830	5	14	25	11	2	0	0	0	0	0
831	76	117	65	14	7	1	1	1	1	0
836	70	90	95	12	5	1	0	1	0	832
837	46	141	85	4	12	1	0	5	3	0
838	80	124	97	19	1	7	1	2	10	2
839	82	157	127	17	11	1	0	73	1	245
Total	359	643	494	77	38	11	2	82	15	1079

¹Publications appearing in print in FY2002. Another 188 manuscripts have been submitted for publication.

²Committee totals include 64 editorships

³SRMs/RMs (Certificates issued)

⁴Calibrations were performed for over 338 customers

Division Key:

- | | | | |
|-----|-------------------------------|-----|--|
| 830 | Laboratory Office | 837 | Surface and Microanalysis Science Division |
| 831 | Biotechnology Division | 838 | Physical and Chemical Properties Division |
| 836 | Process Measurements Division | 839 | Analytical Chemistry Division |

SRM – Standard Reference Material

SRD – Standard Reference Data

Lastly, economic impact studies, conducted by outside experts, investigate the impact of CSTL products and services on the U.S. economy, thereby providing a quantitative tool for measuring the influence and reach of our programs.

Evaluations of the economic impact of NIST's metrology programs in specific technical areas are carried out through NIST-commissioned studies, performed by external contractors. These studies provide both qualitative assessments and quantitative estimates of the economic impacts resulting from the several categories of technology infrastructure that NIST provides to U.S. industry. Quantitative estimates are provided either as benefit-to-cost ratios or as rates of return to the nation (social rate of return). The results of these impact assessments not only respond to the need to measure and analyze current and past performance but also contribute to future strategic planning. Practically, only a few of these in-depth analyses are possible each year. In FY2002, we completed a study of our NIST Traceable Reference Materials (NTRM) program, described in more detail immediately below. In addition, we summarize some of the results of earlier impact studies, with updated information on subsequent activities and benefits accrued since the publication of each report.

NTRM Gas- Mixture Standards:

The NIST Traceable Reference Materials (NTRM) program was created to help address the problem of increasing needs for reference materials with a well-defined linkage to national standards. An NTRM is a commercially produced reference material with a well-defined traceability to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. The NTRM concept has been implemented in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the "Emissions Trading" provisions of the Clean Air Act of 1990.



NTRM

The NTRM program was created by NIST in collaboration with the U.S. Environmental Protection Agency (EPA) and the specialty gas companies (SGCs) to increase the availability of NIST-certified reference materials. Under the program, SGCs follow NIST technical guidance to manufacture SRM-equivalent standards and submit these standards to NIST for certification. Once certified, the NTRMs are the functional equivalent of SRMs and are used to assay the large volume of secondary reference standards demanded by consumers to meet regulatory requirements. For this impact study, SGCs were surveyed, as well as end users such as electric utilities, transportation equipment firms, petrochemical firms, commercial laboratories, and government agencies. Since NTRM program inception in 1992, 15 specialty gas companies have worked with NIST to certify over 8600 NTRM cylinders of gas mixtures that have been used to produce approximately 500,000 NIST-traceable gas standards for end-users, with a market value of about \$140 million. Over the past decade, this process has been and continues to be an integral component of the high accuracy reference gas supply chain. According to the economic impact study published in August 2002, the social rate of

return of this program is 225%, with a benefit-to-cost ratio estimated to be 24 to 1, and a net present value of \$56 million.

Cholesterol Standards:

Chemical metrology is at the heart of accurate medical diagnosis and the development of measures to improve our health and ensure long life. In the U.S. more than one trillion dollars are spent each year on health care, which is about 14 % of the U.S. GDP. More than 20 % of these expenditures are for measurements.



Over the last three decades, NIST, in cooperation with the College of American Pathologists (CAP), has developed a series of highly accurate and precise methods for a number of clinically important serum constituents, including cholesterol. These methods are recognized by the international clinical laboratory community as "definitive" and have been used to certify a series of cholesterol SRMs. The first pure crystalline cholesterol (SRM 911) was introduced in 1967. Using the definitive method, serum cholesterol SRMs were developed in 1981 (SRM 909) and again in 1988 (SRMs 1951 and 1952). These SRMs have led to a steady decrease in the measurement uncertainty from 18% relative in 1969 to about 6% in 1999. The economic consequences of NIST's Cholesterol Standards Program are experienced at several levels of the supply chain from manufacturers, to network laboratories, to clinical laboratories that ultimately deliver medical services to the consumer. The results of this study indicate that NIST has played an important economic role in support of a national effort to monitor, measure, and control cholesterol levels, thereby contributing to the reduced level of heart disease. The economic impact study estimates a benefit-to-cost ratio of 4.5, and a social rate of return of 154 %. The net present value was calculated to be more than \$3.5 million. This report was published in September of 2000, and the timeline extended from 1986 to 1999.

Recent Developments: NIST Cholesterol SRMs remain an important part of the traceability chain for clinical laboratories and manufacturers of cholesterol measurement kits. Approximately 500 SRM units were sold per year over the past three years. These SRMs also serve as important "high order" reference materials, providing measurement traceability for in vitro diagnostic (IVD)

These Cholesterol SRMs have continued to play a critical role in the steady decrease in the measurement uncertainty from 18% relative in 1969 to about 6% in 1999, and 3% today.

products. This level of traceability will soon be required for IVD products to be imported into European markets, as specified in the EU IVD Directive set to go into effect in December of 2003. Since U.S. based companies supply 60 % of the IVD products sold in Europe, it is critical to U.S. industry that NIST maintain and expand SRMs that provide the required traceability.

Sulfur in Fossil Fuels:

The sulfur content of fossil fuels is one of the most important intrinsic factors that determine fuel prices. The accurate determination of the sulfur concentration in fossil fuels is required as a result of environmental regulation that places increasingly lower limits on their sulfur content and the imposition of large fines for non-compliance. At every stage in the process (mining, transportation, buying and selling, and combustion) the sulfur content of both oil and coal must be determined in order to meet buyer and seller specifications that are dictated in large part by government environmental regulations. The efficient and cost effective movement of coal and oil from the mine and well to power plants and refineries requires precise and accurate determination of sulfur content in two or more laboratories. For equity in trade and the efficient production of energy, it is mandatory that instrumentation in these laboratories be calibrated using accurate standards.



NIST has certified the sulfur content in about 30 coal and fuel oil SRMs to an accuracy of better than ± 0.1 % relative, using isotope dilution mass spectrometry. These SRMs provide industry with the primary calibration materials needed for instrumentation used in routine measurements. SRMs also provide industry with a strong traceability link to NIST for such measurements, whether they be for setting the price of fuel or for demonstrating compliance with environmental regulations. Surveyed industry representatives indicated that NIST SRMs have decreased the level of uncertainty

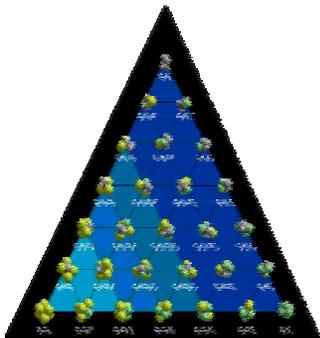
associated with their measurements of sulfur content. This reduction has led to economic benefits throughout the supply chain. Included in the measures of economic benefits are improvements in product quality, production efficiency, and reductions in transaction costs and sulfur emissions to the environment. This study, published in February 2000 estimates a benefit-to-cost ratio of 113, and a social rate of return of 1,056 %. The Net Present Value was calculated to be more than \$400 million. The time period studied was from 1984 to 1999.

Recent Developments: The petroleum refining industry faces a June 2006 deadline to comply with stringent limitations on the sulfur content of highway diesel fuel. By this date, most refiners must meet a 15 $\mu\text{g/g}$ or parts per million (ppm) standard for at least 80% of the highway diesel fuel produced, with a 500 $\mu\text{g/g}$ (ppm) cap on the remaining 20% of their production. By 2010, all highway diesel fuel must meet a 15 $\mu\text{g/g}$ (ppm) cap. In addition, the industry expects that the EPA will propose similar regulations for the non-road diesel fueled vehicles. The benefits to the industry in the 2000 Economic Impact report were derived from improved production efficiency in producing diesel fuel that complied with the 500 $\mu\text{g/g}$ (ppm) sulfur cap. The new cap of 15 $\mu\text{g/g}$ (ppm) sulfur puts additional constraints on the industry that translate to higher potential cost avoidance by the use of SRM standards. NIST is in the process of releasing a new diesel fuel SRM with a sulfur concentration of 11 $\mu\text{g/g}$ (ppm) that will meet the new need of the industry.

A new twist on existing standards: New environmental regulations have capped Hg emissions at 26 tons starting in 2010 and reduced to 15 tons by 2018. There has been value-added to NIST's eight natural coal SRMs that were originally certified only for S and now have been reanalyzed and certified for Hg. These new certifications will take on significant importance since they will be used increasingly by the electric power industry to ensure compliance with these new regulations.

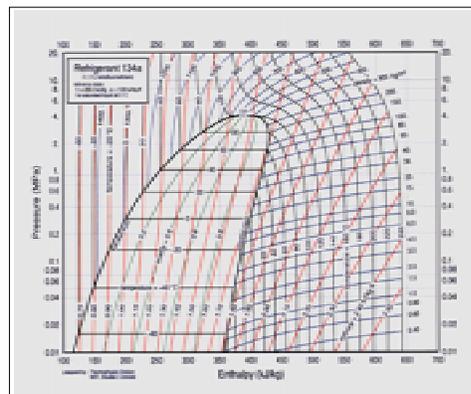
Alternative Refrigerants:

Occasionally, an accelerated R&D program must be undertaken to respond to industry needs that are constrained by set deadlines. With the timetable imposed by the Montreal Protocol of 1987 as



an incentive to develop new alternatives to CFCs, NIST engaged in research that would allow industry to make the switch to alternative refrigerants in a timely and economical fashion. NIST began by identifying the basic requirements for new refrigerants according to the new rules, and then started research on determining the physical properties of such candidate alternatives. NIST's most effective form of information dissemination has been the REFPROP program, a computer software package that is available through NIST's Standard Reference Data Program. The REFPROP program enables

manufacturers and users of alternative refrigerants to model the behavior of refrigerant mixtures in their respective manufacturing processes, a key method in developing CFC replacements. A comparison of industry benefits with the funding stream of NIST's research program estimated a social rate of return of at least 433 %, and a benefit-to-cost ratio of 4 to 1. This report was published in January 1998, and the period studied was from 1987 to 1996.

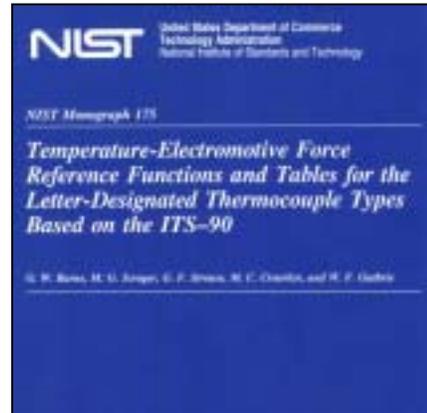


Recent Developments: The NIST REFPROP database has been updated several times since the economic assessment, and version 7.0 was released in August 2002. This database continues to be the primary technology transfer vehicle for the continuing work of CSTL on refrigerants. The latest version includes additional fluids and mixtures, improved property models, increased calculational speed, and an enhanced user interface with additional properties and calculation and plotting options. Its position as the *de facto* standard in the refrigeration industry is stronger than ever. In addition to direct use by engineers in industry (it is the number two seller among the databases distributed by the NIST Standard Reference Data Program), it provides the core property routines for the CYCLE-D and REFLEAK databases distributed by NIST as well as packages distributed by several refrigerant and equipment manufacturers to their customers. The *de facto* status will likely become official in the coming year: a draft ISO standard on refrigerant properties specifies the same models (and thus the same property values) as REFPROP. Although REFPROP is not explicitly specified in the standard, the ISO Working Group recognized the high quality and wide use of REFPROP and adopted the same models. A new title for version 7 of REFPROP (Reference Fluid Thermodynamic and Transport Properties) with "Reference Fluid" replacing the "Refrigerant" of earlier versions indicates our direction: we will merge the NIST12 and NIST14 databases into REFPROP. Thus, a single database would encompass refrigerants, cryogenic fluids, hydrocarbons, and simple inorganics.

DuPont phased out their own "Refrigerant Expert" package in 1999, and pointed their customers to REFPROP and CYCLE-D, and REFPROP has been licensed for use with the ASPEN process simulation package.

Thermocouples:

The NIST thermometry program includes both calibration services and research on thermocouples. Thermocouples are among the most commonly used sensors for monitoring and control of manufacturing processes. The annual sales of thermocouple products sold by the U.S. thermocouple industry (suppliers of wire and thermocouple assemblies) into the U.S. market are approximately \$280 million. The incorporation of these devices into higher levels of product structures across a broad base of domestic industries affects a much larger portion of the manufacturing sector, estimated to be on the order of \$80 billion.



Benefits were estimated based on surveys and interviews of the thermocouple industry and did not include the much larger, though diffuse, community of device users. Participants were asked to estimate the additional expenses that would have been incurred if NIST were to cease to provide primary calibration services. NIST expenditures included research on the fundamental and infrastructural aspects of thermocouple principles, measurement, and test methods as well as calibration services. This study, published in July 1997, conservatively estimated the social rate of return to be 32 %, and a benefit-to-cost ratio of 2.95.

Recent Developments: Currently, the ITS-90 relies on the fundamentally more accurate high temperature platinum resistance thermometer to define the temperature scale in the range of 631 °C to 1064 °C. In this range, prior to the introduction of the ITS-90, less accurate type S (platinum-rhodium vs. platinum) thermocouples were used to define the temperature scale. Work at NIST based on the new scale definition led to new reference functions with improved accuracy for both type S and R thermocouples. Even more importantly, the new definition of the ITS-90 enabled the determination at NIST of highly accurate reference functions for pure element thermocouples, such as gold versus platinum and platinum versus palladium. In the range from 0 °C to 1000 °C, gold versus platinum thermocouples provide an order of magnitude more accuracy than the previous type S thermocouples commonly used as reference standards in industry.

Gold versus platinum thermocouples are now readily available from several U.S. manufacturers.

The next sections of this Report will delve into each of CSTL's 14 programs individually, highlighting major activities and accomplishments in these areas for FY2002. Following the highlights of the 11 customer-focused programs, this Report addresses the three goal-aligned programs. Most of the details of the projects listed in the Measurement Standards, and Data and Informatics Sections are presented in the context of their impacted industry sector, and a reference is supplied to provide a more coherent view. CSTL, however, also works on the cutting-edge of metrology and develops tools to anticipate next generation needs. These tools are not necessarily identified with a particular industry sector, and therefore are presented here in the program title: Technologies for Future Measurements and Standards, which reflects CSTL's third strategic goal.

Technical details of these activities can be found on CSTL's website or by contacting the principle investigators listed in the Report.

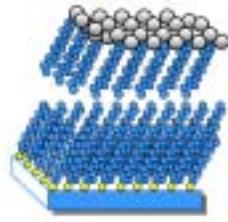
<http://www.cstl.nist.gov/>



B. CSTL Programs



Automotive and Aerospace



Biomaterials



**Pharmaceuticals and
Biomanufacturing**



Chemical and Allied Products



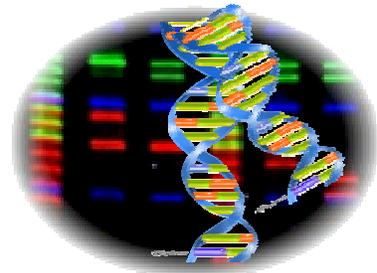
Energy Systems



Food and Nutrition



Environmental Technologies



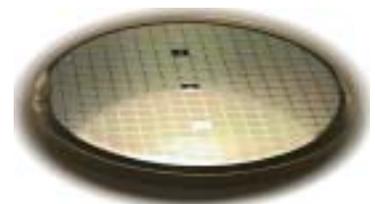
**Forensics and
Homeland Security**



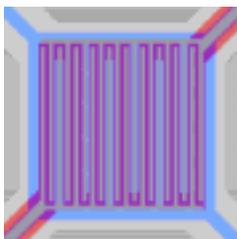
**Health and Medical
Technologies**



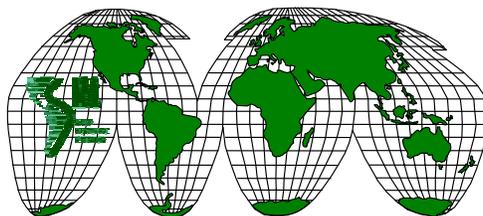
**Industrial and Analytical
Instruments and Services**



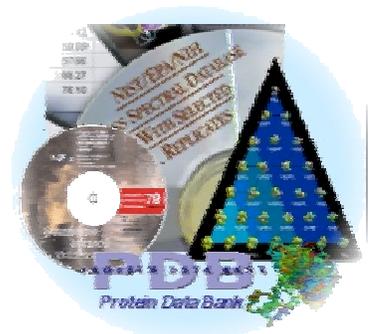
Microelectronics



**Technologies for Future
Measurements and Standards**



Measurement Standards



Data and Informatics

1. Automotive and Aerospace (Transportation)



CSTL provides standards for emissions testing and flow measurements to help the transportation industry meet regulatory requirements. CSTL also works with the industry to provide physical and chemical property data that can be used

In 2004, California's zero-emission vehicle law will take effect, calling for 10% of an automaker's vehicles sold in that state to be zero-emissions vehicles.

in sophisticated computer models for next generation combustion engine design, and provides a new generation of chemical standards and reference data to support advanced materials such as super alloys for high technology jet engines.

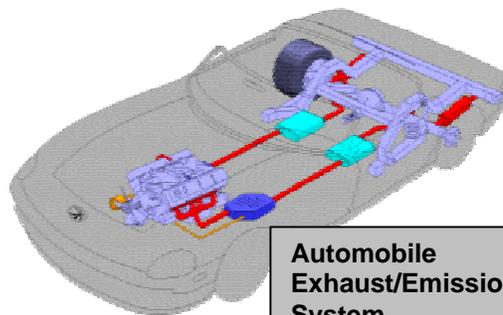
SRMs for Low Level Automotive Emissions

W.J. Thorn III (839)

Stakeholders in the American Industry/Government Emissions Research (AIGER) group are working together to facilitate the automobile industry meeting more stringent 2003 Federal Tier II and California LEV II emission regulations. New more efficient catalysts and computer optimized combustion parameters have reduced tailpipe pollutants to very low $\mu\text{g/g}$ (ppm) levels. These emission levels are further diluted by air into constant volume sampling bags or through the use of new mini-diluter technology while performing the Federal Highway Test on a chassis dynamometer. Development of lower concentration nitric oxide SRMs has been given the highest priority by AIGER members and specialty gas suppliers.

NIST and the Motor Vehicles Manufacturers have worked together since 1975 to develop sixty gaseous Standard Reference Materials (SRMs) against which all EPA mandated fuel economy and mobile source emission measurements must be traceable, by federal law.

In 1998, NIST anticipated the AIGER need for significantly lower nitric oxide standards by partnering with Scott Specialty Gases to solve the instability of the lowest existing SRMs ($5 \mu\text{g/g}$ and $10 \mu\text{g/g}$) and to produce new stable nitric oxide standards between $0.5 \mu\text{g/g}$ and $1.25 \mu\text{g/g}$. These new infrastructure standards have exhibited excellent stability for more than 3 years. In 2001, AIGER members including the U.S. EPA, California Air Resources Board (CARB), General Motors, Ford, and Daimler-Chrysler requested that NIST develop two new nitric oxide SRMs at $0.5 \mu\text{g/g}$ and $1.0 \mu\text{g/g}$. With the assistance of AIGER member funding, NIST and Scott Specialty Gases



Automobile Exhaust/Emissions System

This work has resolved the technical issues related to the stability of low-level NO, and provided AIGER members with interim standards. NIST will also provide SRMs in the near future to help industry meet lower emission regulations.

collaborated to gravimetrically blend 40 cylinders of each new nitric oxide concentration, 80 cylinders total. NIST then performed a 100% analysis on the 80 mixtures to verify that each cylinder's nitric oxide concentration is within $\pm 1\%$ relative. Fifty-four of these mixtures have now been given to NIST to be certified over the next two years as new SRMs, while the remaining twenty-six mixtures were distributed to AIGER members who ordered them, to serve as interim standards until the new SRMs are available in December,

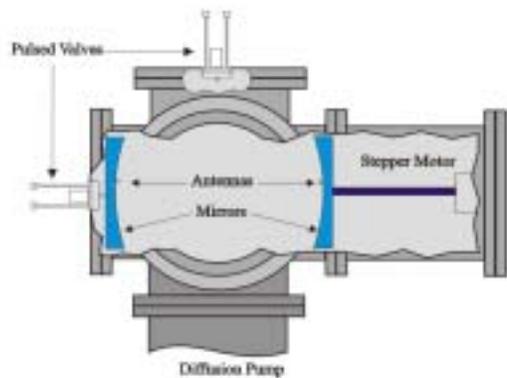
2003. Discussions are ongoing with AIGER to upgrade the NTRM protocol to allow NTRM gas standards to replace NIST SRMs for non-reactive emission measurements. This could free NIST resources to work on standards of other AIGER priority pollutants such as 0.1 µg/g methane.

Critically Evaluated Fourier-Transform Microwave Spectroscopy for Real-Time Measurements of Automotive Exhaust Emissions

R. Bousquet, R. DaBell, and P.M. Chu (839)

The high spectral resolution and high sensitivity of Fourier-transform microwave (FTMW) spectroscopy suggest that the technique can provide real-time response and unambiguous identification of vapor phase analytes with permanent dipole moments. The principal goal of the present program is to critically evaluate the technique's potential as a reliable and robust tool for quantitative measurements of trace gases. Current work has focused on characterizing and improving the signal intensity stability and optimizing the instrument for gas samples with air as the primary balance gas. This project was originally initiated to address the automobile industry's need to identify and quantify oxygenates at trace levels in auto exhaust emissions.

Fourier-transform microwave (FTMW) spectroscopy is being investigated as a quantitative analytical technique to meet the need for an improved analytical tool that provides real-time measurements of trace-level vapors. These tools are critical for addressing pressing industrial issues such as global climate change and the detection of hazardous emissions.



Cutaway view of FTMW spectrometer

FY 2002 work has focused on characterizing the instrument response over extended periods and optimizing the system performance. Initial evaluation revealed that measured peak positions are reproducible permitting definitive species identification. The signal intensities, however, varied substantially over long measurement periods (12 h), preventing reliable quantitative measurements. After several modifications, the signal intensity drift has been reduced to $\leq 10\%$ over 48 h. The maintenance of the instrument had been significantly reduced as well. Additional modifications in the sample delivery system have further improved the system's sensitivity for samples prepared in nitrogen-based matrices compared to previous work, which required rare gas based matrices. As work to optimize the instrument

performance continues, an effort will also focus on evaluating the capability of FTMW spectroscopy to measure analytes in gas matrices that more closely match the specific applications, such as systems with high concentrations of carbon dioxide and water.

Success of this work will also impact many other critical applications including the detection of chemical warfare agents.

SRMs for Hydrogen in Titanium

R.M. Lindstrom, R.L. Paul, and H.H. Chen-Mayer (839)

Hydrogen embrittlement can be a serious problem for many metals, leading to massive structural failures in extreme cases. Therefore the control of hydrogen in the manufacturing process is crucial. Hot-extraction measurement methods used in industry need to be calibrated with accurate working standard materials.



Industrial analytical methods for hydrogen employ hot extraction in vacuum or inert gas, followed by detection of the hydrogen by gas chromatography or mass spectrometry, for example the ASTM Standard Test Method E1447 for hydrogen in Ti alloys. These methods in practice require independently produced, matrix-matched metal standards for calibration, and reference materials for continued validation of the analytical procedure.

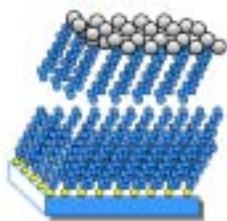
To respond to this need, NIST began the development of a Hydrogen in Titanium SRM. In the SRM preparation, titanium alloys are loaded with a known quantity of hydrogen by carrying the reaction out in a closed system with known volume. The first Standard Reference Material SRM 2453, Hydrogen in Titanium (100 mg of hydrogen per kg of titanium) has been prepared by this method. The amount of hydrogen added to the metal was measured by the volume of gas absorbed and also by the mass gain. Prompt-

The aerospace and, increasingly, the automotive and consumer-goods industries employ titanium alloys because of their excellent combination of high strength, light weight, and high temperature properties. NIST SRMs help ensure that fabricated components in fact have these desired properties.

A combination of well-controlled preparation procedure and neutron based characterization gives assurance that these SRMs are traceable to fundamental parameters.

gamma activation analysis (PGAA) and neutron incoherent scattering (NIS) were used to measure hydrogen in the metal before and after loading. Two additional bracketing materials are required to check calibration linearity and provide check samples at levels significantly above and below the critical level of ≈ 100 mg/kg. SRMs 2452 and 2454 are in preparation at target mass fractions of 50 mg/kg and 200 mg/kg, respectively.

2. Biomaterials



The biotechnology industry is making significant advances in the areas of biomolecular research for use in new environmentally friendly materials and process technologies. CSTL supports these efforts by developing measurement methods, standards and tools for quality control, as well as standards for calibration of specialized bio-based equipment. In addition, advances in tissue engineering continue to grow rapidly requiring standards to assure the integrity of tissue-engineered products during development, storage or shipment.

The total market for the regeneration and repair of tissues and organs is estimated to be \$25 billion worldwide.

Biomarkers of Oxidative DNA Damage Used to Detect Genetic Changes in Tissue Engineered Skin

H. Rodriguez, P.E. Barker, C.D. O'Connell, and M.M. Dizdar (831); P. Jaruga (UMBC, MD), and M. Birincioglu (Inönü University, Turkey)

Fundamental to many tissue-engineered devices are issues of inflammation associated with how biological cells respond to a given matrix or when inserted into the body. It has become increasingly clear in recent years that oxidative damage to DNA plays an important role in a number of disease processes. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) and liquid chromatography/mass spectrometry (LC/MS) have emerged as new techniques for the measurement of modified nucleosides in DNA. Using gas chromatography/mass spectrometry (GC/MS), a total of five genomic DNA biomarkers in tissue-engineered skin (TestSkin II[®], Organogenesis, Inc) was screened and the levels of damage compared to control cells, including neonatal human dermal fibroblasts, neonatal human epidermal keratinocytes, cultured HeLa cells, and commercially available calf thymus DNA. The biomarkers consisted of FapyAdenine, FapyGuanine, 8-OH-Guanine, 5-OH-Uracil, and 5-OH-Cytosine. For 8-OH-Guanine (a free base), its nucleoside form (8-OH-dGuanosine) was also monitored using LC/MS.

Scientific studies have shown that free radicals are culprits causing many diseases of the older population. The most common biomarker for assessing free radical-induced oxidative stress in living cells is oxidative damage to DNA.

Results showed that the level of oxidative DNA damage was found to be at background/endogenous levels (approximately 1 to 10 modified molecules/10⁶ DNA bases). Nearly identical results were obtained when measuring the nucleosides with LC/MS. Accuracy of the measurements was achieved using stable isotope-labeled analogues of modified DNA bases as internal standards. The results show that the obtained tissue-engineered skin did not contain any elevated levels of oxidative DNA damage. In order to assure that such composite materials are free of genetic changes that might occur from oxidative stress during the manufacturing, storage, or transportation of the product, we will consider other cellular biomarkers

Oxidative damage to DNA has been implicated in cancer and several neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (Lou Gehrig's disease), Alzheimer's disease and Parkinson's disease. Furthermore, the accumulation of oxidative damage to DNA especially in non-dividing cells has been postulated to be responsible for the degenerative effects of aging.

Organogenesis Inc.
LIVING TECHNOLOGY



that could be used during the development phase of tissue-engineered materials to ensure that cells have not undergone any inflammatory response during the development or shipment of the product.

Thin Films of Collagen Effect Smooth Muscle Cell Morphology

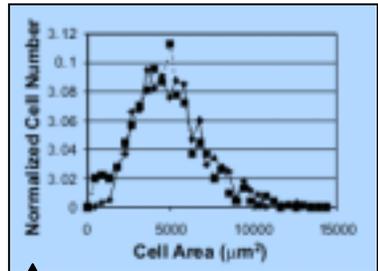
A.L. Plant, J.T. Elliott, and J.T. Woodward, (831); A. Tona (Brown Univ.), and P. Jones (Univ. of Colorado Health Sciences Center)

Collagen is the most abundant extracellular matrix protein in the body, and it is an essential component of many tissue-engineered devices. It can assume different molecular and supramolecular structures, depending on the conditions under

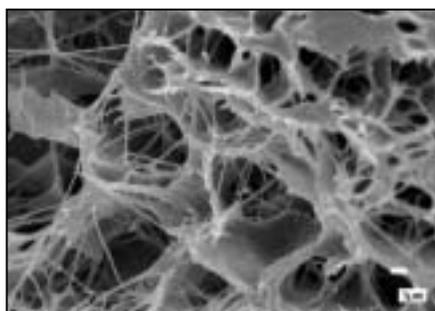
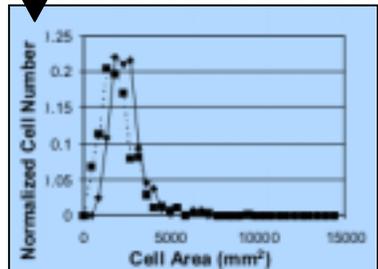
which it is prepared for cell culture. Standardized protocols for collagen use in cell culture studies do not currently exist, and rarely are the final matrices characterized. Furthermore, the cellular response to different forms of collagen is poorly studied.

CSTL researchers have developed a reproducible method for applying collagen to surfaces on which cells can be grown, and to characterize the resulting thin films of collagen protein with respect to molecular structure and cellular response. Using automated quantitative microscopic analysis we showed that the morphology and proliferation of vascular smooth muscle cells is determined by the intermolecular interactions of collagen in these thin films.

These researchers have shown that thin films of collagen can be reproducibly formed under conditions that produce either fibrillar or monomeric collagen, which mimic the healthy and diseased artery, respectively.



The size of cells on thin films (solid lines) was compared to cells on thick gels (dashed lines) using automated quantitative microscopy



Next Steps:
Other rugged and reproducible thin films will be developed as essential reference surfaces on which smooth muscle and other cells can be examined for analysis of their intracellular signaling in response to their culture environment.

Biophysics from First Principles

A.M. Chaka (838) and F. Seno (Univ. of Padova, Italy)

An international, multidisciplinary conference on Biophysics from First Principles was organized to bring together theoretical and experimental researchers from physics, chemistry, biological sciences, and industry to foster interdisciplinary approaches to the study of living systems.

Conference on Biophysics from First Principles: From the Electronic to the Mesoscale, September 7-12, 2002, San Feliu, Spain



CSTL researcher Anne Chaka was elected 2002 Program Chair for Psi_k, the European theoretical physics organization. She also served and will continue to serve on the steering committee for the new EURESICO Computational Biophysics: Integrating Theoretical Physics and Biology Conference series.

The principal focus was on the application of theoretical physics – from first principles to classical simulations, as well as statistical approaches – to understanding biological processes. Challenges facing industry and the environment were presented by invited plenary speakers from DuPont (Daniel Kleier, pesticide invention), BASF (Hugo Kubinyi, drug design and virtual screening), and the U.S. Environmental Protection Agency (EPA) (James Rabinowitz, understanding health effects of environmental chemicals). The maximum capacity of 135 researchers from academic and government institutions around the world attended, as well as industrial participants from companies such as DuPont, BASF, Unilever, Toshiba, and IBM.



Session topics included: Understanding Biological Mechanisms; Transition Metal Chemistry; Predicting the Biological Activity of Chemicals; Structure and Function of Biological Macromolecules; and Molecular Interactions and Solvation: Short- and Long-Range Forces.

The conference was deemed so successful and the interdisciplinary need so timely that the European Science Foundation (ESF) has decided to initiate a new conference series entitled “Computational Biophysics: Integrating Theoretical Physics and Biology” to be held every two years.

Physicists and biologists have few opportunities to interact, yet such interaction has the potential to contribute greatly to progress in both fields. No single simulation or experimental approach will provide all the answers, and solving life science problems requires the skills and knowledge from across traditional disciplines. Advances in physics, such as electronic structure and *ab initio* dynamics, have the potential to play a vital central role in the study of biological systems. The size and complexity of biological problems have driven development of classical approaches such as molecular mechanics and force field methods, and the calculation of free energy of solvation. Theoretical models and numerical simulations from both disciplines need to be integrated to achieve the synergy that is necessary to make real predictions.

CSTL’s Computational Chemistry Group will develop plans to address key modeling challenges identified such as integrating time and length scales, validating current simulation methods, mesoscale phenomena, and improving robustness of biological simulations through increased use of first principles techniques.

This conference represents the first time leading researchers in the field of biological simulations (M. Karplus, K. Schulten, M. Klein), experimental biology (C. Dobson, H. Schrempf), theoretical physics (M. Parrinello, R. Car, B. Lundqvist), and experimental physics (B. Kasemo, W. Zinth) have been brought together for a week-long, intensive conference and discussions. Several challenges were identified, such as a continuing mechanism to foster interdisciplinary research between biologists and physicists. The importance of chemistry as a key link between biology and physics was also stressed. The major challenge identified is the integration of time and length scales, particularly to treat complex problems such as protein folding, biochemical pathways, toxicology, and ion transport through membranes.

3. Pharmaceuticals and Biomanufacturing



CSTL provides a breadth of resources that support the pharmaceutical industry including reference data, as well as artifact standards such as optical filters and fluorescence standards used for instrument calibration. CSTL measurements and standards facilitate the drug discovery process, help optimize production of new pharmaceuticals, and ensure quality control in manufacturing processes. In addition, CSTL's fundamental work in enzyme characterization promotes the transition to biomanufacturing leading to more environmentally sustainable manufacturing

Increased demand for new products has replaced price as the primary force in the U.S. pharmaceutical industry's overall growth.

Enantioselectivity of Three Sulfated Cyclodextrins for the Capillary Electrophoretic Analysis of Pharmaceutical Products

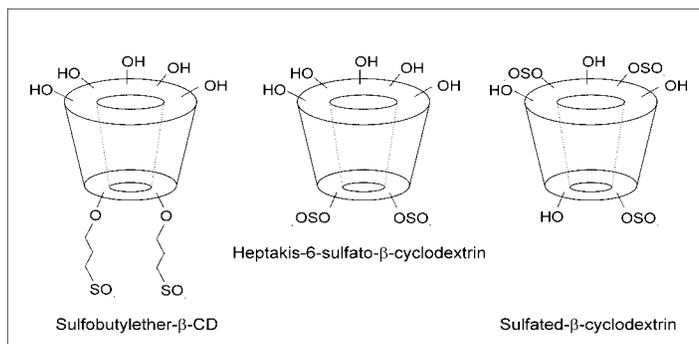
K.W. Phinney (839)

Capillary electrophoresis (CE) is increasingly being used for the determination of enantiomeric composition of chiral drugs. Cyclodextrins (CDs) and their derivatives remain the most widely used chiral additives in CE, and many derivatized cyclodextrins are now commercially available. Several types of anionic CDs have been developed, including a number of CDs bearing sulfate groups. Differing modes of preparation lead to materials that vary in both composition and enantioselectivity. Although this range of enantioselectivity provides some flexibility in method development, it can also complicate the choice of an appropriate chiral additive for the desired separation.

The determination of enantiomeric composition is a critical quality issue in the pharmaceutical industry.

Three commercially available sulfated cyclodextrins having similar numbers of sulfate substituents were selected for evaluation (see figure). The mixed isomer sulfated CD proved to be applicable to the greatest number of the target analytes. Hence, the use of this chiral additive could increase the likelihood of successful method development. On the other hand, the uniform composition of the single isomer sulfated CD could be beneficial for ensuring method reproducibility. The lowest success rate for the target analytes was obtained with the sulfobutylether CD, but a significant degree of enantioresolution was achieved for some of the analytes. The number, type, and position of the anionic substituents clearly play a role in the enantioselectivity of these CDs.

The length of time required to develop analytical methods has remained a bottleneck in the drug development process. Detailed studies of enantioselectivity such as this one provide a knowledge base that simplifies chiral method development.



Structures of sulfated cyclodextrins

Development of an NMR-Based Drug Screening Approach for GPCRs

K. D. Ridge and J. P. Marino (831/CARB); N. G. Abdulaev (CARB/UMBI); and D. M. Brabazon (Loyola College, MD)

Although a crystal structure for the integral membrane G-protein coupled receptor (GPCR) rhodopsin is now available, portions of the cytoplasmic surface are not well resolved and the structural basis for its interaction with the G-protein (G_t) is unknown. The lack of high-resolution structural data for this pharmacologically tractable class of integral membrane protein receptors remains a major gap in our knowledge. Previous studies using soluble mimics of light-activated rhodopsin have shown that grafting defined segments from the cytoplasmic region onto a surface loop of thioredoxin is sufficient to confer G_t activation. To assess whether these mimics can also provide structural insights into the interaction between light-activated rhodopsin and G_t , the ability of a thioredoxin fusion protein comprised of rhodopsin's second and third cytoplasmic loops to bind $G_{t\alpha}$ -subunit ($G_{t\alpha}$) peptides was examined by NMR spectroscopy. Titration experiments show that peptides corresponding to the carboxyl-terminus of $G_{t\alpha}$ bind to this soluble mimic and undergo small but significant structural changes in the bound state. Remarkably, the peptides adopt a C-cap like structure similar to that observed upon the binding of $G_{t\alpha}$ peptides to intact, light-activated rhodopsin. These findings suggest that this functional mimic of light-activated rhodopsin is also a structural mimic for the signaling state and provides a novel solution-based structural assay for screening small molecule inhibitors that potentially disrupt activated GPCR/G-protein interactions.

Comprehensive understanding of the molecular details governing activated GPCR/G-protein interactions is important to the development of drugs that alleviate a number of human diseases.

The approaches developed here offer a promising strategy for "solubilizing" the functions of other GPCRs in order to expedite a structural understanding of G-protein binding and specificity and to the application of drug discovery efforts focused on this interaction.

The Protein Data Bank

T.N. Bhat and G.L. Gilliland (831/CARB); and P. Fagan (TS)

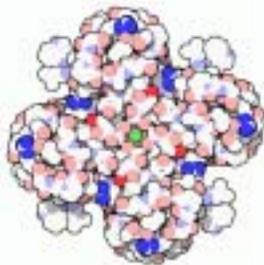
The Protein Data Bank is the single worldwide repository for the processing and distribution of 3-D biological macromolecular structure data. Many secondary sources of information are derived from PDB data. Structural biologists, medicinal chemists, and other biological and biomedical scientists from industry, academia, and private research efforts that engage in protein engineering, structural genomics, rational drug design, protein stability, and other studies of biological macromolecules require information on the structures of biological macromolecules. These studies, which use the three-dimensional structural data, lead to new drugs, new proteins with desired properties for commercial applications, or new insight into biological processes. The coordinates are also essential for bioinformatics efforts since it is well recognized that the three-dimensional structure of a protein is more generally conserved than is the amino acid sequence. The goals of the PDB, the systems in place for data deposition and access, how to obtain further

The PDB provides industry and the research community with three-dimensional structural data of biological macromolecules that can be used for basic research and for countless applications in the pharmaceutical and biotechnology industries.

Research Collaboratory for Structural Bioinformatics (RCSB)

PDB
PROTEIN DATA BANK

<http://www.pdb.org/>



Molecule of the Month
This structure shows the filter portion of a bacterial potassium channel.
PDB, Feb 2003

Efforts of the NIST staff have led to improved capabilities for querying the data, thereby enabling fuller utilization of the data and the resource.

information, and plans for the future development of the resource are described in a special issue of *Acta Crystallographica D* dedicated to crystallographic databases. The article will also be published as a chapter in a forthcoming book "Structural Bioinformatics" to be published by John Wiley & Sons (Philip E. Bourne and Helge Weissig, Editors).

Rapid Identification of Compounds That Inhibit Retroviral RNA-Protein Complexes Using Fluorescence and NMR Spectroscopy

E.S. DeJong and J.P. Marino (831/CARB); C. Chang and M.K. Gilson (CARB/UMBI)

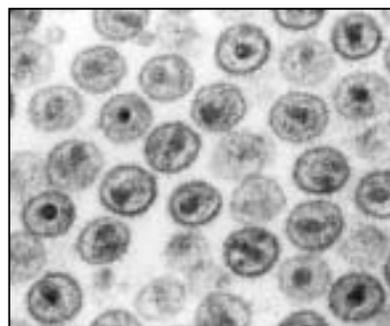
RNA and RNA-protein complexes can provide potentially powerful targets for the regulation of gene expression and inhibition of viral/bacterial infection. For instance, the inhibition of proteins that are involved in mRNA processing, transcription, or retroviral replication could provide powerful new drug targets to combat viral infections, fight cancer, or enhance the effectiveness of existing chemotherapeutic agents. To drive RNA-based drug discovery, general approaches are required for detecting and quantifying nucleic acid-protein interactions that can be used as high-throughput screens (HTS) and for obtaining rapid structural information to guide rational drug design. Using a 2-AP fluorescence perturbation screen, we have identified small heterocyclic aromatic compounds that interact with high affinity (low μM to nM range) with the Rev Responsive Element (RRE) of HIV-1, a potential RNA target for antiviral drugs. A few of the compounds identified through the fluorescence screen have been further shown to effectively compete with an arginine-rich peptide, derived from Rev, for binding to RRE (K_i in the μM to nM range). NMR structural analysis of one of the identified inhibitors, proflavine, bound to RRE has revealed a 2:1 (proflavine:RRE) binding stoichiometry, with the two proflavines stacked one on top of the other in the binding site. NMR experiments also demonstrate that binding of proflavine stabilizes the purine-rich high-affinity Rev binding site of RRE in a manner analogous to the native Rev-RRE interaction.

The development of specific inhibitors of protein-RNA complexes is of significant interest to the pharmaceutical industry because these complexes provide potentially powerful targets to regulate gene expression and inhibit bacterial/viral infection.

NIST researchers have identified a new class of compounds that target the HIV-1 retroviral RRE-Rev RNA-protein interaction, providing new lead compounds in the search for antiviral therapeutics.

NMR structural analysis of one of the identified inhibitors, proflavine, bound to RRE has revealed a 2:1 (proflavine:RRE) binding stoichiometry, with the two proflavines stacked one on top of the other in the binding site. NMR experiments also demonstrate that binding of proflavine stabilizes the purine-rich high-affinity Rev binding site of RRE in a manner analogous to the native Rev-RRE interaction.

HIV 1 Cross Section



Future Plans: Through collaboration with researchers at the National Cancer Institute, we plan to apply our methods to screen large public domain libraries of compounds in a search for new lead compounds against retroviral RNA targets, which will then be optimized using rational design strategies based on NMR structural analysis.

Structural and Biochemical Studies of Enzymes Along the Chorismate Pathway
J.E. Ladner (831/CARB); E. Eisenstein and J. Parsons (CARB/UMBI)

Industrial production of drugs, new biopolymers, and indigo dyes, can be facilitated by increased understanding of the aromatic amino acid synthesis pathway. The increased ability to manipulate this pathway through metabolic and protein engineering will save non-renewable, petroleum-based feedstock chemicals. This study provides a description of the enzymes involved in the chorismate metabolic pathway by solving their three-dimensional structures, modeling the mechanisms of the chemical transformations, and mapping pathway control nodes involved in the biocatalytic conversion of glucose to aromatic hydrocarbons. Only when the detailed three-dimensional structures are known for the enzymes can the precise enzymatic mechanisms and relationships between the structure and the physical properties be predicted. Most

The elucidation of pathways to aromatic biologically active molecules can provide pharmaceutical researchers with a better understanding for the amplification or inhibition of the production of these molecules.

recently, the structure of the product of gene *phzD* from *Pseudomonas aeruginosa* has been solved. The phenazines are biologically active (antibacterial, antifungal, antitumor) aromatic products synthesized mainly by *Pseudomonas* and *Streptomyces* as part of their chorismate pathways. The *phzD* gene product is involved in the pathway in *Pseudomonas* in the production of phenazine-1-6-dicarboxylic acid.



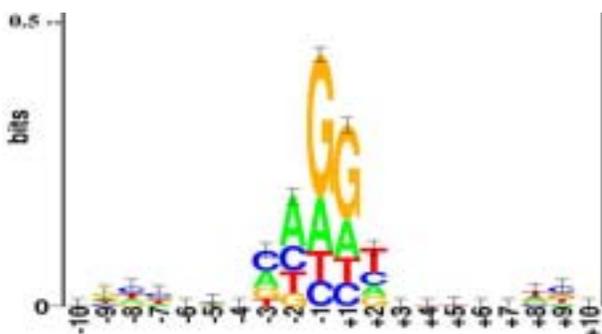
The structure of the product of gene *phzD* has been solved, and the active site of the molecule has been identified.

Target Site of Intron Gain Inferred by a System for Phyloinformatic Analysis (SPAN)
A.B. Stoltzfus (831/CARB); W-G. Qiu (CARB/UMBI), and N. Schisler (Pomona College),

Due to its proven utility, comparative analysis of sequences (comparing homologous genes, proteins, or genomes to derive clues about structure and function) plays a central role in many areas of biotechnology (genome annotation, drug target discovery, biomolecule engineering, medical genetics). The most rigorous methods of comparative analysis are evolutionary ones, based on identification and alignment of homologous sequences, inference of a phylogenetic tree, and phylogenetic reconstruction of changes associated with biologically relevant differences. Due to its complexity and its reliance on human supervision, this type of analysis conventionally is done by an expert, one sequence family at a time. Our goal is to develop a software system that will allow evolutionary analyses of sequence features to be carried out using hundreds of sequence families at a time. Such a System for Phyloinformatic Analysis (SPAN) will provide the bioinformatics community with a rigorous high-throughput method of comparative sequence analysis. The test-bed for development of SPAN is a set of problems regarding the evolution of introns that have withstood a decade of analysis using more conventional methods. On the hypothesis that introns are gained non-randomly, we aim to infer a precise intron "target site", and then use this to predict various aspects of the distribution of introns. In place of the expert judgement that would be used conventionally to tailor an evolutionary analysis for each sequence family, we use automated methods that assess the reliability of information, so as to allow an objective "high-grading" approach in which conclusions are based on only the most reliable parts of the analysis. Based

Comparative analysis of sequences plays a central role in many areas of biotechnology

on this kind of analysis applied to a set of 10 sequence families, we find clear evidence that introns are gained non-randomly at sequences favoring the nucleotide CAG[^]GT.



Data from ten sequence families comprising 677 genes were collated, the sequences were multiply aligned, and family trees were inferred. The resulting data sets were loaded into the SPAN database and augmented with probabilities of ancestral intron states estimated using a Bayesian method developed at CARB. These procedures are not fully automated as yet. Database tools were developed to identify patterns of intron gain. In place of the expert judgement that might normally be used in this

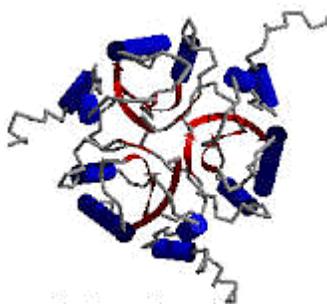
kind of analysis to tweak alignments and trees, and to ignore dubious data points, our system allows us to implement an objective “high-grading” approach based on explicit measures of the reliability of alignment columns, phylogenetic tree branches, and reconstructed events of intron gain and loss. The resulting intron “target” pattern shown here is based on the most trustworthy parts of a large set of data. Preliminary results suggest that this pattern can account quantitatively for patterns in the distribution of intron phases that previously were poorly understood.

This system will be useful in deciphering genome organization, since it will lead to a much greater understanding of one of the central features of eukaryotic genome organization, namely the splitting of genes into introns and exons.

Theoretical Studies of Enzyme Mechanisms

M. Krauss (831)

Class B lactamases are a prime defense mechanism of pathogens against lactam antibiotics and are of great interest medically. Knowledge of the structural and energetic details of the reaction path on a molecular level would be useful in the design of inhibitors. The reaction mechanisms of two enzymes, zinc lactamase and chorismate mutase, that are representative of their class were studied theoretically. The reaction mechanism of the di-zinc lactamase enzyme has not been determined before at the molecular level either experimentally or theoretically. An *ab initio* quantum



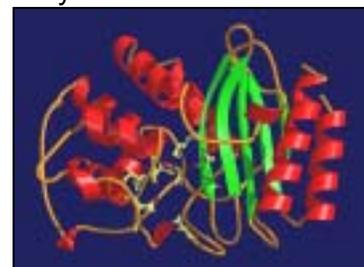
Chorismate Mutase

mechanical and molecular mechanics (QMMM) theoretical method for calculating reaction paths in an enzyme has been applied to chorismate mutase in collaboration with scientists at the

NIH. This enzyme has been the focus of many theoretical studies but the questions on details of the structure and energetics along the reaction path have persisted.

Global optimization and then *ab initio* dynamics on the fundamentals of enzyme reactivity are required to compare the same enzymes from different organisms to ultimately understand specificity of a specific enzyme in a given organism.

Zinc lactamase possesses a flexible flap that forms part of the active site in binding of the substrate. This flap has to move approximately 10 Å and the theoretical solution requires a combination of standard molecular dynamics codes and chemical intuition. The reaction path for the hydrolysis of the antibiotic lactam is calculated to involve highly polarized waters connected in hydrogen bonded networks in



Zinc Lactamase

the highly ionic active site. A range of reactive intermediates is described for the reaction. It is shown that the intermediates can form by proton transfers if the initial hydrolysis products are not removed from the active site of the enzyme by rapid solvation.

The *ab initio* QMMM theoretical method for calculating reaction paths in an enzyme allows for the optimization of the entire enzyme providing the means for accurate calculation of the reaction path and also new insight into the interactions between the active site and the protein environment. One new insight is the observation that the protein environment around the enzyme active site does not change appreciably during the reaction. In another aspect of this study we have calculated the kinetic isotope effects of an enzyme reaction in the active site of the enzyme for the first time.

CSTL scientists have used theoretical studies to gain insight into the reaction mechanisms of medically important enzymes.

4. Chemical and Allied Products



CSTL provides reference data, theoretical models, and artifact standards that are important to the chemical industry. These standards are used for process design and quality control, and help ensure traceability of field measurements to reliable, world-recognized standards.

Often referred to as the keystone industry because nearly every sector of the manufacturing economy uses its products, the U.S. chemical industry ranks among the world's leading industries, accounting for an estimated one-quarter of total world chemical production.

Critical Benchmark Standards for the Cement Industry

J.R. Sieber and A.F. Marlow (839); B.S. MacDonald and C.N. Fales (TS); S.D. Leigh and J.H. Yen (ITL); D. Broton (Construction Technology Laboratories); and S. A. Wilson (USGS)

For several years, CSTL researchers have worked with Construction Technology Laboratories and the United States Geological Survey on SRM projects to benefit the cement industry. These materials represent cement compositions produced around the world with materials obtained in

NIST, ASTM International, and the cement industry have long been partners in the creation of SRMs and research into the characteristics of cement and concrete products. SRMs underpin product quality control for the cement industry worldwide and have done so for over 50 years.

North America, Europe, and the South Pacific. Variety of composition is important because there are many cement products for a myriad of uses. In addition, product quality labs use SRMs with instrumental methods like X-ray fluorescence (XRF) and atomic absorption as well as classical chemical methods of analysis. Portland cement SRMs are used worldwide because they are required by ASTM International in ASTM C



114-99 Standard Test Methods for Hydraulic Cement. ASTM C 114 requires at least six SRMs; therefore, it was necessary to replace the SRM 1880 series.

The final four portland cement SRM renewals were completed this year: SRM 1881a Portland Cement blended with slag and fly ash, SRM 1886a White Portland Cement with Low Iron, SRM 1888a Portland Cement, and SRM 1889a Portland Cement Blended with Limestone. All renewal SRMs are certified for more constituents and properties than the original issues. The work was described in a paper accepted for publication in *Cement and Concrete Research*: "Standard Reference Materials for Cements", J. Sieber, D. Broton, C. Fales, S. Leigh, B. MacDonald, A. Marlow, S. Nettles, and J. Yen.

Due to strong reliance of the industry on XRF for elemental analysis, the members of this project team convinced ASTM International to form a task group to create a new method specifically for XRF analysis of hydraulic cement.



A modern cement plant in Chanute, Kansas.

Critical Benchmark Standards for the Metals Industry

J.R. Sieber, A.F. Marlow, J.D. Fassett, G.C. Turk, R.M. Lindstrom, M.L. Salit, and M.R. Winchester (839); and J.R. Rumble (TS)

The metals industry is implementing a system for product quality control based on ISO/IEC 17025:1999, *General requirements for the competence of testing and calibration laboratories*. Product control laboratories gain accreditation by demonstrating traceability to national reference materials using international standards. Producers must also comply with performance benchmarks imposed by customers. They demonstrate comparability to customer labs and other producer labs by participating in proficiency test programs. Producers and customers are looking to creators of standards and certified reference materials to provide the infrastructure with which to establish the proficiency of all parties. Industry labs need help with certified reference materials, better analytical methods, and uncertainty estimates.

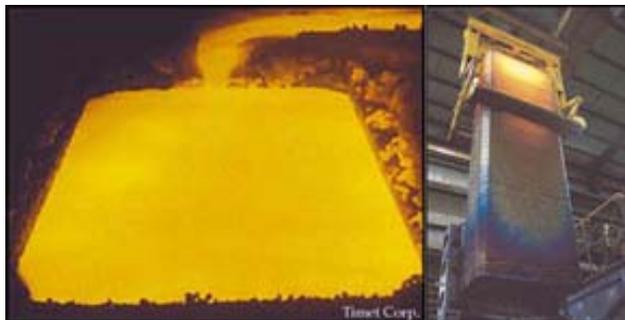
The development of benchmark standards requires in-depth knowledge of the industry and close cooperation with industry representatives through ASTM International, commercial reference materials producers, and corporate laboratories. NIST gains industry input on prioritization of SRM projects and helps commercial producers meet the increasing demand for reference materials.

Five SRMs for metals, alloys, and related materials were completed in FY 02. These SRMs benefit the copper, steel, and aluminum segments of the industry and their customers in aerospace, automotive, electrical, semiconductor, and construction industries.

Accomplishments

- SRMs C1251a, C1252a, C1253a, Phosphorus Deoxidized Copper (completed)
- SRM 699 Alumina (Reduction Grade) (revision of SiO₂ value, completed)
- SRM 133b Cr-Mo Steel (renewal)
- Representation in ASTM Committee E01 on Analytical Chemistry of Metals, Ores and Related Materials for prioritization of SRM requests and method development.
- Assisted the Interlaboratory Analysis Program of Analytical Reference Materials International by providing training and guidance for CRM production.
- Established traceability of X-ray fluorescence and atomic emission spectrometry to the mole.

The level of interaction between NIST and industry will be maintained with emphasis on solving the problem of high demand for an ever-growing variety of new certified reference materials versus high development costs, long lead times, and low sales prospects for any single reference material. NIST scientists will continue to develop matrix-independent methods and work to transfer the technology to the metals industry.



Electron Beam Cold Hearth Refining of specialty metals. In this case, Ti bars are created in a continuous refining process.

Cylinder Filling Ratios for Compressed Liquids

M.L. Huber and D.G. Friend (838)



As the UN Committee of Experts on the Transport of Dangerous Goods performs its mandated study on safety issues related to international commerce, the Department of Transportation (DOT) provides input related to domestic industrial requirements and the domestic regulatory environment. NIST has been asked to assist in the development of cylinder filling ratio guidelines to be used in domestic and international commerce of certain fluids.

The filling ratio definition we used in this work is the ratio of the mass of a fluid to the mass of water at 15 °C that would completely fill a pressure receptacle (this definition is in the draft of UN Model Regulation on the transport of hazardous materials). Depending on the fluid involved, there are different restrictions placed on the filling ratio to ensure safety. For example, it is important that cylinders exposed to high temperatures that may be encountered during storage or transportation do not develop high pressures that could exceed safety limits. Therefore, it is important to be able to determine the filling ratio accurately for a wide variety of fluids. For this project, models were developed to compute the filling ratio using several different methods. When available, high-accuracy equations of state, such as those in NIST Standard Reference Databases, were used. For fluids where dedicated, high-accuracy equations of state were not available, a predictive model based on the theory of extended corresponding states was used. This method has been used successfully in the past for modeling hydrocarbons, cryogenics, and refrigerants. The results were verified by comparison with experimental data where available and existing or proposed international or domestic guidelines.



CSTL researchers provided dependable values of the filling ratio for a wide variety of industrially important fluids such as refrigerants, hydrocarbon gases, welding gases, semiconductor processing fluids, odorants, and other commercially available fluids.

This project provides a reliable model for the calculation of the filling ratio for liquefied gases required for the safe handling of gas cylinders. The results are expected to be incorporated into a UN Model Regulation and to be used in international and domestic commerce of certain hazardous materials.

Double-Sinker Densimeter for High-Accuracy Fluid Density Measurements

M.O. McLinden (838) and C. Lösch (Rubotherm Präzisionsmesstechnik, Germany)

Density as a function of temperature and pressure is one of the most important properties for the determination of a thermodynamic equation of state of a fluid. Density is also vital in fluid metering applications in research and industry. A new apparatus for high-accuracy fluid density measurements over wide ranges of temperature, pressure, and density has been completed and put into operation (−180 °C to +250 °C; 0 MPa to 40 MPa; 5 kg/m³ to 1700 kg/m³). This new apparatus extends our capabilities to higher temperatures and lower densities and reduces the uncertainties by an order of magnitude. These capabilities will be used to provide data on key industrial fluids and mixtures and develop Standard Reference Materials (SRMs) for fluid density.

The new double-sinker densimeter developed by CSTL researchers defines the new best-in-the-world instrument for wide-ranging, high-accuracy P-ρ-T measurements. Furthermore, it provides an absolute measure of density, i.e., no calibration fluids are needed.

especially useful for the measurement of complex mixtures and vapors near saturation. The density measuring system was developed in collaboration with Rubotherm, Inc. of Bochum, Germany. Their components were integrated with the bulk of the system, which was fabricated in the NIST Instrument Shops. The magnetic suspension coupling has proven stable and reproducible to better than 5 μg; yielding a reproducibility in density of 0.0005 kg/m³ (i.e., 5 ppm or less for gases of moderate density as well as liquids). The volumes of the sinkers have been determined to an uncertainty of 20 ppm by comparison to silicon density standards. The final uncertainty in the density depends on both the weighings and the sinker volumes, and we are confident that we will achieve our goal of 0.01 % (100 ppm) uncertainty in density. Although originally designed for measurements on low-density gases, the apparatus has proven capable on liquids with densities as high as 1700 kg/m³.

This new instrument (pictured) employs an Archimedes (buoyancy) technique using two sinkers immersed in the fluid of interest. Sinkers and magnetic suspension coupling comprise the heart of the densimeter. The electromagnet at the top hangs from the balance and levitates the permanent magnet below it. The use of two sinkers leads to a cancellation of effects, such as surface adsorption, which can affect the accuracy of single-sinker techniques, making this instrument



Environmental Control of α-Cr₂O₃ (0001) Surface Chemistry

A.M. Chaka (838), N. Harrison (Imperial College, U.K.), A. Wander (Daresbury Laboratory, U.K.), and X.-G. Wang (GM Research Laboratories)

Chromium oxide is very important for a wide variety of technological applications such as catalysis and surface passivation of metal alloys like stainless steel. Chromium is also added to aluminum alloys because it will segregate to the surface, oxidize, and provide an almost universal foundation for primer and coatings for subsequent surface engineering. Despite its importance, the surface structure and stoichiometry of chromium oxide is not well understood because its insulating character renders analytical techniques based on electron spectroscopy problematic. It is also difficult to obtain clean, well-ordered surfaces for investigation under ultra-high vacuum, let alone interpret experimental observations of systems under industrial conditions. Hence, there is a need for theoretical models that can identify the most likely surface structures, and predict how they will react under realistic conditions in a complex environment. These models can then be used to guide experiment and interpret complex results.

Understanding and controlling surface chemistry of chromium oxide has a broad impact on the catalyst, automotive, steel, and aluminum manufacturing industries.



We have developed a method of *ab initio* thermodynamics that enables us to link 0 K density functional theory electronic structure and vibrational calculations to finite temperatures and pressures to calculate the free energy of a surface in equilibrium with multiple species in the environment. The resultant phase diagram of the α -Cr₂O₃ (0001) surface in the presence of H₂O, and O₂ indicates a complex surface chemistry with an extreme sensitivity to environmental conditions, which can account for much of the variability and controversy in previous studies. Upon exposure to sufficient oxygen pressure, exposed Cr atoms on the surface react to form strong chromyl Cr=O bonds. This chromyl group is extremely stable and can explain how oxygen poisons the surface of chromia catalysts and passivates the surface of stainless steel, which is primarily chromium that has segregated to the surface. The calculated phase diagram also indicates that this chromyl group can be removed by low oxygen pressure and high temperature, which is consistent with experimental observations of catalyst regeneration and susceptibility to corrosion in stainless steel after exposure to heat and vacuum.

The phase diagram of the α -Cr₂O₃ (0001) surface in equilibrium with O₂, H₂O, has been calculated from first principles, and is consistent with experimental observations. Formation of a strong Cr=O bond upon exposure to oxygen can account for the passivation of stainless steel and oxygen poisoning of catalysts.



Alcoa is very interested in using this technique to engineer surfaces of chromium-aluminum alloys. Hence, we will be extending this technique to calculate the phase diagrams for mixed-metal oxide surfaces and to identify processing conditions that favor formation of desirable surfaces. In addition, due to the great sensitivity of the chromium oxide surface structure and bonding to the environment, we plan to explore the potential of this oxide as a chemical sensor.

Gas Chromatography Retention Index Library: A Major New Resource Nears Completion ***J.K. Klassen, P.J. Linstrom, and S.E. Stein (838)***

CSTL researchers have nearly completed a comprehensive gas phase retention index library for capillary columns. These data are abstracted from the literature and evaluated using structure-based predictive algorithms. Data entry and evaluation software has been developed specifically for this program, including specialized methods for selecting reference values to convert relative retention data to more useful retention index values. Estimation methods based on these data, along with various data comparison routines have been developed, tested, and employed for finding suspect values in the collection.

NIST's new Gas Chromatography Retention Index Library contains approximately 70,000 data points for 12,000 compounds on widely used nonpolar capillary columns.

This collection will assist in the confirmation of identifications made by matching acquired mass spectra against NIST library spectra in two ways. First, for those compounds with reference retention data, a check against the measured retention index will either improve the reliability of an identification or exclude a potential false positive result. For compounds not having measured values, this collection provides the basis for a structure-based estimation scheme.

The evaluated data collection will be provided along with the NIST Mass Spectral Library to confirm the identity of compounds in chemical analyses. These data will also provide a basis on which to build well-tested predictive methods. These estimates can assist analysts in making identifications of compounds not represented in the Mass Spectral Library. Only nonpolar stationary phases are included in this first version, and polar phases will be added over the next year. Also, all relative retention times will be converted to retention indices by linking selected measurements to retention index values already in the collection. This collection will be regularly updated to reflect the latest

values available in the literature. Both these data and estimation algorithms will be made available to users of the NIST Mass Spectral Library.

High-Throughput Liquid Transport Properties Measurements in Membranes and Films
C.D. Muzny, S.L. Outcalt, J.L. Scott, and L.A. Watts (838); B.J. Bauer and R.C. Hedden (MSEL)

Measurements of liquid, vapor, and gas diffusion and sorption in thin layer films are critical for development of techniques to predict membrane transport properties. These measurements provide a means to compile the effects of both chemical and structural subgroups in the material, and ultimately, to delineate rational design criteria for mass separating agents such as membranes and polymeric adsorbents. A high-throughput apparatus for the measurement of the diffusivity and solubility of a variety of test components in a wide variety of membranes is under development. This technique employs an array of sixteen test cells that are individually temperature controlled and that can be loaded with differing solvents, test solutions and membranes under analysis. A critical component of this method is to have easily detectable, standard test compounds that probe different physical and chemical characteristics of the membranes. Toward this end a set of test fluorophores has been identified and produced in large quantities. Automation of the experiment, a key to any high-throughput method, is proceeding using the techniques of robotic sample delivery and fiber-optic sample detection systems.

NIST researchers provide critical data for the characterization of membranes and films according to their species-specific transport properties. These data will then be available for use in the rational design of separation processes.

Although polymeric and inorganic materials are used in membrane and adsorptive separation processes, a significant barrier to the optimum use of existing materials and development of new materials is the lack of predictive capabilities for the transport properties of mixtures in any selected material. This data production effort will facilitate the development of the quantitative structure-property relationships necessary for the development of the predictive methods.

The selection of test fluorophores and their production in quantities large enough for the experiments under design was a major accomplishment. After testing for fluorescence properties and stability, G4 PAMAM dendrimers that have their sizes modified by the attachment of polyethylene glycol surface groups of various molecular weights were chosen as the key test compounds. These were then produced in gram quantities via size-exclusion chromatography (SEC) in a set of four different sizes, each fluorescently labeled with a different color for easy separation and detection. Tests of fluorescent response concentration linearity, solubility in water and ethanol, and size validation were performed. The automation of the high-throughput apparatus has been completed via the use of a robotic injection system. Cells with individual solvent flow control can have small quantities of test fluorophores injected via this robotic injector. Sample detection is then accomplished through fiber-optic connection to a high-sensitivity fluorescence detector. A control system capable of simultaneously controlling the temperature and fluid flow on both sides of the transport cells is in place. Final system tests are currently underway. The availability of both the data and predictive methods will impact all industries that currently use membrane-based separations including the water purification industry, the pharmaceuticals industry, and the chemical industry.

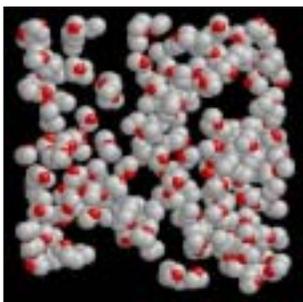


Industrial Fluid Properties Simulation Challenge: Benchmark Standards

D.G. Friend, J.W. Magee, A.M. Chaka, R.D. Mountain, R.D. Johnson III, L.A. Watts, E.W. Lemmon, R.D. Chirico, M.O. McLinden, A. Laesecke, and T.J. Bruno (838)

The First Industrial Fluid Properties Challenge was sponsored by the Computational Molecular Science and Engineering Forum Group (CoMSEF Group) of the American Institute of Chemical Engineers (AIChE) in order to promote and to advance the status of molecular simulation for the calculation of properties of interest to industry. CSTL has had a key role in organizing the Industrial Fluid Properties Challenge, together with industrial participants from such companies as Dow Chemical, BP, DuPont, etc. In particular, NIST has coordinated activities related to establishing benchmark standards against which competition entries will be compared. Questions posed by the competition were prioritized by the industrial participants and included the calculation of certain vapor-liquid equilibrium properties, densities, and viscosities. A total of twelve supporting documents were created, involving about 35 benchmark points with uncertainties.

Molecular simulation is widely perceived to be a tool of the future in the determination of thermophysical properties of interest to industry. In some contexts, it is currently considered as an alternative to measurements or conventional theoretical approaches. However, this tool has limited value because of the absence of a critical evaluation of its capabilities: no one knows how good the results are. A workshop on this subject was held at NIST in 2001, and the framework for the simulation challenge was established.



In some cases, the benchmarks were based on a careful evaluation of published or unpublished experimental results. For other challenge questions, experimental measurements were conducted for the density and viscosity of specified pure fluids and mixtures conducted at Dow Chemical and at NIST. The NIST measurements, in particular, required analysis and purification of available specimens, extension of the temperature/density range of a vibrating tube densimeter apparatus, and establishment of calibration points at high densities using a new double-sinker densimeter.

Contest entries were evaluated in a double-blind protocol, and the announcement of results and discussion were held at a special symposium at the National AIChE Meeting in November 2002.

Contest champions in three categories were selected:

- 1. Andreas Klamt of COSMOlogic GmbH & Co.;**
- 2. Huai Sun of Aeon Technology, Inc; and**
- 3. Marcus Martin and Aidan Thompson from Sandia National Laboratories.**

The simulation contest will serve to assist in the transfer of the technology to prospective industrial users, to identify strengths and weaknesses of the various techniques, to begin the process for systematic development of force fields, and to initiate general protocols for the assessment of property prediction techniques.

Plans are being considered for a second challenge to be completed in 2004; experiences gleaned from the first competition, as well as suggestions from industry and practitioners will serve as input. The paths toward systematization of force field development and evaluation of algorithms will be identified as this project progresses.

Measurements, Modeling and Data for Pressure-Driven Membrane Separations
C.D. Muzny, K. Benko, and J.L. Scott (838); M. Chapman-Wilbert and K. Price (U.S. Bureau of Reclamation); and J. Ranville (Colorado School of Mines)

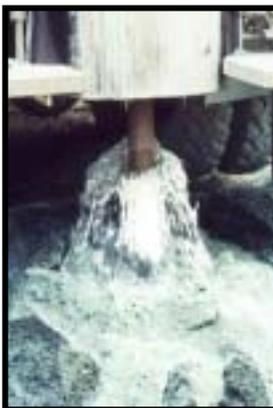
Analysis techniques for the standardization of membrane performance testing in pressure-driven separations are under development. These techniques include methods for the characterization of both the membrane and the complex mixture to which the membranes are subjected for separations testing. The membrane is characterized by a novel liquid-liquid porosimetry apparatus for the measurement of pore size distributions, while the standard fouling mixtures are characterized by field flow fractionation. By producing a standard fouling mixture that includes particulates and a range of large organic species, we are able to determine membrane performance under a variety of realistic conditions. These standard test solutions and procedures will provide industry with a uniform method for pressure-driven membrane separation performance testing. A reverse osmosis membrane module test bed is under development for the validation of the developed test solutions under realistic conditions.

Researchers develop standard methods for the determination of membrane performance in pressure-driven separations. The ultimate goal is to provide a standard test mixture for testing membrane fouling along with standard procedures for evaluating membrane performance.

Improved processes for obtaining specialty chemicals, pharmaceuticals, and advanced monomers using environmentally benign processes, and more economic ways to recover, reuse, and supply water are examples of important industrial and municipal uses of membranes.

As part of this development, it is necessary to have standard techniques for the evaluation of both the membrane and the test solution. The membrane must be evaluated for chemical, physical, and structural characteristics important in the development of rational design criteria for separations for a wide variety of

industries. A unique liquid-liquid porosimetry apparatus for physical characterization of membranes that we previously developed has undergone extensive testing during the past year. This testing has included membranes for ultrafiltration, nanofiltration, and reverse osmosis. The final validation of this technique is still underway, but preliminary results indicate it will be useful for membranes with pore sizes from 2 nm to 1000 nm. This includes virtually all membranes of current technological relevance. The characterization of the standard fouling solutions via field flow fractionation has continued. Validation and standardization methods for this technique have been developed in order to provide repeatable and reliable data for standard solution characterization. As a final test of the overall goal of providing standards for pressure-driven membrane performance testing, a small-scale membrane module test bed is under development. This will have the capability of testing the performance of spiral wound, reverse osmosis modules under the standard conditions being separately developed and characterized. The final production of a membrane test bed will be the major effort of next year.



Notre Dame Radiation Laboratory (NDRL)/NIST Solution Kinetics Database on the Web
T.C. Allison, R.E. Huie, and P. Neta (838)

The NDRL/NIST Solution Kinetics Database has been made accessible on the Internet at (NIST Standard Reference Database 40) and is one of the few databases with this type of information available. A powerful search engine is part of the web site allowing rapid searching of the database, and subsequent links to additional NIST databases will make this a particularly important source of information on free radical chemistry in solution to such areas as physiological and radiation chemistry.



The rates of free radical reactions in solution are important in many parts of chemistry and biology, particularly with the increased understanding of their role in the causes and cures of disease and in aging.

The Solution Kinetics Database contains over 23,000 rate constants, primarily for free radical reactions. More than 11,000 unique species in more than 150 solvents or mixtures of solvents are included. The data have been abstracted from nearly 4,000 literature sources.

<http://kinetics.nist.gov/solution>

Reference	Reaction	Substrate	Rate constant	Detail
1179N207E70001002	$\text{AcOH} + \text{HO} \rightarrow \text{HO} + \text{AcOH}^{\cdot}$	Acetone radical cation	8.73×10^4	Detail
1179N207E70001003	$\text{AcOH} + \text{HO} \rightarrow \text{HO} + \text{AcOH}^{\cdot}$	Acetone radical cation	2.784×10^4	Detail
1432M000E0000002	$\text{Acetone} + \text{HO} \rightarrow \text{HO} + \text{Acetone radical cation}$	Acetone radical cation	8.73×10^4	Detail
1432M000E0000003	$\text{Acetone} + \text{HO} \rightarrow \text{HO} + \text{Acetone radical cation}$	Acetone radical cation	2.784×10^4	Detail
1432M000E0000004	$\text{Acetone} + \text{HO} \rightarrow \text{HO} + \text{Acetone radical cation}$	Acetone radical cation	8.73×10^4	Detail
1432M000E0000005	$\text{Acetone} + \text{HO} \rightarrow \text{HO} + \text{Acetone radical cation}$	Acetone radical cation	2.784×10^4	Detail
1432M000E0000006	$\text{Acetone} + \text{HO} \rightarrow \text{HO} + \text{Acetone radical cation}$	Acetone radical cation	8.73×10^4	Detail
1432M000E0000007	$\text{Acetone} + \text{HO} \rightarrow \text{HO} + \text{Acetone radical cation}$	Acetone radical cation	2.784×10^4	Detail

The database was converted from an older format to one more appropriate for use in conjunction with a web environment, and a web site was established. The web site incorporates useful information and links in addition to the implementation of the database. A search engine was integrated into the web site allowing for fast and accurate searching of the database. Results of queries are returned in a useful summary form with access to full details available with a single click. All records are indexed such that data are intuitively linked (e.g., one can go directly from a single reaction record to all records reported within a journal article).

Over the next fiscal year, the database will be linked to the NIST Chemical Kinetics Database (gas phase) and the NIST Chemistry WebBook and *vice versa*. This will provide maximum linking of similar data, which will lead to a more user-friendly experience for those using the database. A new tool for data entry will be developed to enable distributed data entry to facilitate new data projects. The first of these will be to add reactions of phenoxy radicals to the database; alkoxy radicals will follow these. In developing a new data entry tool and gathering new data, the types of information contained in the database will increase.

Next Steps:

An expanded definition of database fields will be used as the basis for an XML schema for solution kinetics data. The web site will have the ability to return data in the XML format for further processing by the database users. This functionality will ultimately be implemented as a web service allowing automated queries of the database.

Photochemical Reduction of CO₂ Catalyzed by Metalloporphyrins

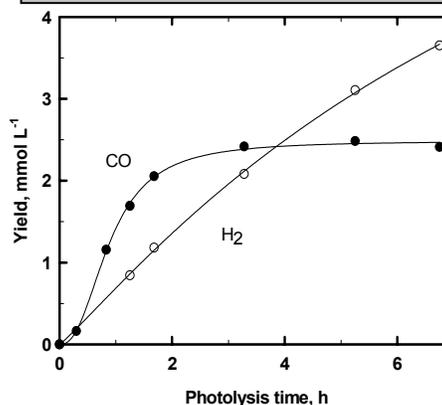
P. Neta (838); and J. Grodkowski (Institute of Nuclear Chemistry and Technology, Warsaw)

The accumulation of CO₂ in the atmosphere from fossil fuel burning leads to global warming. Various strategies are being considered to mitigate this problem. One of the more promising is to mimic nature and carry out the photochemical conversion of CO₂ into useful products, a strategy of photochemical reduction. Reduction of CO₂ can lead to the formation of various useful chemical compounds, such as CO, HCOOH, CH₂O, CH₃OH, and CH₄, which can either be used as fuels or as chemical feedstocks. Iron and cobalt porphyrins and related metal complexes have been proposed as catalysts for the photochemical reduction of CO₂. In order to successfully design production systems based on these catalysts, detailed fundamental information is needed on their photochemical and chemical properties, including their stability under operating conditions. Our approach has been to utilize them in model photochemical systems, to demonstrate formation of CO and/or HCOOH from CO₂, and then to examine the mechanism of catalysis and to determine the relevant rate constants by pulse radiolysis. This fundamental investigation into the photochemical reduction of CO₂ in the presence of metal porphyrins provides the Department of Energy with the information required for them to decide how and if process design based on homogeneous photocatalysis should go forward.

CSTL researcher leads the development of kinetic and mechanistic information on the elementary reactions involved in the photochemical reduction of CO₂ and provides strategies for the reduction of CO₂ into fuel or feedstock materials.

These researchers have found that iron and cobalt porphyrins (MP) catalyze the photochemical reduction of CO₂ to CO in dimethylformamide or acetonitrile solutions containing triethylamine (TEA) as a reductive quencher. In these photochemical studies, the quantum yields were low, and the yield can be dramatically increased by the use of *p*-terphenyl (TP) as a photosensitizer. TP is very effectively photoreduced by TEA which can reduce Co and Fe porphyrins very rapidly to their M⁰P state. While this highly reduced state reacts with CO₂ to form CO, it also reacts with protons (formed in the photolysis) leading to production of H₂ and also to hydrogenation of the porphyrin. After the metalloporphyrin is hydrogenated in several successive steps, it decomposes and the catalytic reaction stops.

Photolysis of iron porphyrin in acetonitrile solution containing triethylamine *p*-terphenyl and saturated with CO₂



Although iron and cobalt porphyrins are able to serve as efficient homogeneous catalysts for the photochemical reduction of CO₂, the decomposition of these substances has proven to be an intractable problem. Thus, we have recommended that there be no attempt to use this approach in subsequent process design.

Metal complexes of similar structures exhibit similar behavior. Attempts to protect the metal complexes against decomposition by embedding them in zeolytic materials did not succeed. It appears that photochemical reduction of CO₂ with metal complexes as homogeneous catalysts suffers from the side reaction due to photochemically produced protons. A more promising route is to investigate heterogeneous systems using semiconductor nanoparticles as light absorbers and catalysts.

Besides being more stable, such systems could lead to better charge separation, possibly leading to the simultaneous reduction of CO₂ and the oxidation of water.

Properties and Processes for Cryogenic Refrigeration

R. Radebaugh, P.E. Bradley, and M.A. Lewis (838); J. Gary and A. O'Gallagher (ITL); R. Gates and L. Reynolds (Univ. of Colorado); and J. Pfothenauer (Univ. of Wisconsin)

The use of cryocoolers in many technological applications has been hampered because of problems such as: short lifetimes, inefficiency, high cost, and excessive vibration. The objectives of this research are (1) to use measurement and modeling techniques for evaluating and improving performance of cryocoolers and their components, such as heat exchangers and pulse tubes, (2) to develop new and improved refrigeration and heat transfer processes for the temperature range below about 230 K, and (3) to provide a database on material properties at cryogenic temperatures. A book chapter on the thermodynamics of regenerative refrigerators was completed and reviewed this year. The book chapter lays the foundation of thermodynamics for oscillating fluid systems such as Stirling, Gifford-McMahon, and pulse tube refrigerators, and will aid the entire cryocooler industry to improve the performance of their cryocoolers.

Cryocoolers are required for many technology areas, including the cooling of infrared sensors for surveillance and atmospheric studies, the cooling of superconducting electronics, magnets, and power systems, the cooling of cryopumps for clean vacuums in semiconductor fabrication processes, the liquefaction of natural gas, and many other existing and potential applications.

Development of our regenerator test apparatus has begun with components designed and fabricated. A prototype pulse tube neon liquefier to provide 500 W at 30 K was developed and tests performed on several geometry options. A low temperature of 30 K has been obtained, but

NIST researchers and collaborators have provided industry with new tools to improve the performance of cryocoolers, particularly those operating at temperatures below about 20 K. This work on two-stage pulse tubes at 30 K will aid the superconductor industry in the development of superconducting systems that can be marketed to a larger customer base. Our materials properties database is now getting more attention and is useful to all industries dealing with cryogenic temperatures.

for liquefaction of neon a no-load temperature of about 20 K is required. Further tests and data analysis are underway under a CRADA with an industrial sponsor. Under another CRADA a 15 K two-stage pulse tube refrigerator was designed for use with a thermoacoustic driver. We have assisted the sponsor in the fabrication and testing of the system. Under a third CRADA we have designed and have partially fabricated a test two-stage pulse tube refrigerator for cooling

superconducting motors and for comparisons with our models. A microscale heat exchanger of parallel plate geometry was optimized, developed, and tested for use in a Joule-Thomson cryocooler for the Air Force. We have added Young's modulus of materials at cryogenic temperatures to our web site as well as a complete set of properties data for many new materials.

http://www.cryogenics.nist.gov/NewFiles/material_properties.html

The NIST Mass Spectral Library: A Major Update

A. Mikaya, S.E. Stein, D.V. Tchekhovskoi, C.L. Clifton, and Q.-L. Pu (838)

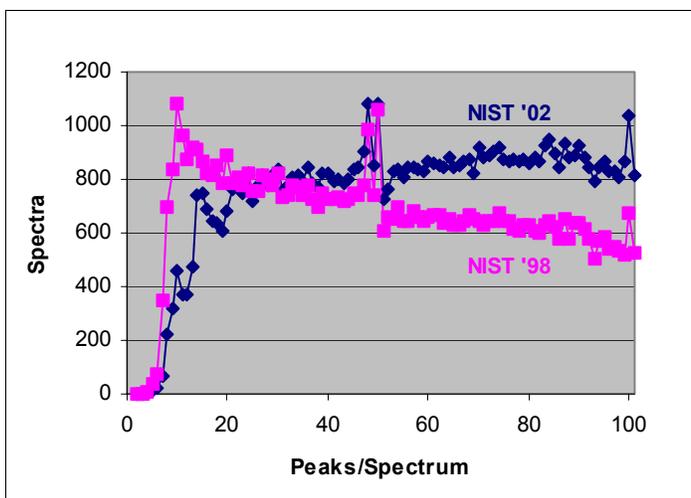
After four years of intensive evaluation and measurement, a new version of the NIST Mass Spectral Library has been completed and delivered to distributors. The number of spectra has

The Mass Spectral Database is a comprehensive, reliable library of mass spectral 'fingerprints' to assist the task of compound identification using the method of gas chromatography/mass spectrometry (GC/MS). Approximately 2,500 new libraries are installed on instruments each year.

increased by over one-third, with each spectrum subjected to a published evaluation method. A variety of quality improvements have been made throughout the library in which names were re-checked, edited and added. Also, 40,000 new CAS registry numbers were added to assist linkage with other chemical databases. A re-ranking of replicate spectra and replacement of lower quality with higher quality spectra has



led to the replacement of thousands of spectra in the main library with higher quality spectra. These enhancements in coverage and quality will improve the reliability of this library for identifying unknown, volatile chemical compounds using the technique of gas chromatography/mass spectrometry (GC/MS).



Over 16,000 spectra formerly in the main library were replaced by either higher quality replicates or newly acquired spectra. This led to a significant change in the distribution of peaks per spectra in the library, as illustrated in the figure.

Spectra for over 54,000 compounds were added to the new version, for a total of 174,948 spectra of 147,198 compounds. This includes large numbers of spectra specifically measured for the library, including derivatives (compounds volatilized through chemical reaction), chemical weapons related compounds, and pesticides as well as replicate measurements for selected compounds. Spectra for 1,735 commercially available compounds of special interest were measured by GC/MS at NIST. All new spectra were subjected to published quality control procedures. Moreover, a variety of quality improvements were made throughout the library. All chemical



IUPAC

names were re-examined to discover errors and to select the best name. IUPAC names were generated by software from structures for approximately 100,000 compounds.

The NIST WebBook - NIST Chemical Reference Data for Industry

P.J. Linstrom and W.G. Mallard (838)

The WebBook is being used by a very wide variety of users, between 10,000 and 20,000 per week, in industry, government and academia. Usage has continued to grow at about 20% per year with over 500,000 distinct IP addresses accessing the WebBook in the last year.

During 2002 the eighth edition of the NIST Chemistry WebBook was released. The WebBook remains one of the most used resources for chemical and physical property data. The fraction of returning users, typically between 45% to 55%, is a good indication that the user community feels that the resource is valuable. For the first time a predictive tool has been added to the WebBook. The well-

established group additivity method for enthalpies of formation as well as heat capacity and entropy has been added as a tool for users who need data for which there are no experimental data. The group additivity tool has been developed using the most recent analysis of the data on the WebBook. Additional new fluids were added for the high-precision fluid properties section. Ongoing work on developing standard protocols for transmission of chemical data has included work with IUPAC and ASTM groups associated with developing standards for data transmission.

<http://webbook.nist.gov/chemistry>

The NIST Chemistry WebBook was voted the winner in the Portals and Information Hubs Category of the ***"Best Chemical Sites on the Web"*** sponsored by: ChemIndustry.com, Inc; John Wiley and Sons, Inc; and the Royal Society of Chemistry

Thermodynamics Research Center (TRC) Comprehensive Program on Critical Data Evaluation

M.L. Frenkel, R.D. Chirico, Q. Dong, G.R. Hardin, and R.A. Stevenson (838); V.V. Diky (Belarussian State University, Belarus); X. Yan and X. Hong (Contractors)

Thermophysical property data evaluation is currently one of the most time-consuming and resource-consuming stages of process design in a number of industries (chemical, petroleum, pharmaceutical, etc.). It has also very significant implications in the analysis of mutual relations of various properties within particular classes or groups of chemical substances, which is traditionally a subject of fundamental research. However, unprecedented growth of the thermophysical data becoming available at present (almost doubling every 10 years) makes it practically impossible to employ traditional (static) methods of data evaluation used over the last 50 years. The main objective of this project is to create new efficient methods and computer tools for the thermophysical, thermochemical, and transport property data collection, critical evaluation, mining, quality control, management, and dissemination and to provide high-quality recommended data in the forms directly suitable for industrial and scientific applications.

The establishment of the TRC Data Entry Facility and TRC Data Quality Assurance Program has dramatically increased TRC capabilities in volume and quality of the 'raw' experimental data collecting, forming the necessary data management foundation for the implementation of the dynamic data evaluation concept. The use of the Guided Data Capture (GDC) at the TRC Data Entry Facility made it possible to establish a rate of data entry at 200,000 data points per year.

The new concept of “dynamic” data evaluation has been developed at TRC. This concept requires the development of large electronic databases capable of storing essentially all the ‘raw/observed’ experimental data known up-to-date with detailed descriptions of the relevant metadata and uncertainties. The combination of these electronic databases with artificial intellectual (expert-system) software designed to automatically generate recommended data based on available ‘raw/observed’ experimental data leads to the ability to produce data compilations dynamically or ‘to order.’ This concept contrasts sharply with static compilations, which must be initiated far in advance of need. Dynamic compilations dramatically reduce the effort and costs associated with anticipating future needs and keeping static evaluations current. The recommended data produced by the deployment of the dynamic data-evaluation concept can rigorously be characterized with their quality assessments providing the ability to propagate reliable data-quality limits to all aspects of chemical process design. The TRC Data Entry Facility was established to support a mass-scale data entry operation for the TRC SOURCE data system. Guided Data Capture (GDC) software was developed at TRC and released from NIST in 2002 to enhance data entry operation both in-house and from the outside sources. The development of the ThermoML, XML-based standard has been completed, to promote interoperability in experimental thermophysical and thermochemical property data collection and management

Future plans include the establishment of a framework of direct data communication, quality assurance, processing, and distribution between major journals in the field and NIST, and the development of the software prototype to implement the Dynamic Data Evaluation Concept for phase equilibrium property groups.

worldwide. The Data Quality Assurance Program has been designed to control the data quality at various stages of data entry, evaluation, and database management. Implementation of this program in 2002 allowed TRC to establish the most complete and reliable data set of critical parameters of organic compounds. The TRC Consortium’s Eleventh Annual Workshop was the second hosted by NIST, and was held to inform the company members on the progress made during the past year.

TRC Thermodynamic Tables



TRC Books



A Standard Chemical Identifier

D.V. Tchekhovskoi and S.E. Stein (838)



IUPAC

The objective of this program is to develop a means of generating a unique identifier for a chemical compound from a digital representation of its chemical structure. It is being developed as an IUPAC project with the intention of establishing it as a standard, international means of representing chemical identity. The structure of the identifier has been established. It has an extensible “layered” structure so that different structural types appear in separate layers. Within an IUPAC committee, a variety of chemical rules have been developed and are now implemented as algorithms that can reliably treat most features of organic compounds. Extensions are underway for inorganic and organometallic compounds.

CSTL scientists have developed, tested, and implemented algorithms for extracting a unique chemical identifier from an input connection table representation of a chemical structure.

The Identifier provides a standard means of communicating and storing the chemical identity of a substance from a structural drawing. Since no such standard now exists, this could greatly facilitate the digital communication of chemistry in a wide range of applications involving the storage and dissemination of chemical data.

The basic methods for construction of the Identifier have been finalized. It is expressed as a series of 'layers,' each containing a specific class of structural information. The layers are: chemical formula, connectivity, isotopic, stereochemical, tautomeric, and charge. This modularization increases the robustness of the Identifier, permits the inclusion of all known structural features and, most importantly, enables extensibility. Creation of an Identifier is done in three steps:

- 1) **Normalization** which applies various structural rules to the input structure to extract only that information required for chemical identification. A key simplifying feature is the elimination of all pi-bonds, radicals, and charges. All of the 'chemical' rules are expressed in this step, including definitions of tautomerization (H-atom migration) and stereochemistry.
- 2) **Canonicalization** generates a unique label for each unique atom in the normalized structure. This uses well-developed 'graph theoretical' methods. This information can serve as feedback to the chemist for error checking.
- 3) **Serialization** transforms the 'canonicalized' structure into strings of characters, one string for each layer. These layers, together, constitute the identifier.

Next Steps: Over the next year, the identifier will be extended to inorganic and organometallic compounds and distributed to a number of organizations for testing. Ultimately, the means for producing the identifier from a chemical structure will be distributed by IUPAC for integration into chemical processing software.

Most of the features involved in the treatment of organic compounds have been implemented and tested. The algorithms were implemented in a test program that was distributed, on request, to well over 60 individuals, who represent a wide range of applications and fields of study. Comments from this testing, along with input from a variety of other source, are being used to complete the processing of organic compounds.

An Efficient Model to Compute the Exact Exchange Potential in Density Functional Theory
C.A. Gonzalez and T.C. Allison (838); E. Ludena, V. Karasiev, and I. Gonzalez (Instituto Venezolano de Investigaciones Científicas, Venezuela); and Y. Simón-Manso (Universidad de Chile)

The use of state-of-the-art electronic structure calculations based on *ab initio* quantum chemical methodologies has become widely popular. Today, scientists in industry and academia make use of this technology to guide complicated experimental work in a large variety of areas including chemical reactivity, catalysis, chemical kinetics, combustion chemistry, environmental chemistry, and organic synthesis. Density functional theory (DFT) has become one of the most widely used methods mainly due to its relatively low computational expense. The electronic energy within the DFT formalism is a functional of the electron density that consists of an "exchange" energy term and a "correlation" energy term. Although, in principle, there exists a universal DFT functional that can describe the electronic problem exactly, the discovery of such functional has eluded scientists around the world for years. In practice, arbitrary forms for the DFT functionals are parameterized in order to reproduce the experimental energetics of a relatively small set of molecular systems. Systematic validation conducted by the Computational Chemistry Group of CSTL indicates that most of these functionals are not transferable to the computation of different chemical properties, where they

CSTL researchers and collaborators have developed and implemented a robust and computationally efficient exchange functional for density functional theory.

show a marked erratic behavior. The exchange functional for density functional theory developed under CSTL leadership is computed variationally through the standard Kohn-Sham procedure and does not require parameters. It is shown that this functional (and its potential) reproduces the “exact exchange” behavior and that it eliminates serious problems such as lack of self-interaction correction, non-variational character, and existence of spurious electron correlation, encountered in most of the exchange functionals used currently by the scientific community.

The results of the work presented here indicate that the major source of error can be traced back to the “exchange” functional. It has been found that these functionals are non-variational and do

The SC- α method has been successfully implemented and tested on a series of atoms and diatomic molecules for which values for the “exact exchange” energies are available. The results show excellent agreement between the SC- α values and the “exact exchange” results.

not eliminate serious self-interactions of the electrons. In addition, the parameterization used to generate these functionals includes spurious electron “correlation.” In this work, we have developed a method called “Self-Consistent Alpha” (SC- α) that eliminates all these problems and does not introduce further parameterization. The SC- α method involves the correction of the DFT exchange energy and its corresponding potential at each Kohn-Sham cycle by a factor computed as the ratio between the Hartree-Fock exchange and the DFT exchange.

The use of the SC- α methodology will allow scientists to compute physical and chemical properties of complicated chemical systems in a reliable and cost-effective manner. The development of this methodology constitutes a major advancement in the area of DFT, a computational chemistry technique widely used by scientists in industry.

Future Plans: Further work leading to validation of the SC- α method in the calculation of different physical and chemical properties of polyatomic systems is under way. In particular, properties such as potential energy barriers critical for kinetic studies of chemical reactions will be studied. A similar study in order to improve the current correlation functionals will also be conducted.

5. Energy Systems



Many CSTL projects impact this industry sector (electric power generation, oil, petroleum and coal, and natural gas) from reference data and models to metering standards and flow calibrations. NIST's accurate and reliable flow standards and databases are critical to this industry both for process optimization and for use in custody transfer applications. CSTL also provides standards that allow this industry to verify compliance with environmental regulations.

Chemical Characterization and Properties of Trace Components of Fuel Gas

T.J. Bruno and W.C. Andersen (838); and A. Abdulagatov (Dagestan Scientific Center of Russian Academy of Sciences)

The U.S. consumes approximately 23 trillion cubic feet of fuel gas each year. Natural gas consists primarily of methane, but 400 additional compounds may be present at varying levels. Most of these materials are naturally occurring (heavier hydrocarbons, sulfur compounds, water,

Researchers provide the natural gas industry with chemical characterization and properties that are critically needed for the efficient operation of fuel gas processing, transmission, and distribution. This is required for custody transfer, calorific value, quality control, safety, and environmental compliance.

carbon dioxide, etc.), and some are added during processing. Liquefied petroleum gas (LPG) consists primarily of propane, but many other compounds are present. The efficient, safe design of equipment operated with fuel gases critically depends upon the properties and chemistry of individual components of natural gas and LPG. Moreover, it has become very clear that the components present at minor levels

(or even at trace quantities) can have a significant impact on the overall properties of the fuel gas mixture.

Recent work has included (1) completion of work on carbonyl sulfide (COS) hydrolysis in propane, (2) the relationship of COS hydrolysis on the Copper Strip Corrosion Test (CSCT), and (3) a comprehensive evaluation of the applicability of the CSCT. Kinetics measurements revealed that the hydrolysis reaction of COS is too slow to be of consequence for the propane industry. Measurements were made at several temperatures, and in the presence of nine catalysts. An Arrhenius plot was developed to provide a predictive approach to the hydrolysis kinetics. In addition, we studied the effect of hydrocarbon diluents in this multiphase reaction system. We determined that the hydrocarbon imposes a diffusion barrier to the reaction, and that a correlation exists between the diffusion coefficient of the hydrocarbon and the reaction rate constant. These kinetics measurements were confirmed with CSCT measurements done on COS mixtures in propane. This evaluation of the CSCT revealed significant operational problems with the test, and we have taken steps to improve the test.



Bruno, T.J. and Lagalante, A.F., "***Process for the Removal of COS from LPG,***" United States Patent 6,334,949, Jan 1, 2002.

Bruno, T.J. and Rybowskiak, M.C., "***Method and Apparatus for Entrainment Mixing of Vapor into Liquids,***" (patent pending).

CSTL work on COS hydrolysis and its relation to the CSCT will change the way the propane industry deals with this sulfur contaminant. The comprehensive review of COS chemistry will replace the earlier 1957 review, and the technology developed during this project will be used in industry and the laboratory.

Thermal Conductivity of Natural Gas Constituents

R.A. Perkins (838)

Natural gas and liquefied petroleum gas are abundant and clean burning fuels that typically consist of mixtures of light hydrocarbons with nitrogen and carbon dioxide. Knowledge of the thermal conductivity of natural-gas mixtures is required to optimize heat exchange surfaces in

CSTL researcher provides the natural gas industry with high-accuracy thermophysical property measurements and reference standard models for natural gas constituents and their mixtures, over wide ranges of temperature, pressure, and composition.

processes for power conversion, liquefaction, separation, and purification. This work focuses on the thermal conductivity of pure propane, butane, and isobutane at temperatures from their triple points to 600 K at pressures up to 70 MPa. New transient hot-wire measurements of thermal conductivity cover the vapor, liquid, and supercritical gas phases with reduced

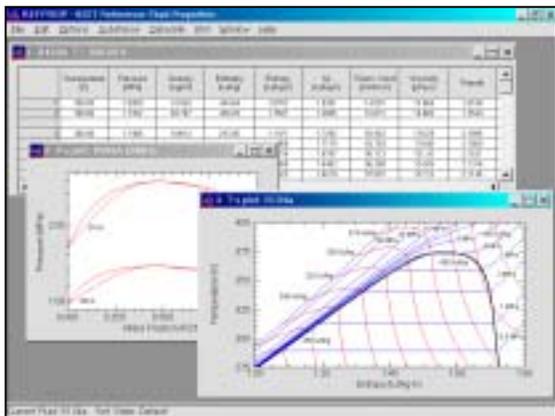
uncertainty and fill regions where data were not available. The thermal conductivity data are used to develop accurate correlation surfaces for these three pure fluids that form the basis of improved models and standards for natural-gas mixtures. These data revealed errors in the best available correlations for thermal conductivity that were greater than 10 % near the critical density of each fluid and again approaching the triple point of butane and isobutane. These new data were used to develop reliable models for the thermal conductivity of these important constituents of natural-gas mixtures. In particular, propane is an ideal reference fluid for extended corresponding states models of the transport properties of natural gas mixtures since its liquid state exists over an exceptionally wide range of reduced temperature.

Natural gas and liquefied petroleum gas are multicomponent blends with a wide variation in composition. Corresponding states models are applicable to ternary and higher-order mixtures and allow a complete description of the thermal conductivity of natural gas and liquefied petroleum gas mixtures. The improved knowledge of the thermal conductivity of propane, butane, and isobutane enables the development of better thermal conductivity models for these fuel mixtures. In addition, these three gases are considered to be “natural refrigerants” and their use has been proposed in alternative refrigerant blends. These improved models for thermal conductivity have been incorporated into the recently released Version 7.0 of the NIST REFPROP database.

The improved models described here were developed in collaboration with the International Association for Transport Properties and are recommended as interim standards. IATP was formerly known as the Subcommittee on Transport Properties of IUPAC.

Release of Version 7.0 of the NIST REFPROP Database

E.W. Lemmon, M.O. McLinden, and M.L. Huber (838)



Industry requires high-accuracy thermophysical property data for the pure fluids and fluid mixtures used as working fluids in refrigeration and air conditioning systems. Property databases are widely needed for the design and analysis of refrigeration systems and are also used for other applications such as flow metering, calibrations, and the design, optimization, and innovation of chemical processes. A major revision of the NIST REFPROP database has been completed and is now being distributed by the NIST Standard Reference Data Program. Previous versions of

this database have become the *de facto* standard for refrigerant properties worldwide. The new version includes additional fluids and mixtures, improved property models, increased calculational speed, and an enhanced user interface with additional properties and calculation and plotting options. Support is provided for using the property routines with other applications, including Excel spreadsheets and Visual Basic programs.

Industrial users now have at their fingertips the most accurate properties available with the updated database entitled Reference Fluid Thermodynamic and Transport Properties (REFPROP, Version 7.0). High-accuracy equations of state for pure fluids and their mixtures have been implemented along with models for the transport properties.

Version 7.0 represents a major revision and upgrade of this database. New features include additional fluids and mixtures. The revision adds the main air constituents nitrogen, oxygen, and argon, and thus, REFPROP 7.0 supplants the NIST 72 database on the properties of air. Ammonia/water mixtures are also included, incorporating a model that had been previously planned as a separate database. Arbitrary mixtures of up to 20 components may be specified, up from the five-component maximum in previous versions. Properties of HFC mixtures are calculated with a new Helmholtz energy model, and experimentally based values of the mixture parameters in this model are now available for 145 binary pairs. The viscosity and thermal conductivity models for 19 fluids have been updated based on recently published data. The new graphical user interface (GUI) retains

Previous versions of REFPROP have become the *de facto* standard for refrigerant properties. The REFPROP subroutines have been embedded in many industrial and commercial applications and provide consistent property values. Version 7.0 provides additional capabilities, increased accuracies, and data for additional pure fluids and mixtures.

the overall look and feel of the Version 6 GUI, but incorporates many enhancements and new features and option with greatly improved speed of calculation, and many more options to prepare plots of thermodynamic surfaces. Additional properties, including dielectric constant, freezing lines, and sublimation lines have been added where data are available. A wide variety of property diagrams may be automatically generated, including pressure-enthalpy and temperature-entropy diagrams and (for binary mixtures) temperature-composition and pressure-composition plots.

Stability of Potential High-Temperature Heat Transfer Fluids

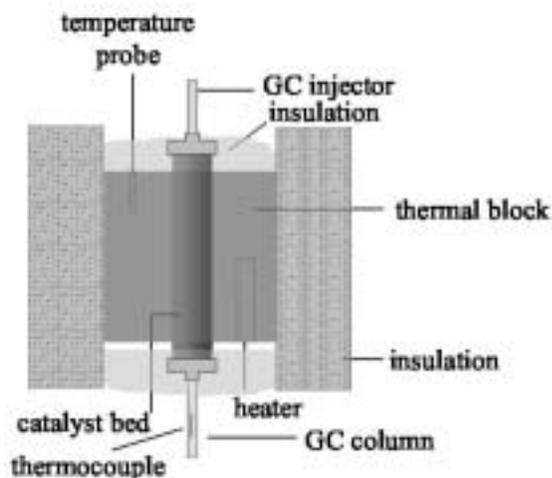
D.G. Friend, T.J. Bruno, and W.C. Andersen (838)

In this solar energy application, heat transfer fluids are subjected to high temperatures and pressures on the order of 315 °C and 4.1 MPa (600 °F and 600 psia). At these temperatures and pressures, the stability of many such fluids is uncertain, especially long term, and in contact with potentially catalytic surfaces. We are working with the National Renewable Energy Laboratory (NREL) to establish the best values for key thermophysical properties of the pentanes and, especially, to determine the stability of pentanes and light aromatics. We are also measuring the kinetics of decomposition at 315 °C and 4.1 MPa in the presence and absence of catalytic materials. The experimental results, based on reaction chromatography and ampoule decomposition kinetics measurements, indicate that these fluids may not be sufficiently stable for the intended application.

The thermophysical properties of light hydrocarbons of between 5 and 8 carbons make this family of compounds attractive as heat transfer fluids for solar panels used in electric power generation.



There has been growing interest in integrating parabolic trough solar systems with organic Rankine power cycles (ORC); this integration of technologies would open a new market niche where solar technologies have been too expensive. Although ORC technology is very mature, the existing plants operate at temperatures below about 200 °C. Preliminary analysis at NREL showed that pentanes (and fluids with similar thermophysical properties) as working fluids could provide high cycle efficiencies. Unfortunately, these fluids had not been tested at high temperatures, and there were concerns about decomposition of the working fluids. NIST was asked to respond to concerns raised by the solar and ORC industry to assist in determining the suitability of pentane isomers in parabolic trough solar systems by study of the stability of the fluids at high temperatures with and without catalytic materials. We have completed our initial efforts to establish key properties for the pentane isomers to be used in cycle design calculations. The stability measurements are based on two techniques: (1) reaction chromatography, a method developed at NIST (see illustration), and (2) ampoule decomposition kinetics measurements. These methods allow the reaction to be measured as a function of temperature in the presence and absence of catalytic surface materials. Results for *n*-pentane, for example (with residence reaction times up to about 10 days), indicate a rate constant for decomposition of about $8.4 \times 10^{-5} \text{ min}^{-1}$. Isopentane, neopentane, and toluene have also been shown to decompose at the conditions of interest. In conjunction with researchers at NREL, several additional heat transfer fluids and mixtures have been identified. Uncatalyzed reaction screening measurements will be performed on two candidates, and continued study depends, in part, on results from these experiments.



The results to date indicate that most of the common fluids examined are not suitable as heat transfer fluids in solar trough applications. Thus, these fluids will not be studied further in laboratory scale or pilot demonstration facilities. However, the integrated technology is still of interest, and alternative heat transfer fluids are being considered.

Properties of Refrigerant Mixtures Near the Critical Point

M.O. McLinden, R.A. Perkins, E.W. Lemmon, A. Laesecke, and J.W. Magee (838)

The refrigerant blends now seeing commercial application as replacements for HCFC-22 in air-conditioning equipment can operate near the refrigerant critical point. This exacts a performance penalty, and this project is supplying improved property data and models in support of the equipment testing and simulation work being carried out in BFRL to elucidate the causes of the performance penalty. Measurements of heat capacity, density, viscosity, and thermal conductivity have been completed. It has also resulted in significant advances in our equation-of-state models and fitting procedures. These results have improved the accuracy of our refrigerant mixture models, which are transferred to industry primarily through the NIST REFPROP database.

Increasingly, refrigeration systems are using refrigerant blends, and the new models developed here have improved the accuracy of HFC mixture properties. These improved models have been incorporated into the recently released Version 7.0 of the NIST REFPROP database.

HCFC-22 is the refrigerant presently used in most residential and small commercial air-conditioners and heat pumps, but it must be phased out under international treaty. Refrigerant mixtures are the likely replacement, and systems using mixtures are now on the market. These blends, typically composed of HFCs 32, 125, 134a, and/or 143a, have much lower critical temperatures than HCFC-22 and normally operate at considerably higher pressures. Under extreme conditions, the blends can operate near or above the refrigerant critical temperature and pressure, with major losses in capacity and efficiency. An understanding of the causes of such losses is a



high priority of the Air-Conditioning and Refrigeration Technology Institute's industry-sponsored 21-CR research program. There are very few property data for the blends near their critical points. This work has focused on the measurement of new data for R-410A (a blend of HFC-32 and HFC-125)

The new model calculates all thermodynamic properties in the liquid, vapor, and supercritical regions with typical uncertainties of 0.1% in density and 0.5% in heat capacities and the speed of sound. Calculations of the bubble or dew point pressure along the vapor-liquid equilibrium boundary have uncertainties of 0.5%.

near its critical temperature. The new data, together with other available measurements for HFC mixtures, have been used to develop improved models for the entire class of HFC blends over wide ranges of conditions. In the course of this effort, significant advances in our modeling and fitting procedures have been made. These include nonlinear fitting techniques and theoretical constraints, resulting in models with fewer parameters that are yet more accurate with improved extrapolation behavior.

Viscosity Measurements and Model Comparisons for Second-Generation Alternative Refrigerant Blends

A. Laesecke (838)

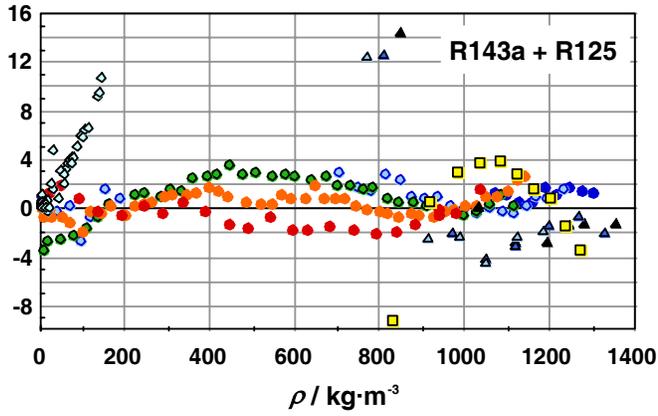
Significant energy penalties have been measured in HVAC systems with next-generation alternative refrigerant blends operating at condenser temperatures that are closer to their critical temperatures. To understand the causes of these energy penalties, working fluids have to be characterized not only at subcritical saturated vapor and liquid states but also at supercritical conditions. Literature viscosity data for such blends and working conditions are very sparse. Accordingly narrow is the basis on which predictive viscosity models for such mixtures have been developed. Also, substantial errors have been identified frequently in literature viscosity data for hydrofluorocarbon systems. Therefore, additional viscosity measurements are needed to build a broader base of validated and reliable data for these important fluids.

The measured viscosity data fill large gaps for R507A and R410A alternative refrigerant blends. They serve as a reference to examine literature data for measurement errors, and they give indications where the predictive model may be improved.

Measurements on the blend R410A in two different viscometers presented an opportunity to discern the susceptibility of this fluid to an applied electric field and to quantify its effect on the results of the torsional crystal viscometer in an effort to extend the application range of this instrument. Wide-ranging viscosity measurements of the blends R507A (0.5 R143a + 0.5 R125 by mass) and R410A (0.5 R32 + 0.5 R125 by mass) were carried out in the torsional crystal viscometer at two subcritical and three supercritical isotherms with pressures up to 68 MPa. The subcritical measurements were conducted at the nominal temperatures 300 K (liquid only) and 340 K (liquid and vapor). Supercritical measurements included the nominal temperatures 360 K, 390 K, and 420 K. For reference, the viscosity of the blend R410A was measured at saturated

liquid conditions between 240 K and 350 K with a sealed gravitational capillary viscometer because in that instrument the sample is not exposed to an electric field. The measurement results were compared with literature data and with values estimated *via* the extended corresponding states model in NIST Standard Reference Database 23 (REFPROP, Version 7).

$$100 (\eta_{\text{exp}} / \eta_{\text{REFPROP}} - 1)$$



This work: ● 300 K ○ 340 K ● 360 K ● 390 K ● 420 K

Heide, Schenk (1996):

▲ 0.752 R125 by mass ▲ 0.50 R125 by mass ▲ 0.241 R125 by mass

R507A (0.50 R125 by mass):

◇ Nabizadeh, Mayinger (1999) □ Fröba et al. (2001)

Besides their industrial relevance, the results of this study laid the foundation for the development of the torsional crystal viscometer to probe flows under combined molecular and electroviscous friction as occur frequently in microfluidic systems.

6. Environmental Technologies



This large industry sector depends on NIST/CSTL for world recognized artifact standards as well as reference data and models to demonstrate compliance with environmental regulations, and for reliable decision-making for environmental remediation, waste disposal, emissions trading and other applications.

The highly fragmented U.S. environmental industry is large and complex encompassing four primary segments: water supply and treatment, solid waste management, air pollution control and environmental cleanup. The global market for environmental technologies is estimated to be \$530 billion.

SRMs for Contaminants on Air Particulate Matter

S.A. Wise, R. Oflaz, J.R. Kucklick, B.J. Porter, D.L. Poster, M.M Schantz, & R.L. Zeisler (839)



The Environmental Protection Agency (EPA) issued new standards in 1997 for air particulate matter (PM) under the national ambient air quality standards. These standards added new regulations for PM less than 2.5 μm ($\text{PM}_{2.5}$), which is the respirable PM fraction, in addition to the existing PM_{10} standards. Research recommendations have already been made by the National Research Council at

To expand the quality assurance capabilities, NIST has entered an agreement with the US-EPA to develop, certify, and issue a suite of SRMs that will be used for the evaluation of analytical methods to provide national measurement traceability and to improve interlaboratory comparability of measurements of today's PM.

the request of Congress and EPA to focus on evaluating what types of particles cause detrimental health effects. To support compositional analyses and other investigations on the fine PM, quality assurance materials are necessary; however, few appropriate materials are currently available to support this research. CSTL is collaborating



Air Sampling Station

with EPA to provide SRMs and quality assurance tools for measurements of inorganic and organic species in $\text{PM}_{2.5}$.

Newly released SRM 2783 air particulate on filter media, provides 0.5 mg of fine air particulate matter on a polycarbonate filter membrane with certified mass loadings for 24 toxic and environmentally important elements. This SRM is intended for elemental analysis using XRF, Neutron Activation Analysis, and other analytical techniques. For organic compounds on $\text{PM}_{2.5}$, we are in the process of collecting a 200-g bulk sample of ultra-fine particulate matter at a site in Baltimore, MD. This PM collection will be completed in late 2003, and the material will be used for preparation of a bulk $\text{PM}_{2.5}$ SRM with values assigned primarily for organic constituents. Because the collection, preparation, and certification of this $\text{PM}_{2.5}$ SRM will require 2 to 3 years, an interim reference material was prepared from a 20-g batch of $\text{PM}_{2.5}$

The interim reference material has been distributed to 18 laboratories as part of a NIST/EPA interlaboratory comparison exercise for the determination of organic compounds in fine particulate matter. The results of this interlaboratory study will be used in conjunction with NIST measurements to assign concentration values for the compounds of interest and will be available to laboratories involved in EPA $\text{PM}_{2.5}$ research programs for use as a control or reference material.

collected earlier at the Baltimore site during different sampling periods in 1998-99 and 2001. This interim reference material has been characterized at NIST for polycyclic aromatic hydrocarbons (PAHs) and nitro-substituted PAHs. Measurements are in progress for other classes of compounds that are of interest because of toxicity and/or as source markers including: alkanes, alkenes, hopanes, sterols, aldehydes, ketones, phenols, acids, and inorganic species.

Gravimetric Results of a Fine Particulate Carbonaceous Reference Material on Quartz-Fiber Filter

G.A. Klouda (837) and J.J. Filliben (ITL)

Gravimetric results have been compiled and analyzed for the production of RM 2784 urban dust on quartz-fiber filters, including process and control blank filters. RM 2784 is on a quartz-fiber filter and is specifically intended for carbon analysis.

Bulk carbon measurements, organic vs. elemental carbon, rely on thermal techniques and therefore require a non-carbonaceous filter. Beyond the need to assign values of elemental carbon (EC), organic carbon (OC), and total carbon (TC) for each method, the results of this exercise are expected to yield information regarding possible mass interval effects, within-filter homogeneity, and filter-to-filter homogeneity.

A randomization plan was designed to set protocols for loading and handling of the filters, and the production of process blank and control blank filters. The distribution of PM loadings and relationships with respect to chamber position, chamber and batch was variable:

average mass loading and standard deviation for 2231 filters was $(1064 \pm 414) \mu\text{g PM}_{2.5}$ per filter with a range of 2763 μg and a minimum and maximum of 92 μg and 2855 μg , respectively. Given the relative standard deviation of mass loading of 39 %, filters identified for a round-robin exercise to assign values for EC, OC, and TC will be randomly selected from eight stratified mass-loading intervals. Two of the most widely used thermal-optical methods, STN and IMPROVE, will be the focus with two of the most qualified laboratories representing each method. The design will be balanced with a strong emphasis on replication and true round-robin sampling.

RM 2784 has been designed and produced especially for the seven EPA "Supersites" that are monitoring fine airborne PM throughout the U.S.

CSTL researchers have determined mass loadings for individual filters produced by a particle re-suspension system.



Gravimetry quantified the variation in loading that is observed here. An intercomparison exercise was designed to utilize accepted thermal-optical analysis protocols to explore possible loading effects.

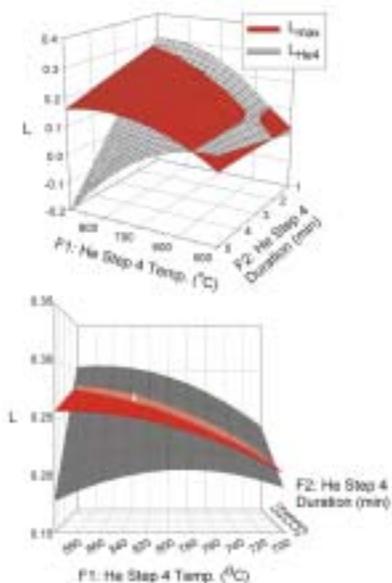
RM 2784 will provide the means to intercompare methods and laboratories for the measurement of elemental carbon in PM. Use of this RM will determine the level of agreement between sites and establish traceability to stated references based on thermal-optical techniques.

Chemometric Optimization of Thermal-optical Analysis in Determining Black Carbon in Atmospheric PM_{2.5}

J.M. Conny, D.B. Klinedinst, S.A. Wight, and J.L. Paulsen (837)

Atmospheric black carbon (BC), a ubiquitous component in fine (<2.5 μm diameter) particulate matter (PM_{2.5}), is responsible for most of the uncertainty in climate change prediction. The chemical, physical, and morphological complexity of aerosol BC presents major problems in assuring measurement accuracy. PM_{2.5} metrology including BC accuracy is also a major concern of industrial leaders currently addressing the new EPA PM_{2.5} emissions standard. Methods based on thermal-optical analysis (TOA) are widely used for ambient air samples; however, different TOA thermal desorption protocols result in wide BC to total carbon (BC/TC) variation. We created three surface models of the following response variables from TOA: BC/TC, maximum laser attenuation in the He phase (L_{max}), and laser

CSTL scientists improve the understanding of the key operational variables in thermal-optical analysis (TOA) by a systematic and repeatable chemometric approach. This is needed by the black carbon measurement community to improve intercomparability and interpretation of results based on measurement accuracy.



TOA response surface maps; lower map is rotated high-resolution view of upper map

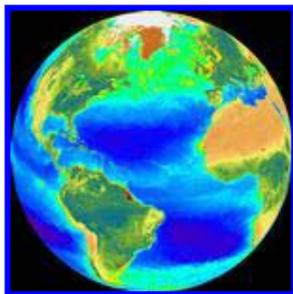
attenuation at the end of the He phase (L_{He4}). A two-level central-composite factorial design comprised of four factors considered the temperatures and durations of all desorption steps in TOA's inert (He) phase and the initial step in TOA's oxidizing (O₂-He) phase. The L_{max} surface was used to optimize the production of pyrolyzed organic carbon (OC char) in the He phase. Unpyrolyzed OC is measured as native BC, causing a positive bias. The L_{He4} surface was used to minimize the loss of char in the He phase, which served as a surrogate indicator for loss of native BC in the He phase, a source of negative bias. The intersection at the L_{max} and L_{He4} surface maxima revealed conditions that minimize potential biases, leading to an optimized thermal desorption protocol. Environmental Scanning Electron Microscope (ESEM) images were used to better understand changes in particle population morphology during the TOA process.

We plan to apply these chemometric techniques to thermal-optical analysis of several U.S. regional PM_{2.5} samples. This will help to fine-tune the optimized thermal desorption protocol from this work, differentiate it if necessary for different types of ambient air samples, and validate robust TOA operational protocols.

This quantitative map of the TOA response surface will help scientists to derive the most appropriate instrumental conditions for accurately measuring black carbon by TOA.

Total Carbon Determination in Buffalo River Sediment Using Isotope Dilution Mass Spectrometry: Comparison of Techniques

D.B. Klinedinst, R.M. Verkouteren (837); and K. Revesz (U.S. Geological Survey)



Accurate measurements of carbon are of great relevance to many sectors of the U.S. economy, and carbon is rapidly becoming an economic global commodity due to its tremendous relevance in global climate change. Unfortunately, there are no SRMs with certified carbon concentration values. While non-certified values exist, they have generally

relied on operationally dependent measurement techniques that are frequently referenced to standards with mismatched matrices. Isotope dilution mass spectrometry (IDMS) is a well-known and “absolute” technique for determining the concentration of a particular analyte, and is especially applicable for complex matrices. IDMS concentration measurements depend entirely on isotope ratio

measurements of a sample, a labeled spike material, and an equilibrated mixture of the two.

Quantitative recovery of the analyte is not required. Here, we utilized ^{13}C -depleted urea and determined mixing ratios with gravimetry. We have now developed, tested, and compared two IDMS methods for carbon using RM 8704 (Buffalo River Sediment). The resulting carbon values are consistent and comparable, but with uncertainties that require further development of the method. Upon successful completion of this project, we will certify high-priority SRMs for carbon to validate carbon sequestration methods in soils. In the near future, these may be used to establish carbon “credits” that offset greenhouse gas emissions.

NIST and USGS researchers investigate a definitive method for certification of carbon in SRMs. This capability is critical for the Nation to meet upcoming challenges in carbon sequestration, and to provide accurate benchmarking materials for many industrial sectors concerned with the carbon content of feedstocks and process residues.

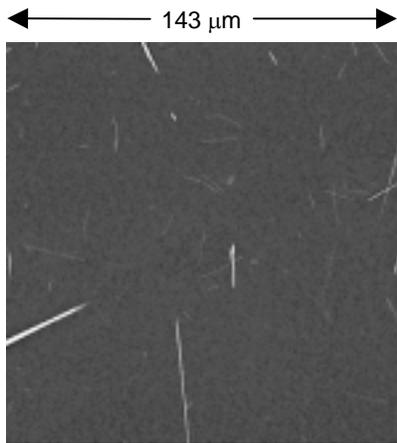
The carbon concentration of RM 8704 (Buffalo River Sediment) was determined with IDMS using urea depleted in ^{13}C as an internal standard. Isotopic ratios were measured using two isotope ratio mass spectrometry techniques: dual inlet/IRMS (NIST) and Gas Chromatography/IRMS (USGS). The mass fraction of carbon in RM 8704 was 0.03279 (0.0079 u_c , $n=3$) and 0.0320 (0.0124 u_i , $n=1$), respectively. The relative difference between the existing RM 8704 carbon reference value and the new NIST result was 1.6%.

Mass Determination of a Population of Asbestos based on Particle Size and Number

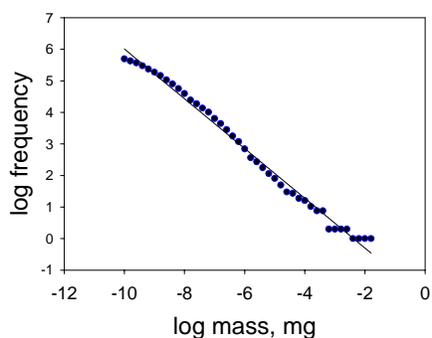
J.R. Verkouteren (837); and A.G. Wylie (Univ. of MD)

The different agencies regulate asbestos on the basis of either mass or particle size/number, however it is clear that a more cohesive approach is needed. In addition, a much lower threshold limit for mass is being considered that is below the detection limit of the standard methods. The new technique described here provides a critical link between particle number and mass and provides the basis for a new method for determining low concentrations of asbestos in bulk materials.

Researchers address a critical measurement issue, which will likely result in new regulations for asbestos.



SEM fiber image and mass distribution of an asbestos fiber population



In this work, the total particle mass of a population of asbestos fibers was determined from 2-dimensional measurements of length and width. A known mass of asbestos was deposited on a filter, and 50 randomly selected fields were imaged on the field-emission-gun scanning electron microscope (SEM) at three different magnifications. The fiber lengths and widths were measured, and the thickness calculated by assuming one-half the measured width. The fibers were binned by length, width, and mass and the total number in each bin over the entire filter was calculated. Overlap in the frequency of bins common to two magnifications indicated internal consistency in the calculation of total number. The frequency distributions were modeled by a power series, consistent with the fractal model proposed for asbestos. The total mass was calculated both by summing the particles in each mass bin, and by integration of the fitted distribution. Although there are very few particles in the large mass bin that contributes greatly to the total mass, their number is predicted correctly by the modeled distribution. Although we have not yet determined the uncertainty of the final mass calculation, the values arrived at by the two different calculation are quite close to the weighed value, lending support to the overall approach. This approach should lend itself to accurate measures of mass concentrations at 0.1%, which is the new threshold limit being discussed. Without such a

The long-term impact of this work is to provide a new framework for asbestos regulations that relates the hazardous material (defined in terms of mass) with the risk (defined in terms of particle size/number).

method, many new materials might be labeled as asbestos-containing based on the discovery of a few fibers and the inability to convert this correctly to mass concentrations.

SRMs for Contaminants in Soils, Sediments and Tissues

C. Beck II, T.A. Butler, R.R. Greenberg, M. Howard, W.R. Kelly, J.R. Kucklick, R.M. Lindstrom, S.E. Long, E.A. Mackey, J.L. Mann, A.F. Marlow, K.E. Murphy, R. Oflaz, R.L. Paul, B.J. Porter, D.L. Poster, M.M. Schantz, J.R. Sieber, G.C. Turk, R.D. Vocke, S.A. Wise, L.J. Wood, L.L. Yu, R.L. Zeisler (839); and S. Tutschku (Universitat Leipzig, Germany)

Validation of the complete analytical method for environmental measurements requires the use of natural matrix SRMs similar to those matrices typically encountered in the analysis of environmental samples. NIST natural-matrix SRMs for organic and inorganic contaminants in soil, sediment, botanicals, and tissue are necessary for the development and validation of analytical procedures and to provide quality assurance of chemical measurements for these samples. The environmental community, including those responsible for promulgating analytical methods, developing policy, enforcing regulations, and interpreting laboratory data, has long known that risk to biota and humans is dependent upon the form of the metal in the environment not just the total elemental composition. SRMs with values assigned for speciated metals are required to address these measurements needs.

Expectations are that species-based regulation is imminent despite the uncertainties in the underpinning measurement science.

Recent soil, sediment, botanical, and tissue matrix SRMs have been developed to address this need and include: marine sediment (SRM 1941b and SRM 2702), mussel (SRM 1974b) and fish tissue (SRM 1946), pine needles (SRM 1575a), and mine waste-contaminated soil (SRM 2780). SRM 1941b Organics in Marine Sediment and SRM 1974b Organics in Mussel Tissue are renewals of these popular marine matrices for determination of organic contaminants with certified values for over 60 constituents. SRM 1575a Pine Needles (a renewal), SRM 2702 Inorganics in Marine Sediment, and SRM 2680 Hard Rock Mine Waste are intended for the determination of elemental composition. SRM 1946 Lake Superior Fish Tissue is the first frozen fish tissue SRM and is intended primarily for the determination of contaminants such as polychlorinated biphenyls (PCBs), chlorinated pesticides, total mercury, and methylmercury, as well as food matrix components such as proximates and fatty acids. The marine sediment, fish, and mussel tissue SRMs were developed to support the numerous laboratories involved in marine environmental monitoring.

To address the need for reference materials for speciated metals, NIST has provided certified concentrations for methylmercury as well as total mercury in the two marine tissue SRMs (SRM 1946 and SRM 1974b) to complement the existing suite of mussel and oyster tissue SRMs with certified values for methylmercury (SRM 2976, SRM 2977, and SRM 1566b). Reference values were also provided for the renewal marine sediment, SRM 1941b, for mono-, di-, and tributyltin species in addition to total tin concentration.



Abandoned Mine

Another area of concern is the environmental impact of mine waste drainage on the neighboring communities. There are an estimated 500,000 abandoned mines in the western U.S., and these sites commonly contain significant quantities of discarded mine waste, resulting in acidic drainage containing elevated levels of heavy metals. Accurately evaluating the environmental impact of mine waste drainage has been hampered by the variability observed in laboratory methodology used to assess this hazard. The mine waste SRM was developed to support these measurements.

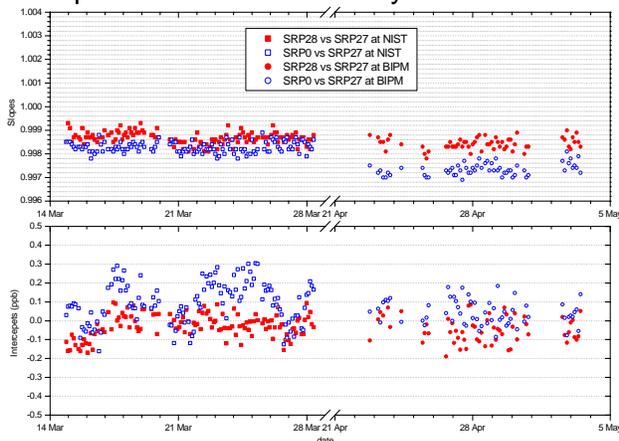
In addition, in cooperation with the EPA, the New Jersey Department of Environmental Protection, and USGS, NIST has begun development of a new SRM to address speciated forms of metals, primarily chromium-VI, in contaminated soils. A contaminated soil known to contain Cr-VI has been collected and is being prepared by USGS for use as the candidate SRM. In addition, a second fish tissue (SRM 1947 Lake Michigan Fish Tissue) has been prepared and measurements are in progress to provide a fish tissue SRM with emphasis on the trace element content to complement SRM 1946, which focuses primarily on the organochlorine contaminants. The stock material used for SRM 2702 is also being used to develop the first reference material (SRM 2703) for solid sampling direct analysis techniques that commonly use small analytical test portions.

Developing an Advanced Primary Ozone Photometer - A Collaboration with BIPM
P.M Chu, J.E. Norris, and F.R. Guenther (839)

The concentration of ozone in the atmosphere remains a significant issue from both scientific and political perspectives. Ozone, at tropospheric levels, is a health concern and contributes to global climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. NIST has supported the ozone monitoring communities for many years with its Standard Reference Photometers (SRPs), however, increasing demands in both the national and international arena, require a new approach. Therefore, the current research effort is focused on coupling state-of-the-art measurement technologies with national primary gas standards to provide an advanced primary ozone standard, which will improve SI-traceability and comparability of global ozone measurements.

Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to ten US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest in ozone measurements has prompted fifteen foreign laboratories to acquire SRPs.

NIST/CSTL supports the growing national and international need of improved ambient ozone measurements by partnering with BIPM to develop advanced primary ozone reference standards and to share the dissemination of secondary or transfer standards. NIST/BIPM partnership follows includes: 1) The transfer the current ozone “national reference photometer” technology to BIPM. 2) Performance assessment of the current technology with respect to current and future ozone measurement requirements. 3) Development of a primary reference standard with capabilities of SI-traceability verification.



Comparison of NIST-constructed Standard Reference Photometers for ozone measurements: SRP 0 at NIST and SRPs 27 and 28 now located at BIPM

During FY 2002, two new SRPs were built, extensively compared to the principal NIST reference photometer (SRP 2), and transferred to BIPM. A 0.5 % calibration bias between SRP 2 and the SRPs transferred to BIPM has been noted and an ongoing effort is dedicated towards identifying and eliminating this bias. To assess the current comparability of global ozone measurements, a pilot ozone key comparison was initiated in the fall of 2002. An advanced photometer demands instrumentation with high sensitivity, accuracy, and precision, as well as rapid time response for measuring chemical species at atmospheric levels (nmol/mol and lower). A number of *in-situ* techniques are being considered, including time-gated laser-induced fluorescence detection, laser absorption spectroscopy, and cavity ring-down spectroscopy for detection of relevant chemical species and

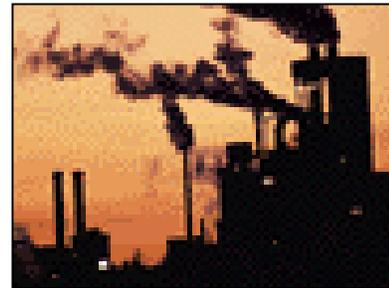
possible contaminants in the carrier gas. Collaborations with researchers developing state-of-the-art instrumentation to measure NO₂, NO, and O₃ at trace levels have been established. During the next year, two additional SRPs will be built for BIPM with participation of BIPM staff to accelerate the transfer of the current technology. Evaluation of potential instrumentation for the advanced primary reference standard will also be initiated.

The advanced primary ozone photometer will provide an internationally validated ozone standard that can be used to anchor all ozone measurements through a defined traceability structure.

Standards to Support Stack Emissions Testing

W.D. Dorko (839)

The Clean Air Act was originally passed in 1977, and from that time through the 1980's the traceability requirements were met by NIST developing Standard Reference Materials (SRMs). NIST developed technical specifications for the Specialty Gas Industry (SGI) to produce required gas mixtures, developed primary standards, and analyzed each of the candidate SRM mixtures for certification purposes. These SRMs were then made available for sale to form the traceability path for emission measurements. In 1990 the Clean Air Act was amended and



part of the new regulation required vastly increased monitoring of the emissions, which required an order of magnitude increase in the usage of calibration gases. The 1990 Amendments also

Stack gas emissions are regulated by the U.S. EPA under stipulations in the Clean Air Act. In order to quality assure compliance the instrumentation used to monitor these emissions must be calibrated with gas mixture calibration standards traceable to NIST.

included arrangements for emissions trading whereby a power plant that emitted less than their "allowable" amount of SO₂ could sell a permit to other companies for the balance. Measurements made against NIST traceable calibration gases are the foundation for emissions trading. Both the emissions trading and the increased monitoring required greatly increased usage of traceable gas standards. NIST did not have sufficient resources to meet this increase within in the context of the SRM program in which NIST invested all of the resources, and performed 100% of the certification analyses. Therefore, NIST worked with the SGI and EPA to develop a new program,

the NIST Traceable Reference Material (NTRM). In the NTRM program, the SGI invests most of the resources and NIST performs about 10% of the analyses for certification. The NIST resources are greatly leveraged with the NTRM program, and the SRM program is maintained.

Since the inception of the NTRM program in 1992 more than a dozen companies have produced and NIST has certified multiple batches of NTRMs. Most of the batches consist of binary mixtures with air or nitrogen being the diluent gas. The analytes are varying concentrations of propane, methane, carbon monoxide, carbon dioxide, oxygen, hydrogen sulfide, nitric oxide, nitrogen dioxide, and sulfur dioxide. The demand for NIST traceable calibration gases cannot even be met with the NTRM program so EPA developed a document describing how Specialty Gas Companies (SGC) could produce "EPA Protocol Gases" that EPA accepts as NIST traceable. The procedure requires that the SGC analyze the Protocol Mixture against either an SRM or an NTRM. This is a mechanism to leverage NTRMs since one NTRM can be used to analyze 100 or more EPA Protocol mixtures. The results are that stack emissions have been maintained at the desired regulated levels, and the desirable ambient air quality has been reached. It has also resulted in a smooth functioning emissions trading program whereby companies that introduce new technology are rewarded and can sell the excess permits to emit to companies that have not yet capitalized for plant improvement. NIST is currently working to develop calibration gas mixture standards for chlorine, hydrogen chloride, and ammonia. The magnitude of the demand for these will not be as large as for the other gases so other traceability mechanisms are being explored outside of the existing NTRM and SRM programs.

Another Approach – Remote Sensing:
NIST is also developing an infrared database to calibrate infrared-based sensors that analyze the emissions from stacks. If this is successful then it will be an alternate method to provide traceability for emissions measurements.

Benchmark Data on Liquid Fire Suppressants

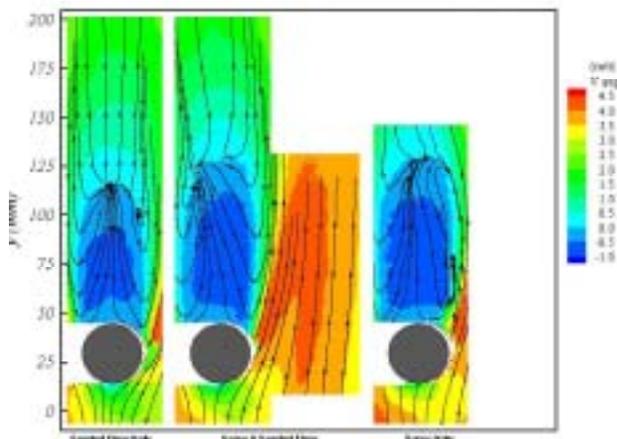
C. Presser (836); J.F. Widmann (BFRL); G. Papadopoulos (Dantec Dynamics); and P. DesJardin (Sandia National Laboratory)

The new generation of non-ozone-depleting Halon alternatives include chemical suppressants that have high boiling point temperatures (i.e., $T_b > 330$ K) and exist in the liquid phase under high-pressure release or in ambient conditions. Release of these agents in a confined cluttered space results in the dispersal of droplets that either travel along ballistic trajectories, move with the convecting flow, or impact nearby solid obstacles. Therefore, an accurate representation of agent droplet transport is crucial for the numerical modeling of fire suppression in confined spaces. To better understand the physics of droplet transport around and behind solid objects, an experiment was designed where controlled grid-generated turbulence was imposed on the air stream.

NIST scientists develop a parametric data set to guide and validate the Vulcan fire modeling efforts underway at Sandia National Laboratory.



Experimental results provide new data for a well-characterized, homogenous droplet-laden turbulent flow field around prescribed obstacles



Results are obtained using particle image velocimetry, phase Doppler interferometry, and visualization techniques. The liquid agents considered in the investigation were water, and two 3M™ fire protection fluorocarbons HFE-7100 (with a boiling point of 334 K), and HFE-7000 (with a boiling point of 307 K).

Particle image velocimetry (PIV) experiments were completed with the assistance of Dantec Dynamics to characterize the impingement and transport of a water spray around a cylinder and body-centered cube arrangement of spheres that represents a more complicate obstacle. Measurements upstream and downstream of a 33 mm cylinder having a 250 W heater and thermocouples to monitor the near surface temperature (see figure) were completed. The cylinder was heated to 423 K to study the effects of a heated surface on droplet vaporization and transport, as a droplet approaches the heated surface. The results indicated that the transport of droplets around the cylinder is highly dependant on droplet size, and spray impingement and surface wetting caused significant cooling of the heated surface.

Plans are underway to initiate measurements with the phase Doppler interferometry system and to obtain spatial profiles of the droplet size and velocity distributions, and droplet number density upstream and downstream of the obstacles for water, HFE-7100, and HFE-7000.

Understanding the correlation of the agent/spray properties, agent atomization methods, and agent dispersion effectiveness will enable the optimization of fire suppression performance of misted liquid systems.



Distribution of Mercury in Seabird Eggs as a Marker of Environmental Contamination in Alaska

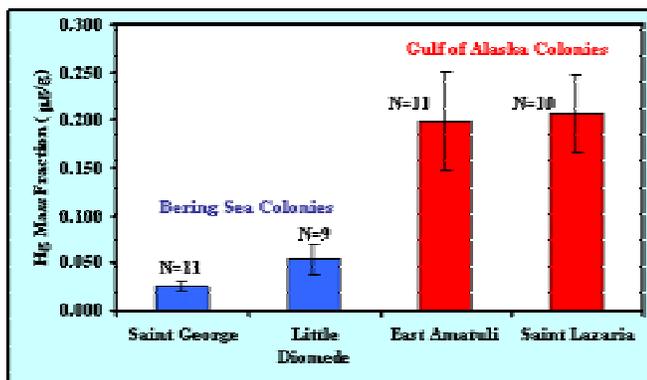
S.J. Christopher, P.R. Becker, S. Vander-Pol, R. Pugh, and R.D. Day (839)

High latitude areas (Arctic and Sub-Arctic) tend to act as a sink for global pollutants such as mercury (Hg). NIST is collaborating with the U.S. Fish and Wildlife Service's Alaska Maritime National Wildlife Refuge (AMNWR) and the U. S. Geological Survey's Biological Resources Division, in the Seabird Tissue Archival Monitoring Project (STAMP). This project is using Alaskan colonial seabirds as markers to monitor the geographic and temporal trends of contaminants in the Alaskan marine environment.

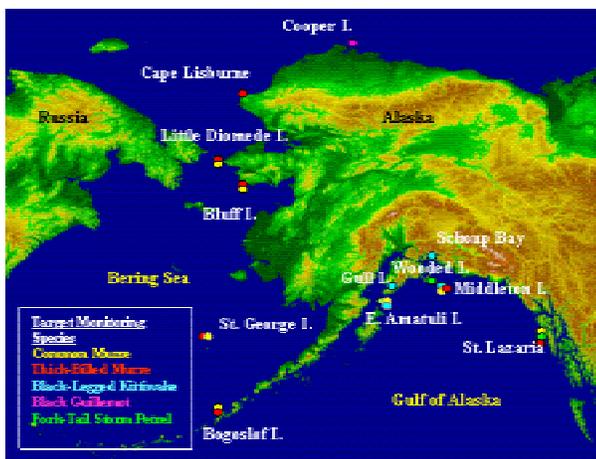
NIST provides support to the expanding STAMP program through the banking of seabird eggs in the National Biomonitoring Specimen Bank and egg contents analyses. Recent analyses of common murre (*Uria aalge*) eggs show the existence of regionally unique Hg chemical signatures.

The purpose of STAMP is to monitor long-term trends in marine environmental quality through the use of colonial seabirds as indicators. NIST's role in STAMP is to provide the quality assurance infrastructure of analytical specimen banking through the National Biomonitoring Specimen Bank and chemical analysis of contaminants in seabird tissues, including eggs, for STAMP's collaborating agencies.

Specimen banking and analytical protocols for seabird eggs were developed and published. Samples from 41 egg specimens from 4 different locations were analyzed for total mercury. Mercury signatures in common murre eggs were readily observed. Hg levels in the eggs were significantly different in the Bering Sea and Gulf of Alaska (see figure), indicating that regionally dependent environmental processes may produce conditions that allow for favorable uptake of Hg in Alaskan seabirds residing in the Gulf of Alaska.



Mercury content (mean \pm 95% C.I.) in 41 common murre eggs plotted as a function of island colony and water body.



Projected sampling sites and species for STAMP

Cutting-edge analytical methods and accurate environmental Hg data produced at NIST were exported to our program sponsors. This research also yields benefits to the numerous researchers studying global Hg cycling and tracking Hg deposition in Alaska. The published Hg data were also provided to researchers in Alaska conducting risk assessments of subsistence foods on Alaska Native communities (murre eggs are consumed by local residents) and the data generated by NIST were a recent contribution to the international Arctic Monitoring and Assessment Programme.

Development and Critical Evaluation of a New ID/ICP-MS Method for Mercury in Sediments and Fossil Fuels

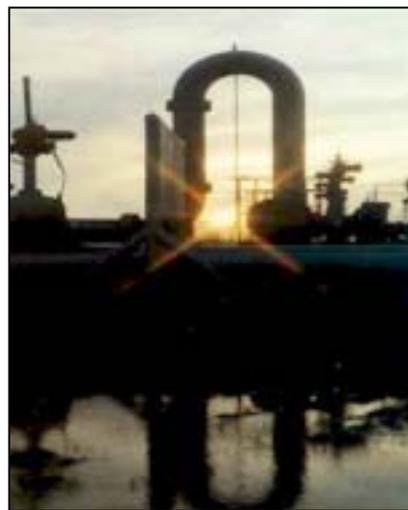
S.E. Long and W.R. Kelly (839)

A method combining high-pressure closed-system sample digestion with isotope dilution cold-vapor generation inductively coupled plasma – mass spectrometry (ID-CV-ICP-MS) has been developed for the determination of mercury in environmental matrices. Refinements to the method, the most important of which has been a dramatic reduction in the magnitude of the procedural blank, now permit accurate determinations to be made at ultra-trace (pg/g) concentrations in any sample matrix. The method is being exploited to make measurements of mercury in complex matrices with much lower expanded uncertainties than previously attainable, and at concentration levels that cannot be matched by other techniques or methods. The development and implementation of this method fulfils an urgent need for more accurate measurements of mercury in environmental materials. Data provided by the method result in a much improved traceability link for industrial EPA compliance monitoring and for calculating anthropogenic mercury releases for risk assessment studies. CSTL will continue to work with EPA, DoE and other interested parties to ensure that these new and unique measurement capabilities for mercury analysis are fully exploited. One application that we are promoting is a study of mercury levels in crude oil feedstocks that are more representative of current refinery processing in North America. These data could be used to assess the contribution of mercury from these sources to the U.S. mercury budget. To date, this has not been assessed with any great reliance, owing to the limitations of existing analytical methods. The provision of a more accurate database will be useful for EPA regulatory decision-making and for strategic planning by the oil industry.

A new analytical method has been applied to the determination of mercury in sediment, coal, and in crude oils representative of the Strategic Petroleum Reserve (SPR) stockpiles. Sales of NIST fossil fuel SRMs have dramatically increased following the addition of certified mercury data using this method.

This work resulted in:

- **Dramatic reduction in the magnitude of the method process blank, now permitting accurate measurements as low as 50 pg/g of sample matrix**
- **Certification of mercury in several NIST SRMs:**
 - **SRM 2693 Coal**
 - **SRM 2702 Inorganic Sediment**
 - **SRM 2721 Moisture and Sulfur in Crude Oil**
 - **SRM 2722 Moisture and Sulfur in Crude Oil**
- **Determination of mercury in crude oil samples from the Strategic Petroleum Reserve (SPR) in support of the DoE**



DoE SPR Well Head

Development and Critical Evaluation of New Methods for Determination of Methylmercury and Butyltin Species in Marine Tissues and Sediment

M.M. Schantz, S.A. Wise (839), and S. Tutschku (Universitat Leipzig, Germany)

An improved method was developed for the determination of methylmercury and butyltin compounds in marine sediment and tissue using microwave-assisted acid extraction or digestion and solid phase microextraction (SPME) followed by analysis using gas chromatography with microwave induced plasma atomic emission spectrometric detection (GC-MIP-AES). Using the SPME-GC-MIP-AES method, enrichment factors for methylmercury and butyltin compounds of 50 to 100 were achieved compared to the typical hexane extraction, and measurements in marine tissue and sediment matrices were possible at 1 :g/kg to 2 :g/kg (methylmercury) and 10 ng/kg to 100 ng/kg (butyltins). The SPME-GC-MIP-AES method was validated using several marine sediment and tissue matrix Certified Reference Materials (CRMs) with certified values for methylmercury and butyltin compounds. The SPME-GC-MIP-AES method was used to measure methylmercury in four marine tissue SRMs ranging from oyster tissue at (13.0 ∇ 1.0) :g/kg to fish tissue at (397 ∇ 13) :g/kg as Hg dry mass. Results from the SPME-GC-MIP-AES method were used in conjunction with results from NIST's instrumental neutron activation analysis (INAA) as well as results from two other laboratories using different analytical techniques to assign certified values for methylmercury in oyster, mussel, and fish tissue CRMs. Mono-, di-, and tributyltin were measured in three sediment SRMs at concentration levels of (0.08 ∇ 0.03) mg/kg to (0.35 ∇ 0.05) mg/kg as Sn dry mass.

The importance of the determination of organometal species, such as methylmercury and tributyltin, is now widely recognized because of the high toxicity to humans and biota exhibited by these compounds. SRMs with values assigned for organo-metals are required to address these measurement needs.

A suite of five marine tissue SRMs is now available with certified methylmercury concentrations ranging from (13.2 ∇ 0.7) :g/kg dry mass to (1380 ∇ 18) :g/kg dry mass. From the lowest certified concentration for any tissue CRM to the fish tissue SRM with a concentration just below an action level of 500 :g/kg wet mass, this suite of SRMs will provide the tools necessary to improve the accuracy and reliability of methylmercury measurements in the marine environment.

NEW SRMS AVAILABLE:

SRM 1566b	Oyster Tissue
SRM 2977	Mussel Tissue
SRM 1946	Lake Superior Fish Tissue

The SPME-GC-MIP-AES method for methylmercury will be implemented for other candidate marine tissue SRMs including SRM 1974b Organics in Mussel Tissue and SRM 1947 Lake Michigan Fish Tissue. The method for butyltins will also be used to provide values for marine tissue and sediment SRMs.

Measurement Quality Assurance Programs for Contaminants in Environmental Samples
M.M. Schantz, J.R. Kucklick, D.L. Poster, S.J. Christopher, and S.A. Wise (839)

Environmental monitoring programs sponsored by the National Oceanic and Atmospheric Administration (NOAA) and the Environmental Protection Agency (EPA) rely on contaminant measurements performed by numerous laboratories and therefore need to assess the quality and comparability of the analytical measurements from the various laboratories. CSTL's Analytical Chemistry Division currently coordinates three quality assurance programs for contaminants in environmental samples.

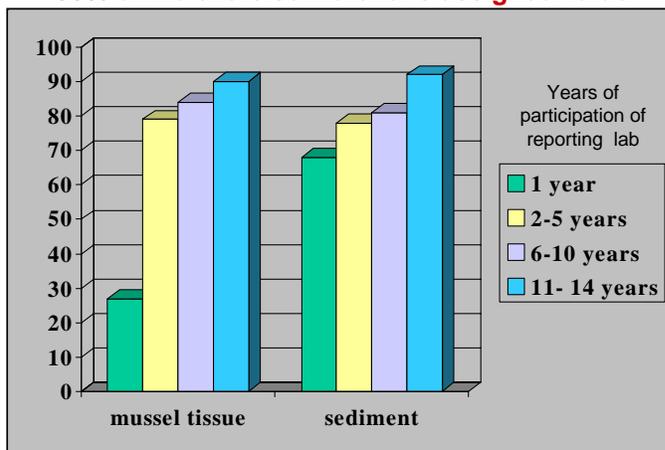
Laboratory measurement accuracy has improved by participation in NIST's QA exercises, resulting in sound environmental decision-making based on analytical measurements of assessed quality and comparability. Participation in these exercises is often a requirement for laboratories involved in federal and state government monitoring programs.

support in the early 1990's with limited interlaboratory comparison exercises; however, in 1999 the program was formalized with annual intercomparison exercises for both organic and inorganic contaminants with over 40 laboratories participating. The *NIST/EPA Intercomparison Program for Organic Contaminants in PM_{2.5} Air Particulate Matter* started in 2000 within the Particulate Matter (PM) 2.5 Organic Speciation Working Group sponsored by EPA and has currently has 25 laboratories participating in two interlaboratory studies for organic constituents in air particulate matter. Through each of these programs, NIST provides mechanisms for assessing the interlaboratory and temporal comparability of data and for improving measurements for the monitoring of organic contaminants in bivalve, fish, and marine mammal tissues; sediment; and air particulate samples and monitoring trace elements in marine mammal tissues.

SRM 1941b Organics in Marine Sediment, SRM 1946 Lake Superior Fish Tissue, and SRM 1974b Organics in Mussel Tissue were all used in QA exercises in recent years and the consensus results from the study were used in assigning the certified concentrations for various contaminants.

The *NIST Intercomparison Program for Organic Contaminants in the Marine Environment* started in 1987 with partial support until 1999 from NOAA and the EPA. Since 1999 this program has continued on a pay-to-participate basis with over 30 laboratories participating in the annual intercomparison exercises for organic contaminants in sediment and marine tissues. The *NIST/NOAA Intercomparison Exercise for Organochlorines and Trace Elements in Marine Mammal Tissues* was established with NOAA

Percent of participant-reported analyte results within 50% of the exercise material's assigned value



Improvement in the accuracy of a lab's measurement capabilities has been shown to correlate with the number of years of participation.

Currently over 75 laboratories participate in these three QA programs for environmental contaminant measurements. Because candidate SRMs are often used as the unknown materials in these exercises, the results are often used in conjunction with NIST measurements as part of the value assignment process for the SRM.

Structure, Adsorptive Separations, and Characterization of Surfactant/Clay Complexes
T.J. Bruno and K.E. Miller (838); and F. Tsvetkov (Daren Laboratories, Israel)

Clays are used in a broad array of applications ranging from pollution prevention and remediation, enhanced oil recovery, the treatment of petroleum liquids, the manufacture of cosmetics and pharmaceuticals, and the synthesis of polymer nanocomposite materials. They are also a critical part of the land environment, and as such, must be considered in any pollutant-soil interaction. An understanding of clay-organic chemical interactions and the effects these interactions have on the structure of clay complexes is a critical issue for future developments in the environment and in clay applications. In order to understand the physical chemistry (both thermodynamics and kinetics) of the clay-solute interaction, we have advanced the application of physicochemical gas chromatography by devising stable clay- and organoclay-coated capillary columns. The capillary column approach we have developed is more efficient, requires lower temperatures, and produces enthalpy of adsorption (ΔH_{ads}) results with a much lower uncertainty than the conventional techniques.

CSTL researchers exploit the unique characteristics of clay platelets in order to produce organic-inorganic composite materials with revolutionary material properties, and to develop of novel chemical separation. They have also used clays as a platform to study the pollutant-soil interaction.

We have completed the production and characterization of the first successful organo-clay coatings for capillary column gas chromatography. We used them to measure the enthalpy of adsorption (ΔH_{ads}) for a family of hydrocarbons on a synthetic layered silicate complexed with cetyltrimethylammonium bromide (CTAB), and also on a synthetic layered silicate coated with octadecane. These substrates are especially significant as surrogate soil systems that can be used to model the interaction of pollutants on soils. In this work, we demonstrated that the ΔH_{ads} is uniformly lower for organoclay than it is for the pure clay surface, and concluded that the organic component "ties up" the most active sites on the clay. As an application of the work with clay and organoclay substrates, we studied the ΔH_{ads} of fuel gas odorants on soil surrogates. This

An immediate impact of the NIST work has been the recognition of differential adsorption of the various fuel gas odorants. The (very common) presence of clay in a pipeline or the leak of a pipe up through a clay soil can have devastating consequences. Our results will allow avoidance of unfavorable odorants, and thereby improve safety.

was motivated by the observation of odorant fading in fuel gas, a suspected cause of which is soil adsorption. We have derived the ΔH_{ads} difference ($\Delta^* \Delta H_{ads}$) between fuel gas components and their respective odorants, and have used this value to predict fading. Our values of ΔH_{ads} complement breakthrough values that have been measured on real soil samples. A significant result is the observation of a high ΔH_{ads} for tetrahydrothiophene (THT), and a relatively low ΔH_{ads} for tertiary-butyl mercaptan (TBM). We extended the work to hydrated clay surfaces and found that the ΔH_{ads} is reduced for thiol odorants, an observation confirmed by breakthrough experiments.

Molecular Simulation of Solute Diffusion (Sodium Chloride) in Supercritical Steam
A.H. Harvey, and R.D. Mountain (838); and J.C. Bellows (Siemens Westinghouse)

A large fraction of electric power is generated in facilities that employ steam turbines, either in the primary power cycle or as a secondary heat-recovery cycle following a combustion turbine. Essentially no experimental measurements exist for the diffusivity of solutes in supercritical steam, despite the importance of these data for understanding deposition in steam turbines and for design of supercritical water oxidation processes. In this work, molecular dynamics simulation was used to calculate the diffusion coefficient of the sodium chloride ion pair at infinite dilution in supercritical steam. Potentials from the literature were used; a polarizable



**Steam Turbine Power Generator
Redding Electric Utility**

CSTL researchers provide the first reliable data for the diffusion coefficient of a solute in steam at turbine conditions. Industrial water treatment experts have estimated that costs to the electric power generation industry of efforts to prevent and repair damage due to these impurities amount to hundreds of millions of dollars per year.

model for the water molecule was chosen to accurately represent water in the vapor phase. The results of the simulations are estimated to be accurate to within 20%, which is a great improvement over current semiempirical estimation methods. The results can be fitted satisfactorily to the form of a simple kinetic-theory expression, allowing extrapolation to conditions other than those simulated. The results from this work now provide the best values available for the diffusivity of sodium chloride in steam.

Our approach is to calculate the diffusivity directly from molecular dynamics simulation, which provides rigorous results if the intermolecular potentials are sufficiently accurate. Good potentials (incorporating polarizability, which is needed for accurate description of water in the vapor phase) are available for water with sodium chloride. Advances in computing power have brought this calculation into the range of feasibility. Collaborations with the CSTL's Computational Chemistry Group are planned to calculate reliable thermochemical data for hydrated silica species and to determine which species will need to be incorporated in our simulations.

This project is one of a series aimed at evaluating the utility of computer simulation methods for problems for which experimental data are not available.

Kinetics and Thermophysical Properties of Ionic Liquid Systems

P. Neta, J.W. Magee, M.L. Frenkel, A. Laesecke, M.O. McLinden, and R.A. Perkins (838); J. Grodkowski (Institute of Nuclear Chemistry and Technology, Warsaw); and G. Kabo (Belarusian State Univ., Belarus)

Ionic liquids are a class of organic salts that are liquid at or near room temperature. They are generally composed of a large asymmetric organic cation and either an organic or inorganic anion. As such, they are nonvolatile and nonflammable. Therefore, they have been proposed as solvents for "Green Processing." The physical properties of ionic liquids may be tuned, for example, by altering the hydrophobicity, and this affects the solubility, reaction rate, and selectivity. However, in spite of the many advantages that these fluids are predicted to offer, fundamental data on their physical and chemical properties are scarce. A physical properties database is being developed at NIST that will provide industry with access to evaluated physical

property data, validated models to interpolate the available measurements, and structure-property tools to predict physical properties not yet measured. Measurements of physical property data are being collected for properties including melting point, density, electrical conductivity, refractive index, viscosity, heat capacity and surface tension. The focus of this data collection is on ionic liquid compounds that are liquid-phase at room temperature and are air- and moisture-stable, since they will have many practical applications.

Ionic liquids, a class of organic salts that are liquid at room temperature, have been proposed as solvents for “Green Processing.” To provide U.S. industry with the knowledge base to exploit these solvents, CSTL researchers have begun to study the physical properties of ionic liquids and their effects on rate constants of fundamental chemical reactions.

A preliminary study has shown that the choice of either the cation or the anion strongly influences the physical properties of the ionic liquid.

Though all possible sources are under consideration to uncover both published and unpublished measurements, it is becoming clear that measurements are limited for most physical properties and are nearly nonexistent for vapor pressure or thermal conductivity. A single compound, 1-butyl-3-methylimidazolium hexafluorophosphate, has been systematically studied, but not its vapor pressure or thermal conductivity. We have initiated experimental measurements with a focus on five physical properties for six high-purity compounds – density, heat capacity, vapor pressure, viscosity, and thermal conductivity. In our labs, we are measuring densities with a pycnometer, viscosities with Ubbelohde-type capillary viscometers, and thermal conductivities with a transient hot-wire apparatus.

The pulse radiolysis technique was utilized to measure rate constants for several oxidation, reduction, addition, and hydrogen abstraction reactions in ionic liquids. In addition to the higher viscosity, significantly lower rate constants for redox reactions indicate that the ionic liquids behave as solvents of low polarity, suggesting a high degree of ion-association. Certain reduction and oxidation reactions are found to behave very differently in ionic liquids as compared with other solvents, both in the rate of the reaction and sometimes even in the direction in which the electron is transferred. These strong effects were found to be due to differences in the energy of solvation of the reactants and products in the various solvents, which affect the driving force of the reaction and thus its rate constant. In the future, the use of ionic liquids for the preparation and stabilization of colloidal metal nanoparticles will be explored.

In most cases the rate constants in ionic liquids are lower than those in classical solvents, in part because of the higher viscosities of ionic liquids, which limits the diffusion rate.

Hydrogen abstraction and addition reactions, in general, are only slightly slower in ionic liquids compared with water and organic solvents.

7. Food and Nutrition



CSTL supports the food industry by providing reference measurements and reference standards for quality assurance and to help ensure compliance with the nutritional labeling regulations. CSTL provides robust metrological traceability for nutrients in food

The U.S. processed food and beverage industry is a major participant in the global market. Almost half of the world's top 50 food processing firms are headquartered in the U.S.

products, contaminants and adulterants in food products, chemical composition and contaminants in dietary supplements/nutraceuticals, and detection of genetic modifications in food products.

Completion of Suite of SRMs to Support Nutritional Labeling Issues

K.E. Sharpless, J.M. Brown Thomas, S.A. Margolis, B.C. Nelson, C.S. Phinney, J.R. Sieber, and L.J. Wood (839)

Nutrition Facts	
Serving Size 1/2 cup (114g)	
Servings Per Container 4	
Amount Per Serving	
Calories 90	Calories from Fat 30
% Daily Values*	
Total Fat 3g	5%
Saturated Fat 0g	0%
Cholesterol 0mg	0%
Sodium 300mg	10%
Total Carbohydrate 13g	4%
Dietary Fiber 3g	10%
Sugars 3g	
Protein 3g	
Vitamin A 80%	Vitamin C 60%
Calcium 4%	Iron 4%

CSTL's Analytical Chemistry Division is working in several areas within the broad category of "food and nutrition" to facilitate compliance with nutritional and dietary supplement labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices. The

CSTL/NIST develops reference materials and analytical methods to:

- **underpin measurements made for compliance with nutritional and dietary supplement labeling laws**
- **provide traceability for food exports needed for acceptance in many foreign markets**
- **improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices.**

composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed; a fat-protein-carbohydrate food composition model developed by AOAC International is a useful way to classify the food matrix. NIST has been working with other federal agencies and the food industry over the past several years to provide a suite of SRMs within this model to address nutrition-labeling issues.

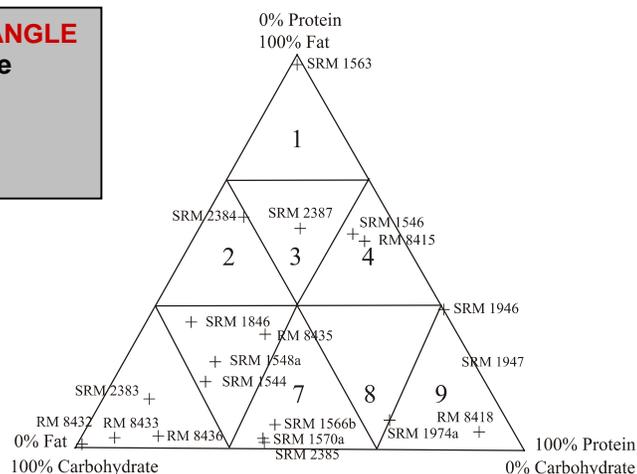
SRM[®]



NEW SRMS COMPLETE AOAC TRIANGLE

SRM 1946 Lake Superior Fish Tissue
SRM 2384 Baking Chocolate
SRM 2385 Spinach
SRM 2387 Peanut Butter

Their positions in the AOAC triangle, along with those of the other food-matrix reference materials available from NIST, are shown. This particular aspect of NIST's food and nutrition program can be expected to decrease. Focus will then shift to more specialized analytes (e.g., individual amino acids, allergens, mycotoxins) in foods, as well as to dietary supplements.



Reference Methods and Materials for the Chemical Characterization of Dietary Supplements

S.A. Wise, K.W. Phinney, L.C. Sander, and K.E. Sharpless (839)

Taxonomically authentic botanical reference materials with assigned values for active and/or marker compounds are needed for quality assurance of analytical measurements associated with the manufacturing process and for the verification of manufacturers' label claims for the dietary supplement industry. Assigned values for contaminants and adulterants are also necessary to address public health and safety concerns. In 2001, NIST, the Food and Drug Administration (FDA), and the National Institutes of Health's Office of Dietary Supplements (NIH-ODS) initiated a multi-year program to develop SRMs and analytical methods for a number of botanical and botanical-containing dietary supplements.

More than 50% of the U.S. population uses dietary supplements, accounting for roughly \$10 billion in sales every year. A variety of botanical-containing products are marketed as dietary supplements; common examples include St. John's wort, ephedra, and ginkgo.

The goal of this collaborative program is to provide SRMs for eight to ten different botanical dietary supplements over a six-year period. Ephedra-containing materials were identified as the highest priority by FDA and NIH-ODS, and we are currently in the process of producing a suite of five ephedra-related SRMs to address this need. We anticipate that the ephedra SRM suite will be completed by September 2003.



FDA and NIH-ODS identified ephedra-containing materials as the first priority for development of dietary supplement SRMs. A suite of materials is being produced, consisting of authentic ephedra plant material, two extracts of the plant material, and two finished products. These SRMs will be characterized for the ephedrine alkaloids, as well as for contaminants (Hg, Cd, Pb, As, and pesticides).

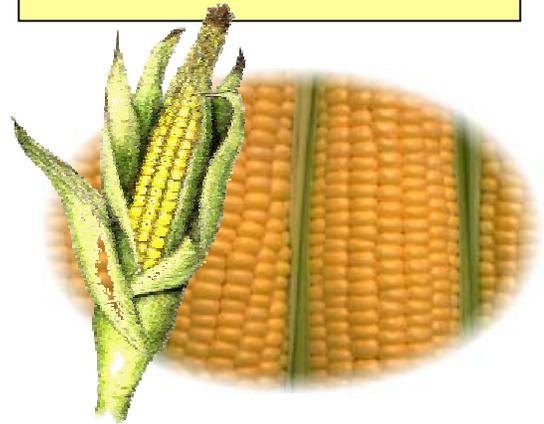
It is necessary to provide SRMs and methods that address two needs in the dietary supplements area: safety and efficacy. Potential health risks may result from contamination (e.g., pesticides, heavy metals), adulteration (presence of unlabeled foreign materials including pharmaceuticals), or variability in product composition (e.g., changes in levels of active constituents). Secondly, product quality and consistency must be maintained, through verification of dietary supplement label claims. These needs can be addressed by the development of analytical methods and reference materials to support chemical measurements for dietary supplements.



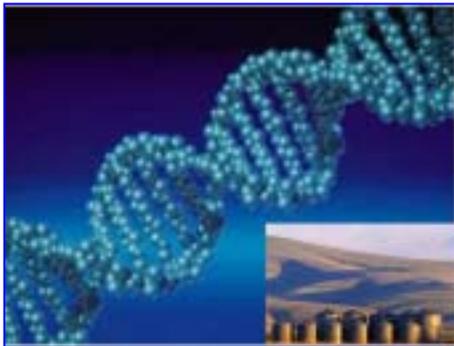
Evaluation of Extraction Methodologies for Corn Kernel (*Zea mays*) DNA for Detection of Trace Amounts of Biotechnology-Derived DNA
M.J. Holden and J.R. Blasic Jr. (831)

Testing for the identification of biotechnology-derived plant material is currently required for compliance with labeling regulations implemented by countries other than the United States. European Union regulations mandate labeling of plant material and food as “genetically modified” if the material is present at a level of 1 % by weight. As a result, testing is important for trade in U.S. agricultural products, especially for the export of two commodity crops, soybeans and corn. Testing involves detection of the specific DNA that defines the modification in the plant genome. The “needle in a haystack” is relevant imagery. The polymerase chain reaction, or PCR, is a powerful tool to detect the target DNA or “needle” by synthesizing many copies of the DNA. Specific and consistent detection is required for accurate identification of a particular genetic modification in a plant sample, especially when the modification is present in trace amounts.

CSTL researchers investigate the quality of genomic DNA, isolated using protocols based on some common principles (binding to matrix or selective precipitation), as material for testing for the presence of biotechnology-derived corn



The first important requirement for success with PCR is the isolation of DNA of high quality and in sufficient quantity to be representative of the sample of plant material. In this study, four investigators in two laboratories (NIST and USDA) evaluated six different methodologies. The plant material from which DNA was isolated consisted of ground corn kernels that contained 0.1



% by weight of biotechnology-derived corn of a specific type known as StarLink. DNA was extracted using five commercial kits plus a published protocol, a variation of a method known as CTAB / NaCl. The particle size of the corn flour used for the DNA isolations was evaluated along with the quality and efficiency of extraction of the DNA from ground corn. The isolated DNA was then tested in three different PCR assays to ascertain the amplifiability of the target DNA. The PCR assays resulted in some “false negative” results, that were most likely due to inhibitors of the PCR process that co-purified with the DNA.

The study highlights several aspects that should be considered when choosing a method for DNA extraction. The sample from which DNA is extracted must be representative of the material from which it is taken. Important parameters in this case include sufficient mass and fineness of the grinding plus the extraction efficiency of the method. Many individual samples must be tested for quality and amplifiability to evaluate a particular method. While several, but not all, methods performed reasonably well, the study showed that additional purification steps are necessary to accurately and consistently detect trace amounts of target DNA. We demonstrated that a commonly

The results of this study are of practical importance for testing laboratories of all kinds that have a mission to identify genetic components of grain. Accurate identification is critical to U.S. international trade in commodity crops.

used criterion for verifying the quality of isolated genomic DNA for PCR, amplification of an endogenous gene (present in all corn DNA), is not sufficient and can lead to false negative results. Inhibitors of the PCR process, that is corn kernel components that are isolated along with the genomic DNA, are likely culprits in false negative results. Our investigations show that acidic polysaccharides act to inhibit the PCR reaction, while starch, a major polysaccharide component of corn kernels, does not. The identification of specific inhibitors and their mode of interference in DNA amplification process should be addressed. The effect of inhibitors on the quantitation of DNA and the efficacy of various processes for removal of inhibitors will be investigated.

Similar studies on DNA extraction should be conducted on relevant plant materials with different compositional analysis. It would be of value because different problems would be encountered. An example is soybean, which contains significant lipid content, not found in corn.

Reference Methods and Standards for Transgenic Proteins in Biotech Foods
D.M. Bunk (839)

There are currently no reference methods for the detection of transgenic proteins in biotech foods. Therefore the reference materials that are now being used have not been validated by methods other than field methods (i.e., immunoassays). Because foreign trade barriers exist for grains based on the amount of biotech grains present, improving the accuracy of field methods for transgenic protein detection could have a substantial impact on international grain sales.

CSTL develops analytical methods based on chromatography and mass spectrometry to detect transgenic proteins without the use of antibodies and immunoassays.



The immunoassays and lateral flow test strips that are currently used in the field method for the measurement of transgenic protein in biotechnology food products rely on antibodies to detect the protein expressed as the result of the specific genetic modification. The use of antibodies imparts high selectivity and sensitivity to protein-based measurements, in addition to making them rapid, robust, and relatively low-cost. However, the highly selective binding of the antibody to the protein antigen can also be problematic. If structural changes in the antigen occur, such as post-translational modifications or degradation occurring during sample preparation, antibody/antigen binding can be diminished, affecting assay results. Measurement procedures that do not rely upon antibody selectivity, must be developed to validate field methods and for the eventual certification of reference materials.

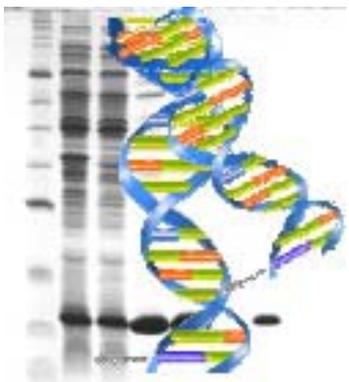


As a starting point to the development of a reference method, a proteomic approach was explored for these measurements. Without the use of antibodies, the transgenic protein in glyphosate-tolerant soy and corn, CP4 EPSP synthase, was detected in crude protein extracts by mass spectrometry after tryptic digestion of the protein extract and multi-dimensional chromatographic separations.

The results of this work demonstrate that transgenic proteins could be detected without the use of antibodies and immunoassays, and provide the groundwork for the development of quantitative reference methods based on chromatography and MS for transgenic proteins in biotech foods.

As a starting point to the development of a reference method, a proteomic approach was explored for these measurements. Without the use of antibodies, the transgenic protein in glyphosate-tolerant soy and corn, CP4 EPSP synthase, was detected in crude protein extracts by mass spectrometry after tryptic digestion of the protein extract and multi-dimensional chromatographic separations.

8. Forensics and Homeland Security



CSTL has provided DNA standards for human identification since 1992; however, over the past year the effort has focused on new methods of analysis to respond to the need to analyze extremely degraded samples. Much of this was stimulated by the need to identify victims from the World Trade Center collapse. CSTL has also had a long history of assessing standards needs and developing new measurement technologies for crime scene investigations in which handguns or explosives are used. Reference standards are used by the forensic community both for quality assurance and to provide legally defensible analyses. CSTL is also active in the development



of microsensor arrays and other measurement technologies for the identification of explosives, as well as chemical and biochemical weapons. CBRNE (Chemical, Biological, Radiation, Nuclear, Explosives) related measurements, methods, and standards represent a major new area of expansion for CSTL.

Y Chromosome Assays and Standards Support Commercial Technologies

J.M. Butler, R. Schoske, P.M. Vallone, J.W. Redman, and M.C. Kline (831)

Y chromosome short tandem repeat (STR) and single nucleotide polymorphism (SNP) markers have a number of applications in human identity testing including typing the perpetrator of sexual assault cases without differential extraction and tracing paternal lineages for missing persons investigations. In order for Y STR systems to become more widely accepted within the forensic DNA typing community, robust multiplex assays are required that will permit collection of information from many sites along the Y chromosome from a very minute amount

New Y chromosome assays will permit more rapid collection of information on the variability of Y STR and Y SNP markers in human populations. A Standard Reference Material (SRM 2395) is under development to aid in reliability of measuring Y chromosome DNA typing results.

of template DNA. We have focused on the design and development of new Y STR multiplexes as well as evaluating various markers in the same reference set of DNA samples.

Commercial Testing Kits:

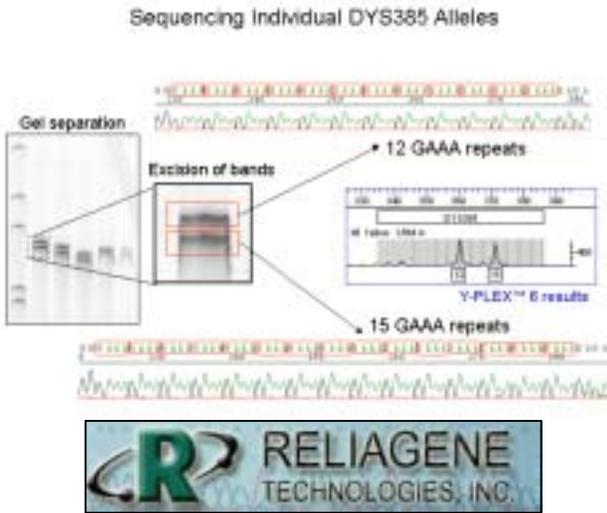
Promega Corporation is developing a 12plex Y STR kit that is a subset of the markers contained within the published NIST Y STR 20plex. Promega is using many of our primers and building upon the information gained in our multiplex development. Orchid Cellmark has adopted the NIST Y STR 10plex and completed population studies and forensic validation with this multiplex assay. Orchid plans to market their kit to members of the forensic DNA



typing community. Both companies are looking to NIST SRM 2395 Human Y Chromosome Standard to define their allele nomenclatures as is ReliaGene Technologies Inc. that currently has the only commercial Y STR kits available. In addition, genetic genealogy companies such as Relative Genetics and FamilyTree DNA are using our 20plex or portions of it in their genetic data collection. For Y SNP assays, we have beta-tested the

NIST is a member of the FBI's Scientific Working Group on DNA Analysis Methods Y chromosome subcommittee and is helping to define the core set of Y STR and Y SNP markers that will be used in future national and international DNA databases.

Signet Y SNP Identification System from Marligen Biosciences, Inc. and provided them with data on the usefulness of their DNA markers.



The new NIST SRM 2395 Y Chromosome, contains extracted DNA from five unrelated and anonymous male individuals with sequence information from over 20 different and commonly used Y STR markers.

Separation and sequencing of alleles for the Y STR marker DYS385, which is duplicated on the Y chromosome gives rise to two bands that must be separated prior to sequencing. The number of GAAA repeats observed in the prototype SRM 2395 samples confirms allele calls by the commercial kit tested.

Developing and Evaluating New Forensic Tests for Probing the Mitochondrial Genome
P.M. Vallone, M.C. Kline, and J.M. Butler (831)

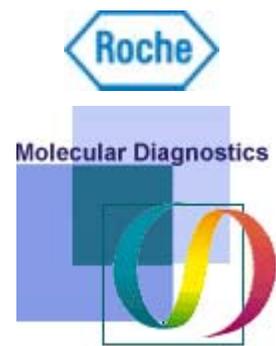
The mitochondrial genome consists of ≈16,569 base pairs. Mitochondrial DNA is maternally inherited and can be employed in forensic investigations. The mitochondrial genome is much smaller than the nuclear human genome (3 billion base pairs). However, thousands (1000 to 5000) of copies of the mitochondrial genome are present per cell versus 2 copies of nuclear DNA. Due to the large number of mitochondrial genomes per cell it is often easier to type samples exposed to harsh environmental conditions i.e. when it is impossible to type the nuclear DNA.

CSTL researchers develop novel multiplex SNP assays for increasing the power of mitochondrial DNA for human identifications purposes; and evaluating “mito-strip” linear arrays from Roche Molecular Systems for rapid determination of HV1 and HV2 haplogroups.

A stretch of ≈ 1100 base pairs in the control region of the mitochondrial genome is commonly analyzed for forensic purposes. The control region is highly polymorphic and is typically analyzed by DNA sequencing methods. Recently a linear array system from Roche Molecular Systems has been developed for rapid determination of common polymorphisms in the control region (specifically HV1 and HV2). Our laboratory was asked to participate in the beta testing of these “mito-strips”.

This work serves to enhance the capability/capacity of mitochondrial DNA in human identification testing. In many cases (such as mass disasters) mitochondrial DNA is the only means to identify an individual.

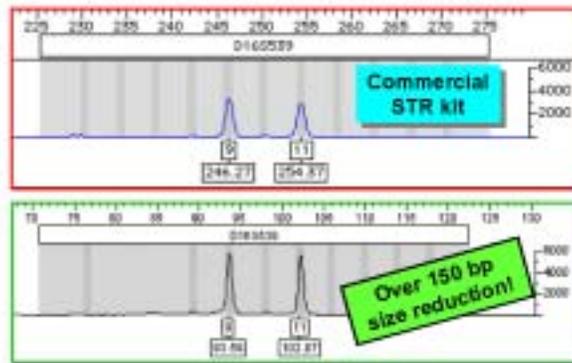
These linear arrays offer forensic DNA laboratories a rapid test for screening samples and excluding ones that do not match prior to the labor-intensive effort of full sequencing.



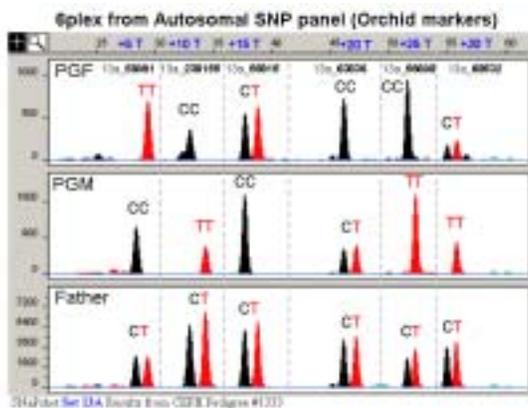
Our original miniSTR assays were developed at the request of Dr. Robert Shaler, the head of the head of Forensic Biology section at the Office of the Chief Medical Examiner of New York (NYC-OCME) who is leading the WTC DNA identification effort. A miniSTR assay dubbed "Big Mini" was provided to the NYC-OCME in January 2002. Based on the groundwork laid with our miniSTRs, the Bode Technology Group has developed commercial miniSTR assays that are being used to enhance the recovery of STR genotypes from WTC samples. Information from over 20,000 bones and tissue fragments is being analyzed to help make the DNA identifications of the WTC victims.



Development of miniSTRs to Aid Testing of Degraded DNA



Comparison of allele calls at the STR marker D16S539. In both cases, a 9-11 genotype was obtained demonstrating concordance between the two assays. The new miniSTR test reduces the overall PCR product size by >150 bp.



NIST has also assisted in the validation of new genetic markers that are being evaluated for their usefulness in human identification. Orchid Cellmark has developed a panel of 70 autosomal SNP markers with PCR product sizes in the range of 60 bp to 80 bp that can produce results on degraded DNA samples. In a matter of a few weeks, the NIST team developed new assays with the commercial markers. Over 500 SNP calls were evaluated and found to be fully concordant between the two different assays.

Results obtained from a multiplex SNP detection assay developed at NIST to evaluate the allele calls produced by the commercial SNP markers. A paternal grandfather (PGF), paternal grandmother (PGM), and father of a family reference set were used to demonstrate expected genetic inheritance with these 6 SNP markers. The father is heterozygous at all 6 loci shown here. SNPs are being considered for use in WTC samples due to their ability to be amplified from degraded DNA as a very small PCR product (60 bp to 80 bp).

Standardization of Sample Preparation Techniques Used in Analyzing Microorganisms by MALDI-TOF Mass Spectrometry

C. Nelson, B.J. Porter, M.J. Welch, B.C. Nelson, E. White V (839); and W. G. Mallard (838)



The rapid and reliable detection and identification of microorganisms has become a crucial issue for homeland security. Current CSTL efforts, supported by funding from the Defense Threat Reduction Agency (DTRA), are focused on standardization of methods for identification of microorganisms using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS). Approaches for sample preparation and data interpretation of spectra obtained using this technique must be critically evaluated to provide further database input and search

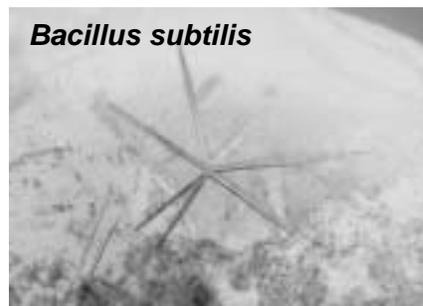
algorithm development. Essentially, proteins expressed by bacteria, both intracellularly and on the bacteria surface, can produce characteristic mass fingerprints useful in distinguishing between organisms, even at the strain level. Slight variations in sample handling have been observed to drastically affect the resulting mass spectra for both Gram negative and Gram positive bacteria, hence the need for determining which techniques

CSTL researchers standardize bacterial sample preparation and analysis by MALDI-TOF MS to facilitate algorithmic development of library searches to assist in the identification of microorganisms.

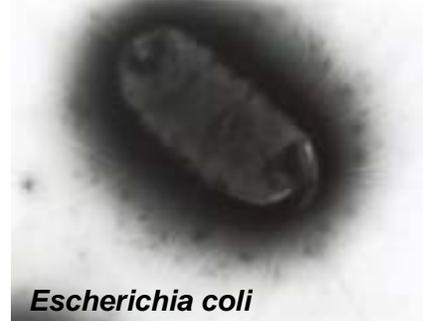


provide the best and most reproducible results. Interlaboratory comparisons of mass spectral data obtained on different instruments showed that common ions could be reproducibly observed when a standard protocol was followed. Standardization of bacterial sample preparation and analysis by MALDI-TOF MS, along with algorithmic development for library searches, will not only aid in the identification of bioterrorism agents for homeland security, but has the potential to impact other areas (e.g., biological weapons treaty violations, environmental, biomedical research, clinical, food industry).

Matrix selection was observed to have the greatest impact on MALDI-TOF mass spectral patterns of the lyophilized *Escherichia coli* (Gram negative) and *Bacillus subtilis* (Gram positive) samples tested. Disruption of lyophilized *E. coli* cells by ultrasonication or extraction of proteins with certain solvents produced mass spectra that were very similar to whole cell analysis, with the exception of several higher molecular weight ions between 20 kDa to 40 kDa present in the former. Data collected on different samples of *E. coli* indicated the presence of common biomarkers among the strains tested, as well as markers that may be specific to each strain. Comparisons of the spectral patterns of two preparations of a single strain of *B. subtilis* from two different sources indicated the presence of many common ions, which may be used to fingerprint the organism, regardless of its origin. An interlaboratory comparison study provided evidence to support the feasibility of MALDI-TOF MS as a rapid technique for bacterial identification in field applications when different instruments are used. Although different laboratory conditions and instruments were used, common ions were observed reproducibly across the laboratories when a standard protocol was followed.

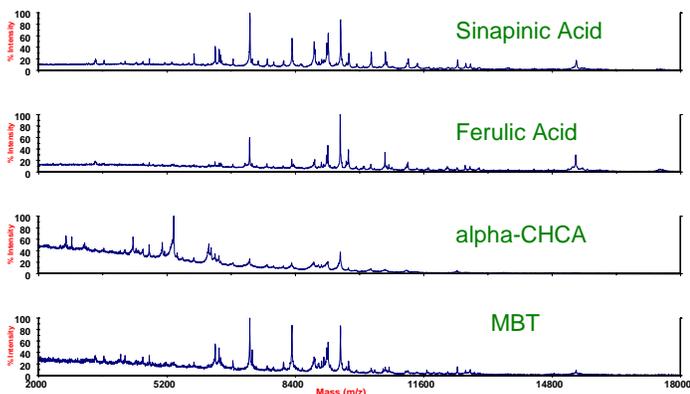


Bacillus subtilis



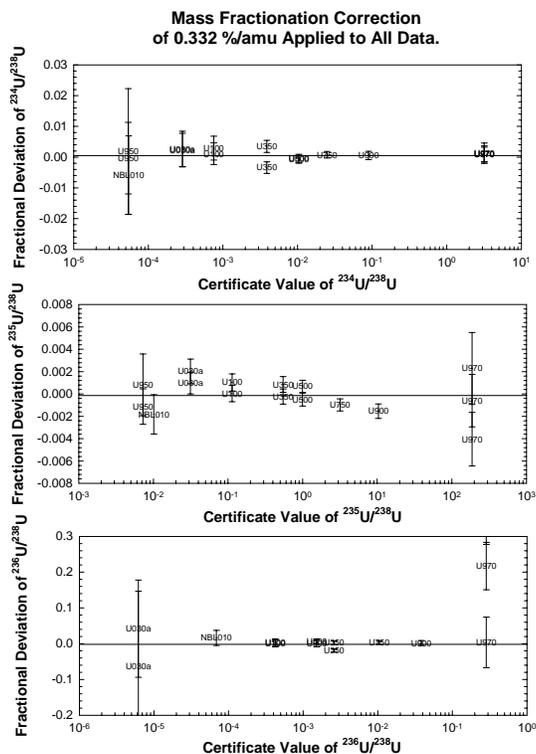
Escherichia coli

With the support of the Defense Threat Reduction Agency (DTRA) Chemical/Biological Arms Control Program, this project is expected to continue for the next 2 to 3 years. NIST will continue to examine the various experimental parameters affecting the spectra of test microorganisms, e.g., matrix, wavelength and intensity of the laser, and the nature of the sample (ionic strength, growth media, etc). Variability due to small changes in the protocol will be examined to determine how exacting the specifications of the standard operating procedure must be. A specific set of standard conditions that produce reproducible spectra under the widest set of normal instrument variations will be determined. Standard operating procedures for the production of spectra will be developed and tested in interlaboratory round robin studies with the goal to create a library of MALDI-TOF MS for a wide variety of microorganisms.



MALDI-TOF mass spectra of whole cell *E. coli* ATCC 9637 (strain W) using four different matrixes: sinapinic acid, ferulic acid, alpha-cyano-4-hydroxycinnamic acid (alpha-CHCA), 2-mercaptobenzothiazole (MBT).

Precision Isotopic Measurements with the NIST High Performance Secondary Ion Mass Spectrometry Facility
A.J. Fahey (837)



Deviation in $^{235}\text{U}/^{238}\text{U}$ isotope ratios from certified values.

The specific isotopic ratios of an element in a sample can, in some cases, be used to determine the history of a sample, its age, or its place of origin. In order for isotopic ratio data to be useful it often must be determined to a precision and accuracy of less than 1 part per thousand. To this end we have characterized the new NIST ims-1270 secondary ion mass spectrometer and its subsystems for high-precision measurements of isotopic ratios.

CSTL researchers measure isotopic ratios with high precision and accuracy in order to characterize microscopic quantities of materials.

Samples of known chemical and isotopic composition are available in the form of U_3O_8 as Standard Reference Materials. These SRMs are unique in that they span more than 4 decades in their major isotope ratio. In order to characterize the ability of the ims-1270 at NIST to measure uranium isotopic compositions precisely and accurately, a selection of uranium SRMs were measured where the abundance ratio $^{235}\text{U}/^{238}\text{U}$ ranged from ≈ 190 (highly enriched) to ≈ 0.7 (natural). Combined with the minor isotopes of uranium, this scale of variation

allows us to nearly span the dynamic range of the pulse-counting detection system. In addition, it allows us to test the reproducibility of the mass fractionation for isotopic ratio measurements on particles.

The figure shows the deviations of each of the measured isotopic ratios for the available uranium SRM materials as a function of the certificate values for each SRM. The data shown was taken over a period of four months from May to September of 2002. A constant mass fractionation was applied to all of the data. Nearly all of the data points lie within one standard error of the mean for each of the isotopes and SRMs. There are two exceptions to this and the reasons for each are well understood. There is a trend in the $^{235}\text{U}/^{238}\text{U}$ values that is apparently dependent on the isotopic ratio. This is due to the fact that there is a non-linearity in the response of the electron multiplier detector used to count the ions. This effect has been documented by others including the group at New Brunswick labs that currently curates these SRMs. The magnitude of this non-linearity is very small, approximately 4 parts per thousand spanning more than 4 orders of magnitude (that is, $<1 \mu\text{g/g}$). The other significant deviation from the certified value lies in the measurement of ^{236}U for the SRM U970. This isotope of uranium, ^{236}U , is the least abundant isotope in all of the uranium SRMs, and in U970 it is the most difficult to measure because of an unresolvable interference due to ^{235}UH . Although the hydride interference correction can be made reliably for most of the SRMs the U970 is unusual because of its very high abundance of ^{235}U , generating a large ^{235}UH signal, and its low abundance of ^{238}U (the ^{238}UH signal is used to correct for the interference of ^{235}UH at the mass of ^{236}U). However, our data indicate that by using the “cold trap” on the ims-1270 sample chamber, we can reduce the hydride interference enough to make an accurate correction.

The measurements reported here show that the new NIST ims-1270 is capable of high-precision and accurate measurement of U_3O_8 particles. The mass fractionation is stable and reproducible and corrections can be made reliably for hydride interferences. This will aid in our support of the International Atomic Energy Agency (IAEA) for monitoring signatories of the Nuclear Non-Proliferation Treaty.



Microfluidic Whole-Cell Biosensors for Early Detection of Toxins in Water Streams
L.E. Locascio, E.A. Waddell, J.C. Travis (839); and A. Suggs, B.J. Love, and N.G. Love
(Virginia Polytechnic and State Univ.)

This collaborative research effort between the NIST and Virginia Polytechnic and State University involves the development of a cell-based microfluidic biosensor that will be used to detect the presence of electrophilic toxins in water samples. Electrophilic toxins flowing from streams into wastewater treatment plants can have a profound detrimental effect on treatment processes by destroying the bioreactor that is at the heart of the plant. Based on earlier work by Love’s group, it was hypothesized that the presence of electrophilic toxins could be monitored by measuring the potassium efflux from whole cells exposed to this class of toxins. The project involves the immobilization of whole cells, *Escherichia coli* K-12, inside microfluidic channels and measurement of cellular potassium efflux in a microfluidic system using a potassium-sensitive optical sensor embedded in the device. Prototype devices will be evaluated using samples spiked with a range of electrophilic toxic chemicals. Ultimately, the device is targeted for the detection of toxic chemicals in wastewater streams, but may also be used to detect toxins in drinking water supplies.

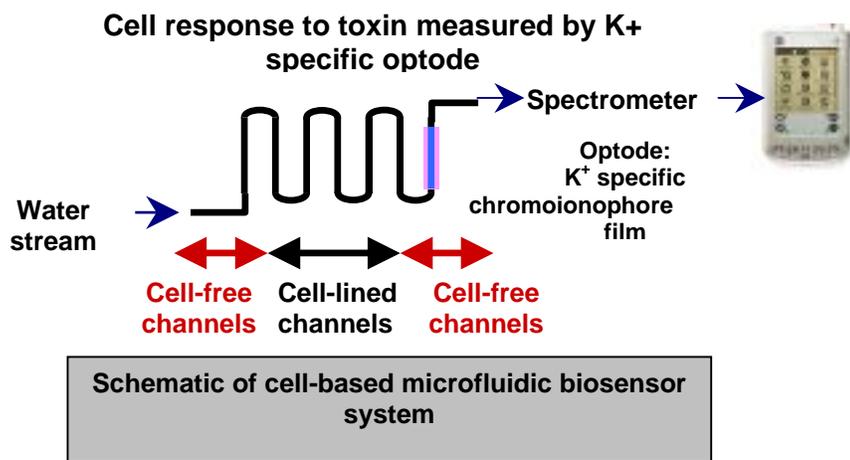
Collaborative research efforts lead to the development of a sensitive and reliable biosensor to detect the presence of electrophilic toxins in wastewater streams.

Different classes of chemicals feeding into water treatment plants have been known to cause bioreactor degradation leading to system processing failures. A recent example of this occurred

earlier this year at the Hagerstown water treatment facility upstream of Washington, DC where millions of gallons of raw sewage were dumped into the Potomac River as a result of chemically induced bioreactor failure.

Electrophilic toxins are one class of chemicals that can impact bioreactor function and cause a chemically induced failure. In this collaborative effort, we have designed a cell-based microfluidic biosensor to detect the presence of electrophilic toxins in water streams entering wastewater treatment plants in an effort to prevent failure of the bioreactor. The approach is to immobilize bacterial cells in a microfluidic system and monitor cell response, in particular potassium efflux, as the water flows through a bed of immobilized cells. In essence, we have created a micro-bioreactor. The behavior of our micro-bioreactor is then used to predict the behavior of the process bioreactor when exposed to the same water supply. A negative response in the microsystem can be used to decide when to divert part of the incoming stream to prevent process failure.

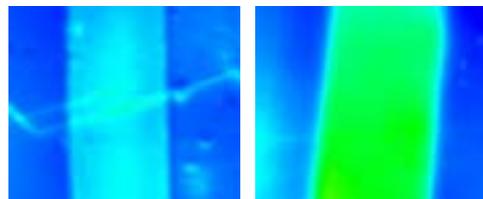
The focus of this collaborative work is the *in-situ* monitoring of environmental toxins that could seriously impact the efficacy of bioreactors used in wastewater treatment processes. A reliable early warning system such as the one here can have a profound environmental and financial impact by detecting the problem before a process upset occurs.



The microfluidic system that we have designed for this work has two main components: (1) a bed of immobilized *E. coli* cells creating the micro-bioreactor; and (2) a reversible potassium sensor. The cells have been immobilized by adsorption directly onto the polymer microchannel surface. It was determined that there was greater binding and greater cell viability if the cells were grown in batch to the late log phase and then seeded in

the microchannel. Cells that were seeded for 4 h to 8 h exhibited a stable biofilm. Overgrowth of the cells in the micro-bioreactor is a concern, however, so we are currently investigating other mechanisms for immobilizing cells to discourage fast growth that could lead to channel occlusion.

The reversible potassium sensor was a polymer film that contained a potassium sensitive ionophore and a chromoionophore that responded to a local pH change with a change in fluorescence intensity. Fluorescence images of the film before and after exposure to potassium ions are shown in the figure. The optode was placed downstream of the immobilized cells and was used to monitor the release of potassium ions from inside the cells in the presence of an electrophilic toxin. Optimization of the optode film and incorporation of appropriate control films are being studied to improve the performance of the device. The goal is to produce a simple and reliable system that can be placed in the field and used continuously.



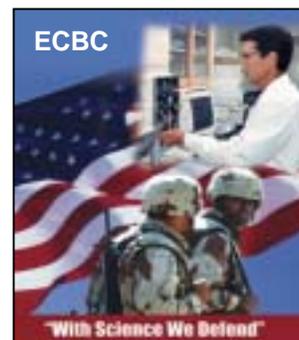
L) Fluorescence image of optode membrane with no K⁺ in solution. R) Image of membrane with 50 mM K⁺ in solution

Chemical Warfare Agent Detection with Microsensor Arrays
C.J. Taylor, R.E. Cavicchi, D.C. Meier, and S. Semancik (836)

MEMS-based (Microelectromechanical System) microsensors were used to detect chemical warfare agents in a project supported by the Defense Threat Reduction Agency (DTRA). The microsensors are based on surface-micromachined 100 μm x 100 μm platforms called microhotplates. The agents are detected by measuring conductance changes in metal oxide thin films deposited on the microhotplates; the sensor response is correlated with the identity and concentration of the chemical agent present.

Microsensors are tuned for specific agents by incorporating metal oxide films of different composition into arrays of sensors, and by selecting different fixed and time-varying temperature programs. Studies were first conducted on simulant agents at NIST to develop appropriate device prototypes for testing the technology on actual sulfur mustard and nerve agents at a surety laboratory.

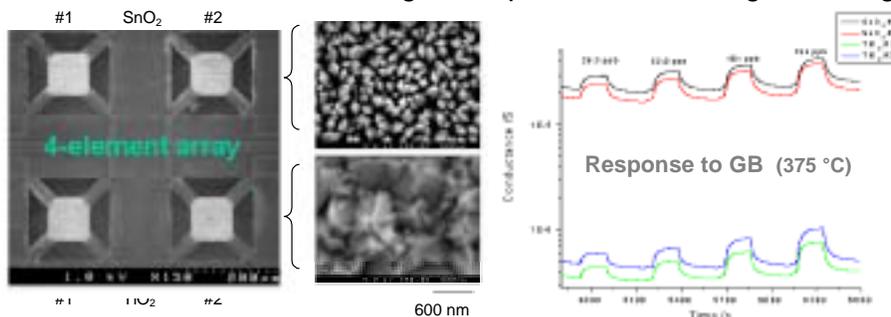
Studies done at NIST and a surety laboratory have established that conductometric microsensors based on oxide sensing films and temperature-controllable MEMS platforms can detect a variety of chemical warfare agents. These types of microsensors may some day be used to warn military personnel and civilians of releases of chemical warfare agents. As part of the work, the sensing performance of 4-element microsensor arrays to the simulant molecules of chloroethyl ethyl sulfide (CEES) - a sulfur mustard simulant, and diisopropyl fluorophosphates (DFP) - a nerve agent simulant, was also evaluated. The simulant experiments were performed to develop sensing materials and operating schemes suited to the classes of compounds used in chemical warfare. The first phase of testing on three agents, sulfur mustard gas (HD) and the nerve agents tabun (GA) and sarin (GB) was completed in experiments conducted at the Edgewood Chemical Biological Center (ECBC).



The NIST microsensor technology offers other desirable features for agent detection including low power consumption, low cost, ease of including redundant devices, and ability to readily integrate on-board electronics for signal conditioning.

Microsensor response signals were generated as changes in resistance (conductance) of thin SnO₂ and TiO₂ films as they were exposed to the agents. The nanocrystalline sensing films were fabricated on microhotplate platforms by chemical vapor deposition, and operated between 20 °C and 480 °C in both fixed- and variable-temperature modes. These tests demonstrated that the devices can detect the agents in the 0.01 μg/g (ppm) to 0.3 μg/g (ppm) range with adequate signal-to-noise. Good stability and reproducibility were observed over 14-hour continuous monitoring runs. Since “LC50”

concentration levels for 10-minute exposures are ≈1 μg/g (ppm) for the agents tested, it is clear that the microsensors are sensitive enough for a practical monitoring technology.



Nano-crystalline tin and titanium oxide sensing films on in a 4-element microhotplate array with conductance response

Verification of Calibration Procedure for Testing Military Gas Masks

R.A. Fletcher, R.L. King, J.A. Small (837); M.R. Winchester (839); J.C. Yang, and G.W. Mulholland (BFRL)

Military personnel wear gas masks for protection against a chemical and biological assault. The recognized potential global threat from chemical and biological agents demands that effective defense measures be taken. An important part of this defense strategy is the testing and verification that assures secure gas mask fits. Currently, the Army determines the gas mask fit quality by measurement and comparison of the ambient aerosol concentration outside of the fitted masks to the aerosol concentration inside. The particles can identify both leaks in the mask and inefficiencies in the filters. This technology is believed to provide a complete diagnostic of the integrity of the mask, the filter, and the fit on the individual.



The Army, working with TSI, Inc., an aerosol instrument manufacturer, has developed the M41 Protective Assessment Test Systems (PATS) to test and verify the goodness of fit for the gas mask on the individual person that is issued the device.

The M41s are tested and verified by the U.S. Army Test, Measurement, and Diagnostic Group using a fit-test calibration stand (test stand). The components of the test stand are an aerosol generator, aerosol electrical charger, a mobility classifier to produce monodisperse 80 nm diameter aerosol, an aerosol dilution system and two condensation particle counters (CPCs). The test aerosol concentration range is 100 particles/cm³ to 10,000 particles/cm³. The CPCs serve as the calibrants in the test stand for the M41s. There are no aerosol concentration standards for the CPCs nor are there certified reference materials available for aerosol concentration standardization. The measurement process currently has no links or traceability to a standards granting organization. The U.S. Army Test, Measurement, and Diagnostic Equipment Activity (USATA) requires the development of an aerosol concentration standard method, traceable to NIST.

CSTL has provided a NIST traceable calibration for the aerosol electrometer. This allows us to verify the voltage response for known femtoampere current inputs. We have finished the calculations to simulate particle loss in the CPC. Electron microscopy has been completed on quantitatively collected 80 nm particles over a concentration range of approximately 200 particles/cm³ to 10,000 particles/cm³. This verification and possible development of a standard method would enable the accurate calibration of Condensation Particle Counters that are currently used to calibrate M41 Protective Assessment Test Systems (PATS) for the Army.

With present technology and the transient nature of aerosols, a certified reference material for aerosol concentration has not been feasible. Therefore, NIST verified the accuracy of the calibrant currently used in the field by comparing aerosol electrometer measurements with those obtained with NIST's primary particle counting approach using electron microscopy.



This work is also directly relevant to homeland security as the procedures developed here are applicable for verification of instruments used to fit test gas masks and protective suits used by first responders and emergency response personnel.

Characterization of Trace Explosive Detection Equipment for Airport Security Applications
J.G. Gillen, R.A. Fletcher, S.A. Wight, S.V. Roberson, E.S. Etz, A.P. Lindstrom, C.J. Zeissler,
and J.R. Verkouteren (837); B.A. Benner (839); and R. Lareau (Transportation Security
Administration)



CSTL is responding to the growing metrology requirements for trace explosive screening by leveraging existing expertise in particle analysis, analytical chemistry and chemical microscopy to build a measurement infrastructure for the continued development, characterization, calibration and standardization of trace explosive detection devices.

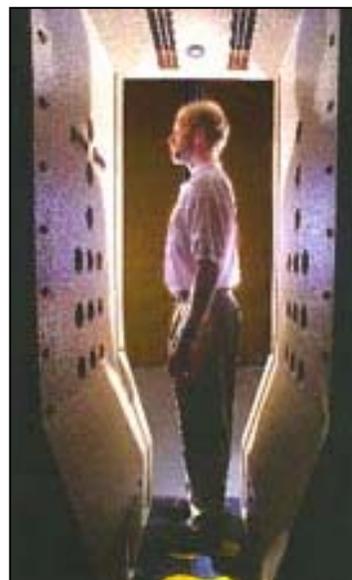
Since the low volatility of most high explosives makes direct analysis of vapors impractical, the majority of explosive detection systems rely on the collection of micrometer-sized explosive particles collected on swipe samples (tabletop units commonly used at airport security checkpoints) or from airborne particle collection (next generation walk-through portal detection systems). Collected explosive particles are thermally desorbed from the collection medium (cloth or steel mesh) into an ion mobility spectrometer (IMS) for identification. The effective collection and subsequent thermal desorption of explosive particles into the IMS is the critical front-end process for the successful and reproducible detection of explosives. In FY02, we initiated a new 3-year research program (through NIST's Advanced Technology Program Funding) to study this process in detail. A complete understanding of the operational parameters and limitations of trace explosive instruments is required for their effective and reliable operation.

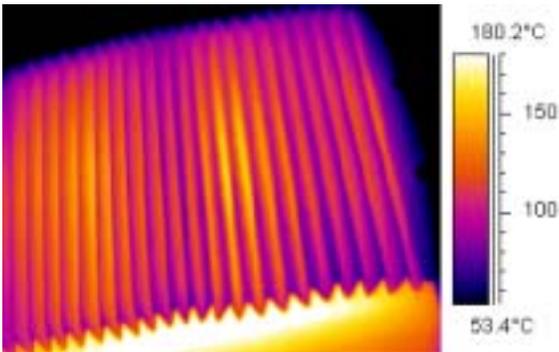
Researchers in CSTL and the Transportation Security Administration's (TSA) Trace Explosive Detection Group have been working to build a chemical metrology program to support the widespread operational deployment and effective utilization of trace explosives detection devices throughout the U.S.

A number of experiments were initiated to evaluate both swipe and portal-based explosive detection systems. A priority has been to study the factors involved in the release and effective collection of explosive particles in portal systems. One current study involves the use of fluorescence optical microscopy to study the release of monodisperse, fluorescently tagged microsphere particles from model surfaces under an impinging air jet (as used in airborne collection systems). Preliminary data suggests that almost no particles with diameters less than $\approx 6 \mu\text{m}$ are liberated under these sampling conditions. This information constrains the size of particles likely to be sampled using airborne collection techniques and will aid in the design of next generation explosive detection systems. The particle size

Current aviation security legislation requires the unprecedented utilization of trace explosives detection systems for screening of baggage, cargo, and passengers at US airports in support of Homeland Security.

collection efficiency of the metal collection filters used in portal systems is also being evaluated in this work. Another issue addressed this year was determining how to visualize a few individual explosive particles surrounded by millions of ambient aerosols on mesh filter collectors. For this purpose, new approaches for chemically specific imaging of explosive molecules were developed using the NIST cluster secondary ion mass spectrometry technique.



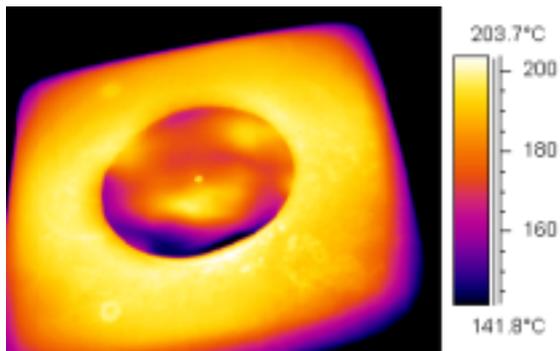


Infrared camera image of stainless steel mesh used in portal explosive detection unit

A second major thrust has been to study the temperature dependence of explosive particle vaporization. In these experiments, secondary ion mass spectrometry, Raman microscopy, and scanning electron microscopy have been used to study the temporal variations in chemical composition and morphology that occur during heating of individual explosive particles. The figures show infrared camera images of two types of collection media used in commercial explosive detectors. The CSTL infrared imaging capability is used to provide precise non-contact and spatially resolved temperature measurements of these surfaces. This information provides a unique method for relating the chemical degradation

information obtained using our microanalytical techniques to the actual temperatures achieved during the sampling process. In the next fiscal year, the scope of the work begun this year will be expanded to include a variety of additional studies. A robust examination of particle generation and collection will be conducted as well as a detailed evaluation of the thermal degradation of various explosives. New analytical capabilities in ion mobility spectrometry, gas chromatography, mass spectrometry, and optical and infrared microscopy are being added to support this effort.

Characterization of the performance characteristics of explosive detection systems is of significant importance for the TSA and law enforcement. Optimized performance will help to ensure the safety of our transportation system, minimize possible security threats and provide a more robust tool for state and local law enforcement applications.



Infrared camera image of swipe collection cloth

Autoradiography Measurements to Quantify Activity of Co-60 Particles
C.J. Zeissler (837); and R.M. Lindstrom (839)

The physical nature of particulate matter presents special measurement challenges for forensic analysis of dirty bomb debris. For instance, conventional biological standards cannot be utilized. Standards for biology consist of a limited list of radiotracers in thick, tissue slices or gene separation gels. Particles, however, act as point sources, whose positional geometry can significantly influence the measurements. Another challenge is that the activity of particles in environmental dusts cannot be adjusted as one might in biological tracer studies, so that special treatment has to be given to signal-to-noise problems. Dirty bomb debris may include beta-emitting particles of Co-60, Cs-137, Sr-90, etc. Individual particle analysis enables the collection of a variety of discrete chemical, isotopic, and microstructural data on each particle in a population. These data may be used to deconvolve sources and histories. In contrast, information obtained from conventional methods such as gamma spectrometry or

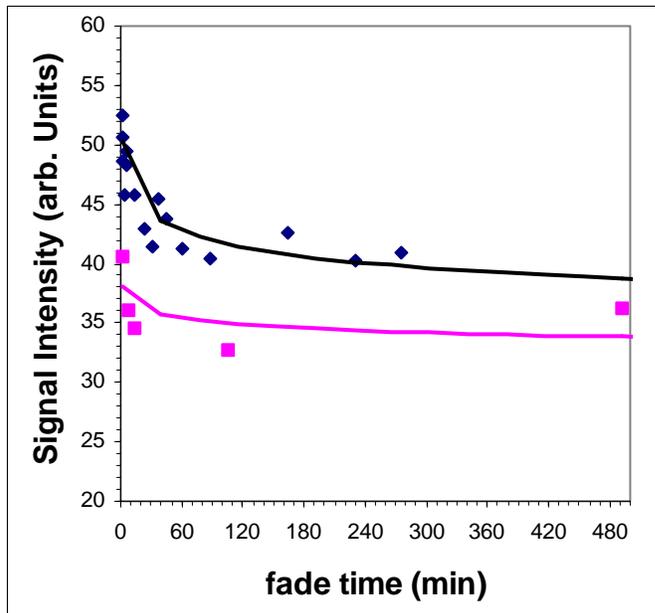
CSTL researchers are developing an autoradiography measurement approach to support forensic analysis on dirty bomb debris at a level that falls over one order of magnitude below conventional detection capabilities.

radiochemistry provides a measurement averaged over the whole sample, and does not provide additional forensic information available in the microstructure or morphology of each particle.

The digital autoradiography method has been shown to detect Co-60 nondestructively at a level at least one order of magnitude below low-background gamma spectrometry methods. Calibration down to 10 mBq has been achieved. Detection down to about 1 mBq or lower appears to be possible, but has not been verified at this stage of the project. Measurement uncertainty that is not due to counting statistics is approximately 10%. The difference in spatial resolution between two different detectors has been evaluated. This year we studied the fading characteristics of two phosphor detectors, measurement variability, phosphor calibration, spatial resolution, Co-60 detectability, and ease of adoption. In the future, we plan to produce working standards and a measurement protocol for potential use by national forensic authorities.

A nondestructive, sensitive characterization method that preserves discrete particle information can have great forensic utility. Conventional methods should continue to be sufficient for conventional detection and characterization, but they have limited sensitivity and forensic value. With this new microcharacterization method, previously undetectable debris on dirty bombs or residues from alleged manufacturing locations could be used towards the identification of terrorist activities. Ultimately, such information can be used to prevent future manufacture or deployment of dirty bomb devices.

The purpose of this research into autoradiography measurements is to develop a quantitative, nondestructive and discrete measurement capability for particles that emit beta radiation at or below mBq levels. Single particle detection and characterization measurements of a particle population are applicable to forensic dirty bomb investigations.



Fading curves for two autoradiography phosphor detectors using a 3 Bq Co-60 source.

New Measurement Technologies and Standards to Aid in Crime Scene Investigations
W.A. MacCrehan (839); M. Layman, J. Secl, and A. Dorsey (George Washington Univ.)

Measurement technology and a quality assurance standard are being developed for the forensic investigation of crimes using guns and improvised explosive devices. A simple protocol was developed for the collection and analysis of the organic gunshot residue (OGSR) from hair using a fine-toothed comb. A series of weapon firing studies with both mannequin-supported wig hair and human shooters showed the hair combing approach to be highly successful. Currently, there are no quality assurance materials for the validation of analytical procedures for explosives determination or for the testing of laboratory proficiency.

CSTL researchers made compositional measurements of the organic additive content on the candidate smokeless powder Reference Material, RM 8107 Additives in Smokeless Powder, which is being developed to help forensic investigators achieve better information from the collection of gunpowder residues at shooting and explosives crime scenes.

In an effort to support homeland security, we are developing a gunpowder reference material, RM 8107 Additives in Smokeless Powder. Gunpowder is the most common active ingredient in improvised explosive devices (IED) such as pipe bombs. Forensic investigators recover incompletely burned powder residues from crime scenes such as the Atlanta Olympic Park bombing (7/27/96), and these residues are analyzed for their additive composition as a means of categorizing the powder against known powder samples. A NIST smokeless powder reference material can be used by forensic laboratories to validate their analytical methodology and test the performance of laboratory personnel, assuring the accuracy of their smokeless powder compositional identifications.

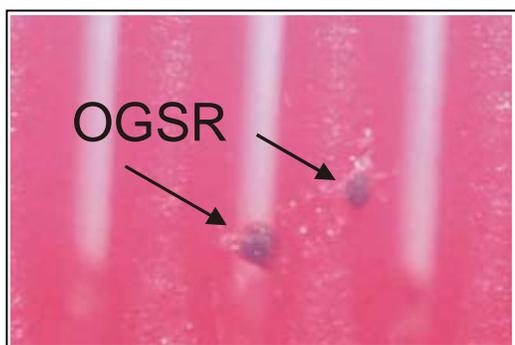


Image shows OGSR particles collected by combing a revolver shooter's hair.

In our approach, the chemical composition of gunshot and explosives residues collected at crime scenes are determined by micellar capillary electrophoresis (CE). During the past year, we have made progress toward addressing two issues in successful gunpowder residue analysis: effective collection of gunshot residues and accurately determining the composition of explosives residues. A simple protocol for OGSR collection was developed using a fine-toothed comb (for the collection of fleas from pets) and direct analyte ultrasonic solvent extraction in a plastic zip-enclosure bag. This new OGSR analysis approach was evaluated in a series of weapon firing experiments using mannequin-supported human wig hair, which allowed us to determine successfulness in simulated shooter and victim

scenarios. Four weapons were tested including a revolver, semiautomatic pistol, shotgun, and a carbine. In the majority of OGSR combing samples, the gunpowder additive nitroglycerin was successfully detected. The method also proved effective in detecting OGSR in human shooter testing. In the future, we intend to work toward the development of analytical measurements and standards for the analysis of the high explosives, such as plastic explosives, that are more commonly encountered in international terrorism investigations.

Reference Methods and Standards for Alcohol and Drugs of Abuse Testing
M.M. Schantz, J.V. Goodpaster, L.T. Sniegowski, S. Tai, and M.J. Welch (839)

Standard Reference Materials (SRMs) for use in the forensic sciences include: SRMs for blood alcohol determinations (SRM 1828a Ethanol-Water Solution), for measurements of drugs of abuse in urine (SRM 1507b THC-9-COOH; SRM 1508a Benzoyllecgonine; SRM 2382 Morphine Glucuronide; SRM 8444 Cotinine; SRM 1511 Benzoyllecgonine, Morphine, Codeine, THC-9-COOH, Phencyclidine; and SRM 2381 Morphine and Codeine), and in hair (SRM 2379 Cocaine, Benzoyllecgonine, Cocaethylene, Amphetamine, Methamphetamine, and PCP; and SRM 2380 Codeine, Morphine, Monoacetylmorphine, and THC). SRMs 2379 and 2380 for the determination of drugs of abuse in human hair were completed in FY02 and will provide this growing measurement technology with a mechanism for assuring the accuracy of the methods used to determine drugs of abuse in hair.

Illegal use of controlled substances remains a serious problem in the U.S. Millions of tests are run annually to determine whether a person has ingested or possesses controlled substances. The incidence of false positives and false negatives from these tests impact public safety as well as the reputation of the individuals tested. It is therefore critical that such tests be traceable to accurate standards.



Recently ACD, in conjunction with the NIST Office of Law Enforcement Standards (OLES), completed the evaluation of the specificity and sensitivity of spray reagents used by law enforcement officers in the field to test for the presence of drugs of abuse. Varying amounts of the target drugs were applied to the test strips supplied with the reagents and then treated with the sprays. Volunteers were asked to rate whether or not a positive response could be seen as a test of the sensitivity of the tests. Various substances that might be encountered by law enforcement officers were tested for interference with the drug tests. In general, the drug spray reagents performed in agreement with the manufacturer's claim. In addition, three new SRMs are under development in response to feedback from the forensic community as to their most immediate needs for standards. The development of these new SRMs was initiated with support from OLES and include (1) an updated and redesigned SRM 1828b for blood- and breath-alcohol testing (SRM 1828a will be replaced by a set of nine solutions that will cover the concentration ranges of importance to both blood and breath alcohol testing), (2) an SRM designed for qualitative analysis of materials from an arson investigation, and (3) an SRM for drugs of abuse in human serum to complement the current SRMs for drugs of abuse in urine because forensic laboratories often receive blood instead of urine.

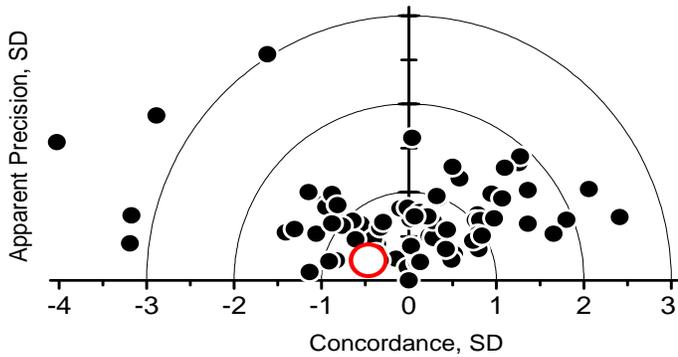
With the completion of these SRMs, the law enforcement community will have several new tools to assure the accuracy of the methods used to detect drugs and substance abuse.

Development of Graphical Tools for the Analysis of Data from Interlaboratory Comparisons
D.L. Duewer (839) and M.C. Kline (831)

For any experiment to have optimal utility, data and information must be transformed into knowledge and understanding. Forensic interlaboratory studies present a particular communications challenge since the audience potentially interested in the results (including the public, legal profession, participants, and agency sponsors) has inherently diverse backgrounds. Graphs that simply but accurately summarize results help meet this communications challenge. From anecdotal evidence, our graphical reports from earlier studies have led to improved forensic DNA measurement systems.

The MSS3 was initiated in late 2000, data were accepted until October 2001, and data entry and validation was completed as of June 2002. This interlaboratory challenge study was designed to help the forensic human identity community make more effective use of their limited resources by illuminating the relationships between their measurement of the concentration of DNA in extracted samples and the quality of their multiplex STR “fingerprints”. Analysts from seventy-four forensic laboratories participated. Graphical tools, such as the “target” plot to the left, have been used in individualized reports sent to each participant as well in the study’s format summary documents. Each comparability (difference from consensus) is estimated from results reported for seven different samples, centered on the sample’s consensus value and scaled to the interlaboratory standard deviation. Comparability has two aspects: concordance (average difference from consensus, plotted along the horizontal axis) and apparent precision (standard deviation about the average difference, plotted along the vertical axis).

The third NIST-sponsored Mixed Stain Study (MSS3) was designed to help the forensic human identity community make more effective use of their limited resources by illuminating the relationships between the quality of their DNA concentration measurements and of their initial DNA “fingerprint” efforts.



The best comparabilites are at the target’s center; the large red hole denotes a participant reporting values with excellent apparent precision and very good – if slightly low – concordance. One of the major findings from MSS3 is that participants reporting DNA concentration measurements with unusually poor apparent precision or unusually high concordance values typically expended more analytical effort to produce DNA fingerprints of adequate quality.

This "target" plot displays DNA concentration measurement comparabilities for the MSS3 participants.

9. Health and Medical Technologies



CSTL provides quality assurance for the healthcare industry by providing the appropriate chemical and physical reference standards, developing reference methods and databases, and also by working closely with the clinical and medical community to transfer the accuracy of the highest level of metrology to high-throughput clinical laboratory measurements. Measurements are responsible for up to 15% of

Healthcare costs amount to ~ 14% of the GDP, an estimated \$1.5 trillion. By 2007, healthcare spending as % of GDP is projected to reach over 16%.

the \$1.3 trillion annual costs of healthcare in the U.S. A significant portion (25% to 30%) of these measurements are performed for non-diagnostic reasons, such as re-tests, error prevention, and detection.

NIST Assists US IVD Industry in Meeting the Requirements of European Directive 98/79/EC W.F. Koch (830); and W. E. May and M.J. Welch (839)

European Directive 98/79/EC on in vitro diagnostic medical devices (IVDs) requires that products be traceable to “standards of higher order”, e.g., nationally/internationally recognized certified reference materials. It further states that such devices “must be designed and manufactured in such a way as to provide adequate stability and accuracy of measurement within appropriate accuracy limits” and that “the accuracy limits have to be specified by the manufacturer.” Implementation date of this Directive is December 2003. At present, reference methods and/or reference materials are available for only a limited number of the several hundred analytes that are measured in medical laboratories on a

The recently enacted European Directive 98/79/EC on in vitro diagnostic medical devices (IVDs) requires that products be traceable to “standards of the highest order”, e.g., nationally/internationally recognized certified reference materials (CRMs). Because approximately 60% of the IVD products on the European market (\$5.6 billion in 1998) are imported from the USA, this is a very important trade issue.



daily basis using IVDs. Furthermore there is currently no internationally recognized procedure for stating accuracy claims in this arena. Since approximately 60% of the IVD products on the European market (\$5.6 billion in 1998) are imported from the USA, this is a very important trade issue.

In response to this EU directive and at the urging of the US medical device industry, NIST organized and hosted a special workshop on Measurement Traceability for Clinical Laboratory Testing in November 2000. Out of this workshop grew the concept and the need for an international body to coordinate and prioritize the development of reference methods and reference materials for this industry (one of the few that still maintains a positive trade balance). The Joint Committee for Traceability for Laboratory Medicine, JCTLM (hosted by the BIPM) is the result. (NIST arranged and chaired a special pre-JCTLM meeting of US industry representatives to develop a coordinated US position on industry’s response to metrologically traceable reference materials and methods for clinical analyses.) The main purpose of the JCTLM is to actively involve the IVD industry, professional organizations, government, and national and international standards developing organizations in the solution to establishing the means and processes for assuring measurement traceability and reference systems for the healthcare community. NIST played the pivotal role on the organizing and steering Committee for this new



BIPM

organization. After several preliminary and planning meetings, a full organizational meeting was held in Paris in June 2002. NIST was a proactive participant and was appointed chair of one of two working groups within the JCLTM. NIST hosted the first Working Group 1 meeting in August 2003, actively engaging US industry involvement, and is proceeding in the planning of its second meeting in late March.

To respond to the accuracy limit provision of the directive, NIST organized and hosted a meeting of key industry representatives on January 7, 2003 to develop a short course on this topic to be webcast to the whole industry in early April.

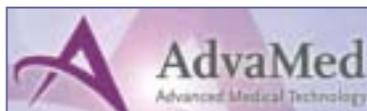
Concurrent with these efforts within JCTLM, NIST is working closely with the US IVD industry in developing practical guidance on the calculation of the uncertainty of calibrator values for in vitro diagnostic manufacturers, in order to respond to the accuracy limits provision of the Directive.

Standards for in vitro Diagnostics and International Trade

W.E. May, D.M. Bunk, J.D. Fassett, W.R. Kelly, S.E. Long, K.E. Murphy, B.C. Nelson, M.B. Satterfield, L.T. Sniegowski, S. Tai, R.D. Vocke, and M.J. Welch (839)

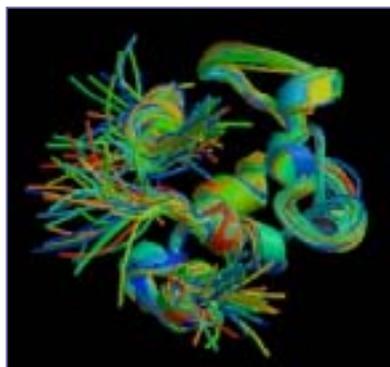
The accuracy and traceability of the data from medical tests are becoming increasingly important. Typically, medical guidelines are derived from clinical studies where medical outcomes are correlated with medical test results. Such data are often collected using many different laboratories and instruments, in different parts of the world, and at different times. However, effective use of such data will require that any differences observed be attributable to the clinical parameter(s) being measured and not to the measurement processes.

Valid decision-making requires that a medical test result for an individual patient – from a different laboratory at a later time – be correlated to the clinical study data for the broader population.



In addition to reliability and cost concerns, there is an increased urgency for developing internationally accepted reference methods and certified reference materials for new diagnostic markers. ADVAMED, representing the U.S. IVD Industry, has asked NIST to work with our counterparts in Europe and the Asia-Pacific Region to provide the reference materials and methods of “higher order” that are urgently needed to comply with the requirements of the EU IVD Directive, described in the previous article. Without timely completion of these standards, their access into the European market will be seriously jeopardized. Through the Joint Committee for Traceability for Laboratory Medicine (JCTLM), NIST, the EU and AP laboratories have agreed to share the workload involved in developing and

maintaining the more than 100 standards needed for industry compliance with the EU IVDD. NIST has been asked to provide about 40% of the standards based on our current capabilities and U.S. market share. Currently NIST has developed and is maintaining high-accuracy reference methods for 26 clinical diagnostic markers including a number of electrolytes (calcium, chloride, lithium, magnesium, potassium, and sodium), hormones (cortisol, thyroxine) and small organic substrates (cholesterol, creatinine, glucose, urea, uric acid,



**Structure of Cardiac Troponin C-Troponin I Complex
Image from PDB**

triglycerides) to support the national reference system for clinical chemistry. Reference methods have also been developed and SRMs disseminated for a number of micronutrients (e.g., vitamins A, C, D, E, and beta-carotene) therapeutic drugs

(dilatant, lithium, phenobarbital, theophylline, valproic acid) and drugs of abuse (cocaine, delta-9 THC) as well. During the past year we have: (1) critically evaluated isotope-dilution ICP-MS as a replacement to isotope dilution thermal ionization mass spectrometry for serving as the U.S. reference method for electrolytes in blood serum, (2) developed and critically evaluated new reference methods for cortisol, folates, homocysteine, and triiodothyronine in blood serum, (3) certified the concentrations of Cd and Hg in whole blood (SRM 966) and completed analyses for value assignment of 10 toxic elements at trace and ultratrace levels in a new freeze-dried urine reference material, SRM 2670a and (4) evaluated six candidates to serve as a primary reference material for Cardiac Troponin-I (cTnI).

NIST has developed and is maintaining high accuracy reference methods for 26 clinical diagnostic markers. Additional reference methods and standards for new health status markers are in development. Improved accuracy facilitated by this program is leading to better diagnosis, treatment and reduced health care costs.

Toward Comparable Cardiac Troponin I Measurements: Selection of a Primary Reference Material

D.M. Bunk and M.J. Welch (839)

The clinical measurement of serum cardiac troponin I (cTnI) has become an important tool in the diagnosis of acute myocardial infarction (AMI) and myocardial damage. Unfortunately, a ten-fold difference in measured concentrations among assays is common, and as much as a hundred-fold difference in response has also been observed. To address the need for standardization of cTnI assays, NIST, the American Association for Clinical Chemistry (AACC), and the International Federation for Clinical Chemistry (IFCC) have all collaborated to evaluate reference materials for clinical cTnI measurement.

To reduce the large variation among results from clinical cTnI assays, NIST, in cooperation with the AACC and IFCC, is developing a cTnI reference material.



After evaluation of six different candidate reference materials using two round robin studies, a single reference material has been selected. More than 20 different commercial cTnI assays, made by 12 different manufacturers, from the United States, Europe, and Japan, were used to evaluate the candidate materials. This choice was made after evaluation of the troponin preparation for purity, stability, its ability to provide harmonization of assay results, and the

commutability of the material among the 20 commercial cTnI assays used in the study. A cTnI reference material will be used by the assay manufacturers to develop new assays and to validate calibration materials for their current assays.

SRM 2921, purified human cardiac troponin complex, is currently being prepared. Once this material is available, a third round robin

In a recent issue of *Clinical Laboratory News* (Vol 28, No 10, Oct 2002), the selection of a material to be used as a primary cTnI standard was touted as an “important milestone” in the standardization of clinical cTnI assays. *Data from the second round robin demonstrates that the use of one common reference material could reduce interassay variability to 10 to 15%, a substantial improvement compared to the 130% variability observed in the first round robin.*

study will be carried out to assess the ability of SRM 2921 to harmonize the cTnI assay results from patient samples. Future work will also include the development of a reference method for cTnI in serum.



A point-of-care test for troponin I levels following AMI. Photo from Spectral Diagnostics.

New Reference Methods Developed for Cortisol, Folates, Homocysteine, and Triiodothyronine in Human Serum

B.C. Nelson, M.B. Satterfield, L.T. Sniegowski, S. Tai, and M.J. Welch (839)

The issuance of the in vitro diagnostic (IVD) device directive by the European Union has increased the need for NIST to develop new reference methods and SRMs to provide traceability for the US IVD industry so that this industry may maintain its strong position in European markets.

NIST has a strong commitment to promoting accuracy in health-related measurements and providing measurement traceability to the U.S. in vitro diagnostic (IVD) industry through development of reference methods and SRMs.

In the past year, NIST has completed development of reference methods for the determination in blood of homocysteine, a new marker for risk of cardiovascular and other diseases; folates, critical vitamins for suppressing neural tube defects and the effects of elevated homocysteine; triiodothyronine (T3), a marker for thyroid function; and cortisol, a hormone important to endocrine function. These new methods were based upon isotope dilution combined with liquid chromatography/mass spectrometry (LC/MS), LC with tandem mass spectrometry (LC/MS/MS), and gas chromatography/mass spectrometry (GC/MS). The LC/MS method for folate and the LC/MS, LC/MS/MS, and GC/MS methods for homocysteine developed at NIST were extensively evaluated and tested against methods used by the Centers for Disease Control and Prevention (CDC) and elsewhere and found to provide results of high accuracy and precision. The LC/MS/MS method for T3 along with an LC/MS/MS method for tetraiodothyronine (T4) was used in an international comparison with excellent agreement with laboratories in Europe. The LC/MS/MS method for cortisol has been thoroughly tested and it provides unrivaled precision and specificity for this low-level hormone.

These new reference methods and the SRMs that they will be used to certify will provide critical traceability to the IVD industry and will help improve the reliability of routine clinical measurements.

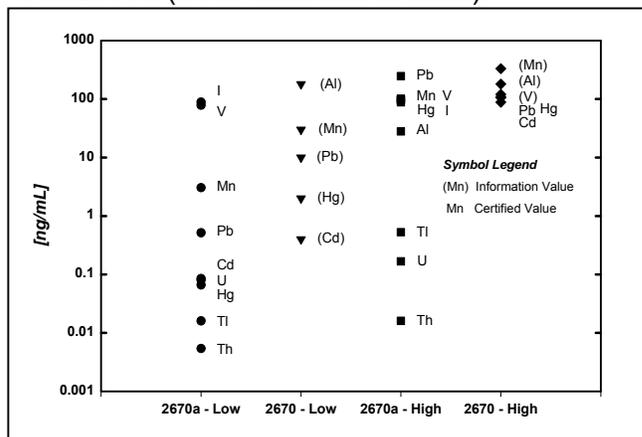
Newly Certified Trace Elements in Urine and Blood SRMs

R.D. Vocke, C. Beck II, O. Ertas, W.R. Kelly, S.E. Long, and M.S. Rearick (839)

Noninvasive methods for obtaining information on human biological function are useful both as screening tools for population studies and for individual diagnoses of medical disorders. Urine and blood are the two most common body fluids used in clinical evaluations of selected trace and ultra-trace element levels in humans. Besides the obvious importance of toxic element markers as indices of occupational exposure (e.g., Pb, Cd, Hg), there is increasing interest within the medical profession of the precious metal levels in such fluids and how that might be tied to surgical implants as well as exposure to automotive exhaust that has passed through catalytic converters. The Centers for Disease Control and Prevention (CDC) is also increasing the frequency of their health surveys in order to establish population baselines for a wide range of elements and releasing these values directly to the public. Such measurements are of great interest to public health officials but are very difficult to make because of the extremely low level of the analytes and are in need of a quality control (QC) material. Our effort in characterizing urine and whole blood materials has been directed particularly towards certifying the elements within the low or normal levels, which have typically been listed as "information values" due to the difficulties in making measurements near the limits of quantitation. We have

Clinical analyses are used both as tools for screening the state of health of a given population as well as for individual diagnoses of medical disorders. Urine and blood are the two most common body fluids used in clinical evaluations of selected trace and ultra-trace element levels in humans.

focused on two reference materials, SRM 966 (toxic elements in frozen whole bovine blood) and SRM 2670a (trace elements in urine).



Summary of Selected Trace Element Concentrations in Urine SRMs

We have completed certification analyses for Cd and Hg in the base level of the whole blood reference material, SRM 966, utilizing a combination of a new technique and instrumentation.

Using an extremely sensitive Hg isotope dilution-hydride generation-ICPMS technique, a certified Hg value of (44.5 ± 3.3) ng/L was successfully measured in the low level of this material. Cadmium was also re-measured using the high sensitivity of the sector-field ICPMS together with isotope dilution mass spectrometry culminating in a certified Cd value of (31.8 ± 18) ng/L.

Both elements in this material had previously been “information values” for Hg and Cd at < 60 ng/L and < 400 ng/L, respectively. We have also completed analyses of 10 elements at trace and ultratrace levels in a new freeze dried urine reference material, SRM 2670a. Selected data are shown in the plot, comparing the values in the new material with the information values (where they existed) in SRM 2670.

The application of new instrumentation and analytical techniques has led to an **increase in the number of elements that are being certified** compared to earlier clinical SRMs.

Critical Evaluation of ID ICP-MS vs. TIMS as New National Reference Method for Electrolytes in Blood Serum

K.E. Murphy, S.E. Long, J.D. Fassett, and M.S. Rearick (839)

Reference methods play a critical role in the measurement infrastructure required to diagnose, treat and prevent disease. Decisions regarding health status are based on measurements of electrolytes, such as potassium (K) and calcium (Ca). The accuracy of these measurements depends on accessible reference methods and standards. We have developed a new reference method for the determination of K and Ca based on “cold plasma” isotope dilution inductively coupled plasma mass spectrometry (ID ICP-MS). Traditionally, isotope dilution thermal ionization mass spectrometry (ID TIMS) has provided the accuracy base and SI traceability for the determination of these analytes in clinical materials. ICP-MS is simpler to implement and more widely available than TIMS. We benchmarked the cold plasma ID ICP-MS methodology against the previously applied ID TIMS definitive method in several ways. First, a direct comparison was performed by using both techniques for ratio measurements of K and Ca on the same isotope dilution samples. Second, an indirect comparison was made through the analysis of Ca in Standard Reference Material 956a Electrolytes in Frozen Human Serum, which had been certified by ID TIMS in 1996. Third, we demonstrated comparability for serum Ca results with other National Metrology Institutes (NMIs) that applied ID TIMS through participation in the Consultative Committee for Amount of Substance (CCQM) Pilot Study, P-14. All sources of bias in the method were considered and a complete uncertainty statement was given. The ID ICP-MS expanded uncertainty based on a 95 % confidence interval for the determination of Ca in P-14 serum samples was ± 0.42 % relative.

High-accuracy reference methods for electrolytes in body fluids play a critical role in the measurement infrastructure required to diagnose, treat, and prevent disease.

The critical evaluation of this ICP-MS-based procedure and our demonstrated ability to achieve comparability with a definitive method meets the demand for accurate and accessible clinical measurement methodology and satisfies traceability and global comparability needs.

Development of a reference method based on ICP-MS takes advantage of modern, widely available instrumentation and realizes a **factor of four improvement in the uncertainty interval**, approaching the capabilities of the definitive method.

Measurement Quality Assurance Program for Fat-Soluble and Water-Soluble Vitamins in Blood Serum

J.M. Brown Thomas, D.L. Duewer, S.A. Margolis, and K.E. Sharpless (839); and M.C. Kline (831)

The Micronutrients Measurement Quality Assurance (QA) Program supports measurement technology for selected fat-soluble and water-soluble vitamins and carotenoids in human serum and plasma. This program was initiated in 1984 in conjunction with the National Cancer Institute (NCI) Division of Cancer Prevention and Control to ensure the long-term reliability and comparability of the measurements made while studying the possible cancer chemoprevention role of these analytes. From an initially NCI-supported core of 10 NCI-grantee laboratories, the program currently involves 45 fee-paying laboratories worldwide. This program provides participants with measurement comparability assessment through use of interlaboratory comparison studies, Standard Reference Materials (SRMs) and control materials, and methods development and validation. Significant improvements in measurement comparability among QA participants have been documented. Many of the participants depend on this QA program to demonstrate their compliance with Clinical Laboratory Improvement Act (CLIA) requirements.

The Micronutrients Measurement QA Program is designed to assess and improve the comparability and reliability of measurements of vitamins and carotenoids in human serum and plasma.



<http://www.cstl.nist.gov/nist839/839.02/qa.html>

Results from the interlaboratory comparison studies help participants in the QA program make accurate clinical and health-care decisions and maintain and improve their measurement comparability.

Serum-based samples with assigned values for target analytes (e.g., retinol, tocopherols, β -carotene, and ascorbic acid) and performance-evaluation standards are distributed by NIST biannually to participants for analysis. NIST provides feedback to the participants concerning their measurement performance and suggestions for methods development and refinement. The comparability of laboratory measurements for target analytes has improved substantially over time. This improvement has been achieved through the development and promulgation of robust measurement technologies, identification and production of suitable reference materials, isolation and identification of measurement system biases, and support and encouragement of long-term within-laboratory measurement quality control efforts. The long-term stability of the target analytes in lyophilized and liquid-frozen reference materials stored at $-80\text{ }^{\circ}\text{C}$ has been documented, as has the utility of a variety of graphical tools for the analysis of interlaboratory measurements. More information about the QA program is available on the web.

The Complete Mitochondrial DNA (mtDNA) Genome Sequence of Human Cell Line HL-60 and Its Inclusion in NIST's mtDNA SRM

B.C. Levin, D.K. Hancock, K.L. Richie (831); K.A. Holland (Gettysburg College); M. Coble and T. J. Parsons (Armed Forces DNA Identification Laboratory); L.J. Kienker (FBI Laboratory); and D.W. Williams (Georgia Bureau of Investigation)

MtDNA is used by the forensic community for human identification and by the medical community for diagnoses of human mtDNA diseases. An mtDNA Standard Reference Material (SRM 2392) prepared by NIST to provide quality control to the scientific and clinical communities when they amplify and sequence human mtDNA became available in December 1999. This SRM includes two human DNA templates (CHR and 9947A) and all the information necessary to successfully conduct PCR amplification, cycle sequencing, gel separation, and data analysis to obtain the final DNA sequence. The sequences of 58 unique primer sets, which allow the sequencing of the entire mtDNA (16,569 bp) with no gaps, are also provided. The FBI recently requested that NIST add DNA from cell line HL-60 which has several evenly spaced polymorphisms in the mtDNA control region and no C-stretch (difficult to sequence) areas. The FBI's Combined DNA Index System (CODIS) program now includes mtDNA sequences from unidentified remains, as well as from relatives of missing persons. In order for authorized laboratories to contribute or examine these indices, the FBI has deemed that certain quality standards must be met. In particular, a positive control from the human cell line HL-60 must be run in

DNA SRMs provide quality control and assurance that laboratories are sequencing unknown DNA samples correctly.



The inclusion of HL-60 DNA in SRM 2392a will enhance this SRM's utility to forensic laboratories, to clinical laboratories that screen patients for mtDNA mutations and diseases, and to toxicologists who screen mtDNA for mutations.

conjunction with their unknown samples. This addition of HL-60 to the NIST SRM 2392 and an interlaboratory evaluation of the HL-60 sequence by three other laboratories have been completed. The new SRM 2392a will provide additional quality control when amplifying and sequencing human mtDNA. Corroboration of the SRM results will provide assurance that unknown DNA samples are also being amplified and sequenced correctly. The primary customers are the forensic laboratories throughout the U.S. and the clinical laboratories that screen the DNA of patients for mitochondrial diseases.

Mitochondrial DNA Mutations in Patients with Myelodysplastic Syndromes ***B.C. Levin (831); and M.G. Shin, S. Kajigaya, and N.S. Young (NIH)***

Recently, a number of papers have been published that equate mtDNA mutations with specific diseases, including cancer, which had previously not been associated with mtDNA mutations. The functional relevance of these mutations (i.e., whether they have a causal relationship or could serve as biomarkers for early detection of the disease) has not been proven. In this research, we examined the mtDNA mutations found in patients with Myelodysplastic Syndrome by amplification and sequencing of the entire human mtDNA (16569 bp) and compared the results with a comparable normal control group. The entire mtDNA genome (16,569 bp) in 10 patients with Myelodysplastic Syndrome and 8 normal controls was amplified by PCR and sequenced. The sequences were analyzed and compared to each other as well as recognized mitochondrial DNA databases. Overall, there was no increase in the number of mtDNA genes

Over 100 point mutations and more than 200 deletions and rearrangements in mtDNA have been associated with disease, and new mutations are being described every year. However, the database of "normal" mtDNA sequences is relatively limited.

harboring polymorphisms or “new” mutations between our patients and normal controls, although there were a few more mtDNA changes resulting in amino acid changes in myelodysplasia (16 in 10 patients versus 9 in 8 controls). Thirty new mutations, all nucleotide substitutions, were found among the ten patients, distributed throughout the mitochondrial genome; five mutations resulted in amino acid changes, but none in the controls. We were not able to confirm previously described mutations in sideroblastic anemia, nor “hot spots” in the Cytochrome C Oxidase I and II genes. Our data do not support a major role for mitochondrial genomic instability in

This work points out the importance of preparing a database of the sequences of the entire mtDNA of normal individuals such as those that are available in the NIST SRM 2392. Without such controls, the importance of mtDNA polymorphisms and mutations may be overestimated.

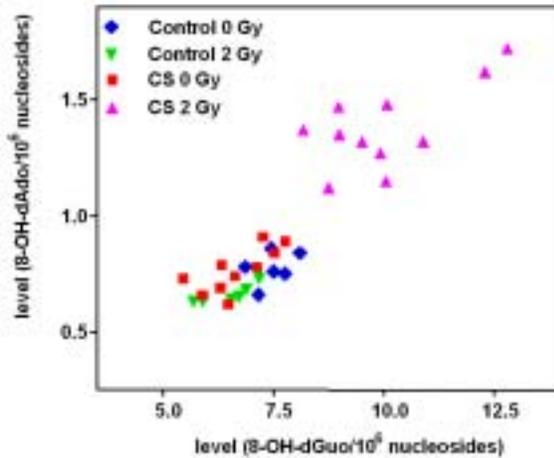
Myelodysplasia, and they fail to confirm previous reports of significant or widespread mitochondrial mutations in this disease. Modest changes in mutation numbers and mitochondrial microsatellites may be evidence of increased mutagenesis in mtDNA, or, more likely, a reflection of limited clonality among hematopoietic stem cells in this bone marrow failure syndrome.

The Role of the Cockayne Syndrome Gene in Cellular Repair of Oxidative DNA Damage
M.M. Dizdar, H. Rodriguez (831); J. Tuo and V. Bohr (NIH); and P. Jaruga (UMBC, MD)

Cockayne Syndrome (CS) is a human genetic disorder with diverse clinical symptoms that include hypersensitivity to sunlight, severe mental and physical growth retardation, progressive neurological and retinal degeneration, and skeletal abnormalities. CS has two complementation groups, CS-A and CS-B. The gene of CS-B (*CSB* gene) encodes a protein (CSB protein), which is known to play a role in the cellular repair of DNA damage, but it may participate in other pathways of DNA metabolism. In this study, the role of the CSB protein in the cellular repair of oxidative DNA damage was investigated.

Studies of DNA damage can provide clues to the pathogenesis of human diseases.

Transformed human cell lines with site-directed mutations in the *CSB* gene were established. These cell lines were then used to study phenotypical changes affected by the mutations, including DNA repair by whole cell extracts and the accumulation of oxidative DNA damage in genomic DNA of cells after exposure to oxidative stress. Cells were exposed to low doses of ionizing radiation such as 2 Gy to cause oxidative stress. It was found that the mutant cells and those with the deleted *CSB* gene had greater sensitivity than wild-type cells to ionizing radiation. The mutant cells had lower activity of DNA repair as shown by determination of activities of DNA repair enzymes. The results showed that the biological functions of the CSB protein in DNA repair might be mediated by distinct functional motifs of the protein. In addition, the accumulation of two well-known products of oxidative DNA damage, i.e., 8-hydroxy-2'-deoxyguanosine (8-OH-dGuo) and 8-hydroxy-2'-deoxyadenosine (8-OH-dAdo) in genomic DNA of cells was measured using the technique of liquid chromatography-mass spectrometry. These products accumulated to a greater extent in mutant cells and in cells with the deleted *CSB* gene than in wild-type cells after exposure of cells to ionizing radiation. This



Accumulation of 8-OH-dGuo and 8-OH-dAdo in genomic DNA of Cockayne Syndrome patients after exposure to γ -radiation

work was then extended to primary fibroblasts from eleven CS patients and six control individuals. These cells were exposed to 2 Gy of ionizing radiation to induce oxidative DNA damage and were then allowed to repair the damage. No significant change in background levels of the aforementioned compounds was observed in DNA of fibroblasts of normal individuals, indicating complete cellular repair. In contrast, cells from CS patients accumulated significant amounts of these lesions, providing evidence for a lack of DNA repair.

The results of this study suggest that the accumulation of oxidative stress-induced DNA lesions in genomic DNA of the CSB patients may contribute significantly to the pathogenesis of the human disorder CS.

Design and Use of a Peptide Nucleic Acid (PNA) for the Detection of the Heteroplasmic Low-Frequency MELAS Mutation in Human Mitochondrial DNA

D.K. Hancock, F.P. Schwarz, B.C. Levin (831); F. Song (CARB/UMBI); and L.-J.C. Song (Georgetown University Medical Center)

Most pathogenic human mtDNA mutations are heteroplasmic (i.e., the mutant mtDNA coexists with the normal mtDNA in the tissue or cell). The mutation level varies with the tissue and is often difficult to detect (especially in blood samples) when very low levels of the mutant exist in a population of wild-type mtDNA molecules.

We are using a simple methodology to detect low levels of the single base pair heteroplasmic MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like Episodes) (A3243G) mutation. We designed, synthesized, characterized, and purified a series of PNAs and tested them to determine the best one and the optimal conditions for blocking the amplification of the wild-type mtDNA while allowing the mutant DNA to amplify. We

A multitude of mitochondrial DNA (mtDNA) diseases have been correlated with single nucleotide polymorphisms (SNPs), mutations, insertions, and deletions. Most of these diseases are neuromuscular, but deafness, diabetes, epilepsy, progressive dementia, hypoventilation, cardiac insufficiency, renal dysfunction, and sudden onset blindness are some of the other symptoms of mtDNA mutations.

determined the optimal concentration of the PNA to be about 2 $\mu\text{mol/L}$. We examined eight MELAS patients as well as control healthy individuals. In all the MELAS patients, we were able to show that mutations that were not visible or barely visible in the sequence electropherogram in the absence of PNA became the dominant peak and

The results of this study will have a considerable impact on the clinical community who screen patients for mitochondrial diseases. It will greatly facilitate the detection of a mutation that was previously extremely difficult to detect and will help to prevent false negative results.

readily detectable in the presence of our designer PNA. The lowest detectable level of the mutation was 0.1%.



Future Plans: NIST plans to develop an SRM for the detection of the MELAS mutation in human mtDNA. This SRM will consist of the specially designed PNA, the primers needed to amplify the region where the mutation occurs, and the optimized protocol to allow any laboratory with PCR and sequencing capabilities to use this SRM and detect the MELAS mutation in blood even if it is not detectable in the absence of the PNA.

Discovery of a Critical DNA Repair Enzyme

M.M. Dizdar (831); P. Jaruga (UMBC, MD); and T. Hazra and S. Mitra (Univ. of Texas)

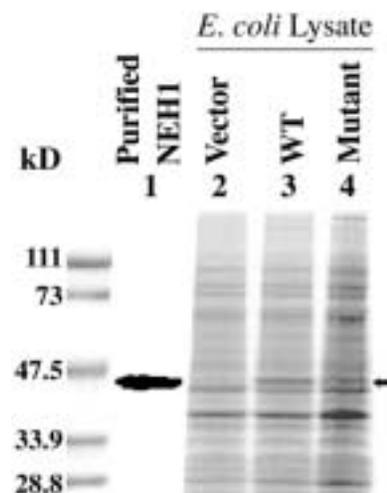
The identification and characterization of DNA repair enzymes might be the first step toward understanding how oxidative stress produces health problems and/or exacerbates the existing diseases. This study was conducted to determine the specificity of one important human DNA repair enzyme, NEH1, using mass spectrometric techniques. In the human genome database, sequences were identified that are similar to those known to code for DNA repair enzymes in the bacterium *Escherichia coli*. Following identification of the sequences, a previously unknown protein was produced. This protein was characterized by various techniques. Mass spectrometric measurements proved this enzyme to function as a DNA glycosylase when tested on oxidatively damaged DNA *in vitro*, and to remove two biologically important lesions from DNA. DNA glycosylases are repair enzymes that are involved in the first step of a complex DNA repair process in cells and remove modified bases from DNA. This glycosylase named NEH1 possesses two characteristics. First, it works on critical portions of human DNA

Detailed knowledge of mechanisms of DNA damage and repair might lead to drug developments and clinical applications including the improvement of cancer therapy by inhibiting DNA repair in drug- or radiation-resistant tumors and/or the increase in the resistance of normal cells to DNA damage by overexpressing DNA repair genes.

that are actually active. Second, when DNA is replicating, cells produce more NEH1 as if in an effort to repair errors caused by oxidative DNA damage before they are fixed in the next generation causing mutations. This coupling of repair to DNA replication indicates that the repair of active genes is different from the repair of the bulk of the genome. NEH1 was found to be highest in the liver, pancreas, and thymus. The paper that describes these results was published in *Proceedings of the National Academy of Sciences* and featured on the cover page of this journal.

Oxidative DNA damage results from normal cellular metabolism and oxidative stress, and is implicated in numerous human diseases from cancer to neurodegenerative diseases such as Alzheimer's disease to the normal aging process.

it works on critical portions of human DNA



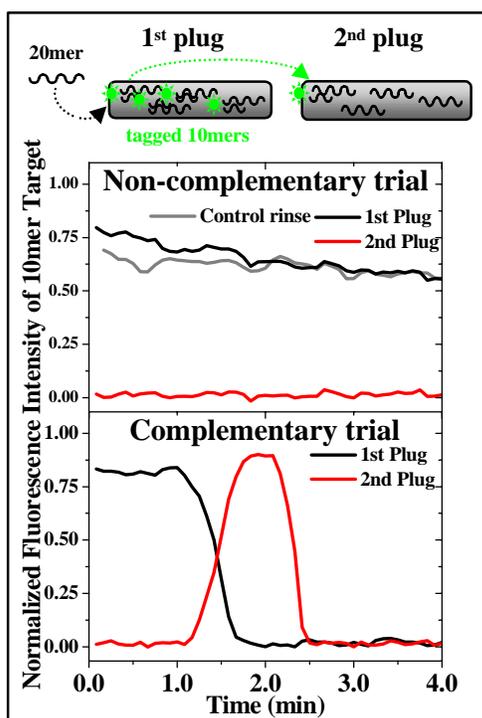
Expression and purification of NEH1. Lysates (15 µg) of *E. coli* expressing NEH1 were analyzed by SDS/PAGE and Coomassie blue staining. The Bio-Rad protein markers are shown on the left. Purified WT NEH1 (lane 1); lanes 2-4, lysates of *E. coli* with empty vector, WT, and Pro-1 mutant NEH1 expression plasmid, respectively. The position of NEH1 is indicated by arrow.

DNA and Protein Gel Plugs for Biological Assays

R.A. Zangmeister, G.A. Thomas, D.J. Ross, and M.J. Tarlov (836); and K. Olsen (Loyola College)

CSTL has devised a new, versatile method for securing biological ligands in microfluidic devices for a variety of biomedical assays. One such assay involves hybridization of nucleic acids for DNA sequence determinations. Single stranded DNA (ssDNA) probes modified on one end with an acrylamide functional group are photo-polymerized with polyacrylamide, creating a stable gel matrix with the ssDNA covalently bound. Complementary single stranded sequences, or targets, that are electrophoretically driven through such probe-containing gel matrices are captured, while non-complementary targets migrate through.

Researchers are developing techniques to improve the analytical sensitivity of microfluidic devices for biomedical assays.



Scheme for competitive DNA assay using probe (1st plug) and capture (2nd plug) gel plugs. Plots of fluorescence intensity measured of tagged 10mer initially hybridized in gel plug 1 during electrophoresis of both complementary and non-complementary 20mer targets.

The high concentration of the probes in the gel plugs coupled with the enhanced mass transfer characteristics of microfluidic channels ensure that targets will encounter a complementary probe, be captured, and thus be detected. Spatially distinct gel plugs each containing a different probe sequence can be formed photo-lithographically in a single channel for simultaneous detection of multiple targets.

In addition, a parallel effort this year demonstrated a conceptually similar approach for antigen capture assays where antibodies were entrapped in photo-polymerized gel-plugs. Specific antibody/antigen interactions using target and non-target antigens were demonstrated with the hydrogel-entrapped antibodies.

Future work is expected to demonstrate enhanced utility of gel-plugs to applications involving catalytic DNA. A novel two-plug probe/capture strategy will be used in conjunction with catalytic DNA for the detection of Pb in water. A paper describing the formation and use of DNA gel plugs was published in *Analytical Chemistry* in 2002.

A new method for immobilizing biological capture ligands in microfluidic devices has been devised with potential for use in disease diagnosis, and high throughput genomics and proteomics.

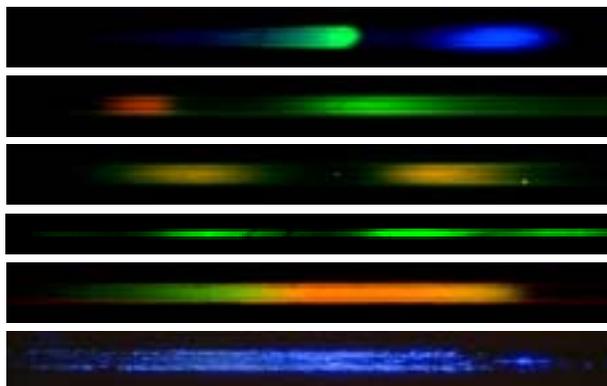
Temperature Gradient Focusing: A New Preconcentration and Focusing Technique for Microfluidic Devices

D. Ross (836); and L.E. Locascio (839)

One of the remaining problems in the field of microfluidics is the need for an effective method of pre-concentration that is suitable for miniaturization and integration into “lab-on-a-chip” platforms. A new focusing method, Temperature Gradient Focusing (TGF), has been developed to meet the outstanding need for effective pre-concentration techniques for microfluidics. Initial experiments indicate that the new method has great promise as a technique for preconcentration as well as for the focusing and separation of a variety of charged analytes including fluorescent dyes, amino acids, proteins, DNA, and colloidal particles. TGF operates by balancing the electrophoretic motion of analytes in a micro-channel against the bulk flow of buffer solution through the channel while applying both an electric field and a temperature gradient along the channel. If the ionic strength of the buffer solution is temperature-dependent, the temperature gradient will give rise to a corresponding gradient in the electrophoretic velocity of a charged analyte in the channel. The bulk flow velocity can then be adjusted so that the total analyte velocity (the sum of the bulk and electrophoretic velocities) is zero at some point along the channel, and all of the analyte will be focused at that point. As a method for analyte pre-

CSTL scientists have devised a new approach to pre-concentration for microfluidic devices. This method has demonstrated concentration factors exceeding 10,000 and can be used for a wide variety of charged analytes.

concentration, TGF out-performs all other methods, with demonstrated concentration factors exceeding 10,000. In addition to analyte pre-concentration, TGF can be used for the separation of different charged analytes in a manner analogous to isoelectric focusing of proteins. TGF is much simpler to implement than isoelectric focusing and has the additional advantage that it can be used with any charged analyte, rather than just proteins. This versatility is shown graphically in the figure with separations of a variety of analytes.



Demonstration of TGF for the focusing and separation of (from top to bottom) small fluorescent dyes, amino acids, proteins, DNA, and colloidal particles.

The TGF method is relatively simple compared with other pre-concentration methods, making it particularly well suited to integration into “lab-on-a-chip” devices.

10. Industrial and Analytical Instruments and Services



Instrument manufacturers depend on NIST/CSTL for physical and chemical standards, as well as NIST databases, for instrument calibration to provide traceability to national standards to the end users of their products and instruments. CSTL work supports both mature analytical applications, as well as the newest technologies such as high-throughput screening, and cutting edge research for next-generation semiconductor manufacturing.

By 2004, U.S. shipments of industrial and analytical instruments are expected to total nearly \$60 billion.

Atomic Standard of Pressure

M.R. Moldover, and J.W. Schmidt (836); and K. Szalewicz (Univ. of Delaware)

Below 300 kPa, the primary pressure standard at NIST is a mercury manometer. Above 300 kPa, piston gauges, commercially manufactured piston-cylinder sets, are the practical widely-used pressure standard achieving measurement uncertainties of a few parts per million (ppm or Pa/MPa). Continued performance improvement necessary to achieve measurement uncertainties significantly below 1 ppm appears to be problematic.

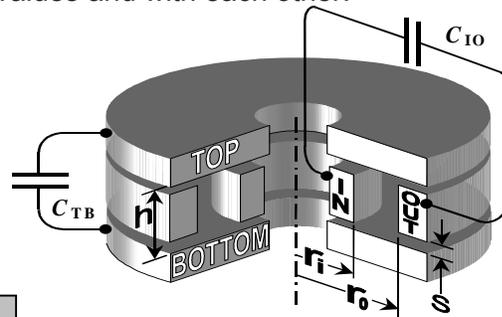
Therefore, a new approach to primary pressure measurement standards above ≈ 300 kPa must be developed if improved performance levels are to be realized. This will be accomplished both by measuring and calculating the dielectric constant $\epsilon(p, T)$ of helium with extraordinary accuracy and will permit the determination of the pressure $p(\epsilon, T)$ from electrical and temperature measurements of helium. The calculation of $\epsilon(p, T)$ from quantum mechanics by several theorists has improved dramatically recently with the variation between the results of all calculations falling below the 1 ppm level. The probability of continued improvement within the next few years is quite good.

CSTL researchers are developing a novel primary standard for pressure in the range 0.3 MPa to 5 MPa. This is accomplished by both measuring and calculating the dielectric constant $\epsilon(p, T)$ of helium with extraordinary accuracy.

Dielectric constant measurements are being improved by drawing on NIST's expertise in electrical metrology to develop a novel, doughnut-shaped, four-electrode, cross capacitor as a pressure cell. In general cross capacitors are extremely stable electrically and far less subject to surface contamination (oxides, adsorbed water, or films of oil) when compared with capacitors using other geometries. The toroidal design has the further advantage of eliminating spurious end effects that complicate the measurements. During FY01/02, the cross capacitor and pressure vessel were tested at 0°C, 30°C, and 50°C by measuring the dielectric constant of helium. All of the measured values of $\epsilon(p, T)$ from two separate cross capacitors of differing designs were consistent with the theoretical values and with each other.



Toroidal Cross Capacitor and Component



This work has the potential to provide the basis for improved determination of the Boltzmann constant. Near-term impacts derive from the significantly improved measurement of dielectric constant for a range of gases, notably the primary constituents of natural gas.

CSTL has also used cross capacitors to measure the dielectric constants of the primary constituents of natural gas including methane, ethane, propane, nitrogen, carbon dioxide, and argon. The measurements span the 0 °C to 50°C temperature range and extend to 7 MPa. In this range, these data are more accurate than any previous

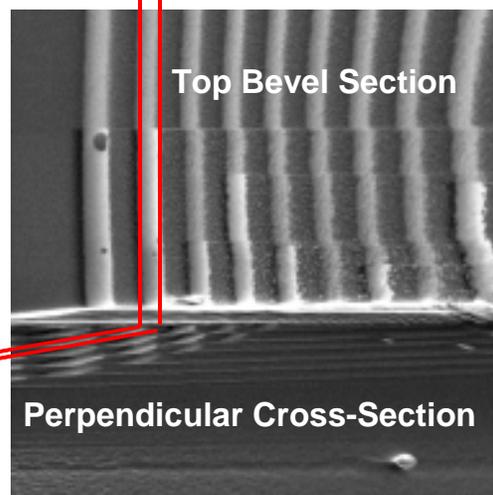
measurements and provide reference data for use in metering natural gas based on heating value. In fact, they are of sufficient accuracy, better than 0.1%, to support new approaches to on-line measurement of the heating value of natural gas with significantly greater precision and accuracy than current methods based on chromatographic constituency determination.

Bevel Craters for Surface Analysis of AlGaAs/GaAs Multilayer Structures **S.A. Wight, J.G. Gillen, and A.J. Fahey (837)**

The lateral wet-thermal oxidation of high aluminum content AlGaAs layers can be used to form buried oxide layers that are widely employed for the fabrication of vertical cavity surface emitting laser structures used in the optoelectronics industry for fiber optic data links, proximity sensors, encoders, laser range finders, laser printing, bar code scanning, and optical storage. The challenge is to analyze the variation in composition of the oxidized layers laterally and as a function of depth. The oxidized portions of the AlGaAs layers ion beam etch much more slowly than the un-oxidized AlGaAs and the interstitial GaAs layers. This complicates depth profile measurements by Auger or secondary ion mass spectrometry (SIMS). A commercial secondary ion mass spectrometer (SIMS) instrument was modified with a custom external raster waveform. This waveform was designed to deliver a variable ion dose. The desired result was a bevel crater, one that ramps down from the top surface at one edge to some depth at the other edge. This produces lateral magnification of the subsurface structure for analysis by static SIMS or scanning Auger spectroscopy. The diagonal slice through the AlGaAs/GaAs multi-layers exposes a larger portion of the oxidized AlGaAs layer for subsequent analysis. The raster profile, ion species, and ion energy were evaluated by scanning electron microscopy and Auger imaging in a commercial UHV Auger microscope. Oxygen was chosen over cesium and gallium as the primary ion species because of the

CSTL scientists are investigating improved techniques for characterization of multi-layer structures used in the optoelectronics industry.

decrease in differential sputtering. The lower energy (3 keV) oxygen was selected over higher energies because it reduced surface roughening. Low energy argon (1 keV) ion sputter cleaning is being used in the Auger microscope to remove the primary sputter ion species because oxygen is an important component in the elemental analysis.



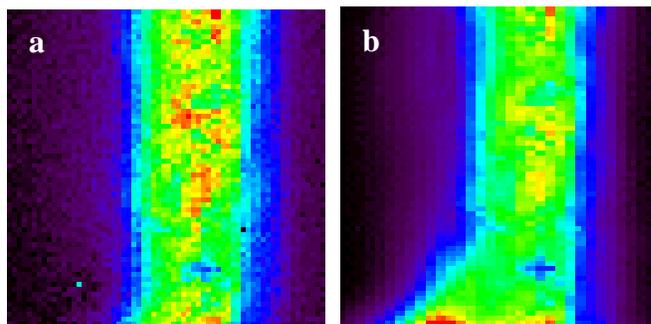
Secondary electron image of bevel exposed oxidized AlGaAs layers. Red lines follow one layer from side section to top bevel section

The development of the bevel crater has facilitated the analysis of the oxidized AlGaAs. The bevel crater technique has also been successfully applied to other multi-layer structures, such as Ni/Cr depth profile standard and KRIS sample.

Chemical Imaging of Polymer Blends with Confocal Raman Microscopy **C.A. Michaels (837)**

Confocal Raman microscopy has been used to study spatial variations in chemical composition and crystallinity for polymer blend films of interest to the industrial members of the NIST/industry polymer interface consortium (Dow Chemical, PPG Industries, Visteon, and MTS Systems). This chemical imaging technique yields data sets consisting of Raman spectra at each (x,y) sample coordinate, with diffraction limited lateral resolution ($\approx 0.5 \mu\text{m}$) and excellent depth discrimination ($\approx 5 \mu\text{m}$). The chemical selectivity of Raman spectroscopy has been exploited to characterize the spatial distribution of a high value fluoropolymer in an acrylic resin film used as a protective coating for metal substrates in building applications. Raman spectra at 785 nm excitation of the film/substrate and film/air interfaces of a blend film (50/50 mass ratio) suggest a preferential depletion of (polyvinylidene fluoride) PVDF at the substrate interface. However, images of film cross sections, as shown in Figure 1(a), where the intensity of the CF_2 stretching band at 799 cm^{-1} has been plotted, reveal no obvious PVDF gradient between the film surfaces. The markedly different surface and air interface spectra, coupled with the lack of a strong CF_2 gradient in the film cross sections, suggest that only a thin, surface depletion layer exists at the substrate interface. Figure (b) is a fluorescence image of the film cross section recorded simultaneously with that shown in figure (a), the difference in the images clearly demonstrating the chemical selectivity of Raman imaging. The sample preparation method used here is expected to result in significant surface roughness, and measurements designed to assess its impact on the spectral maps are ongoing.

CSTL scientists are examining chemical composition and crystallinity of polymer blends of interest to industrial collaborators in the NIST/industry consortium on the Characterization and Modeling of the Interfaces and Interphases of Polymeric Materials and Systems.



(a) 64x64 μm intensity map of CF_2 Raman band of PVDF/PMMA-co-PEA film cross-section. The film/substrate (air) interface is on the right (left) side of the film cross section. (b) Fluorescence intensity map acquired simultaneously with (a).

This technique has also been used to examine the relationship between thermal history and film morphology through spectral characterization of the level and spatial variation in crystallinity for polyethylene (PE) blends. A CH_2 bending vibration at 1418 cm^{-1} results from a factor group splitting due to the crystal field of the orthorhombic crystal structure and is a clean signature of crystallinity in polyethylenes. Mapping of this band intensity (normalized to an internal standard)

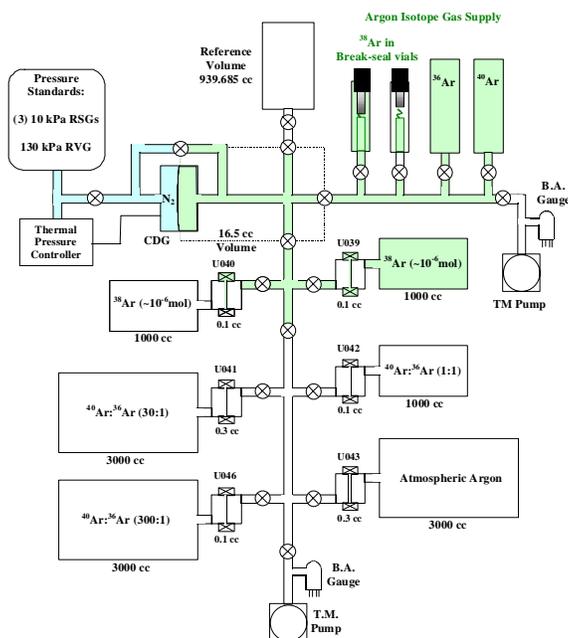
This work was recently presented at a consortium review meeting, held at PPG Industries Coatings R&D Center in Allison Park, PA. Efforts to facilitate the transfer of confocal Raman chemical imaging technology to other industrial partners through sample evaluation and interaction with visiting scientists are ongoing.

yields a crystallinity image with diffraction-limited lateral resolution and depth resolution set by the confocal parameter of the collection optics, typically on the order of $5 \mu\text{m}$. Current work is focused on samples for which the thermal processing has been optimized for production of crystallites with $1 \mu\text{m}$ to $20 \mu\text{m}$ diameters.

Development of High-Precision Isotope Reference Standards
A.P. Miiller (836)

Although conventional K/Ar dating methods are widely used for dating minerals and geologic materials, significant *interlaboratory* disagreements (1 to 2%) frequently exceed *intralaboratory* accuracies by an order of magnitude. To improve calibration of the mass spectrometers used, several high-precision argon isotope spike systems have been developed for the U.S. Geological Survey, each consisting of a reservoir and a Dorflinger pipette capable of delivering the required $(2 \text{ to } 4) \times 10^{-10}$ moles per aliquot. Two systems will deliver pure ^{38}Ar and three will deliver artificial mixtures of $^{40}\text{Ar}/^{36}\text{Ar}$ with uncertainties that are expected to be 0.1 to 0.2 % for both amount of gas delivered and for isotopic ratios of the mixtures. The performance of these systems exceeded USGS's expectations by at least a factor of two. The ultra-low fill pressures (≈ 10 Pa) proved to be the major challenge in calibrating the spike systems with requisite accuracy.

NIST is working with the U.S. Geological Survey to address the need for accurate standards to calibrate mass spectrometers at major argon-dating laboratories and thus resolve significant disagreements.



System used to calibrate the argon spike systems

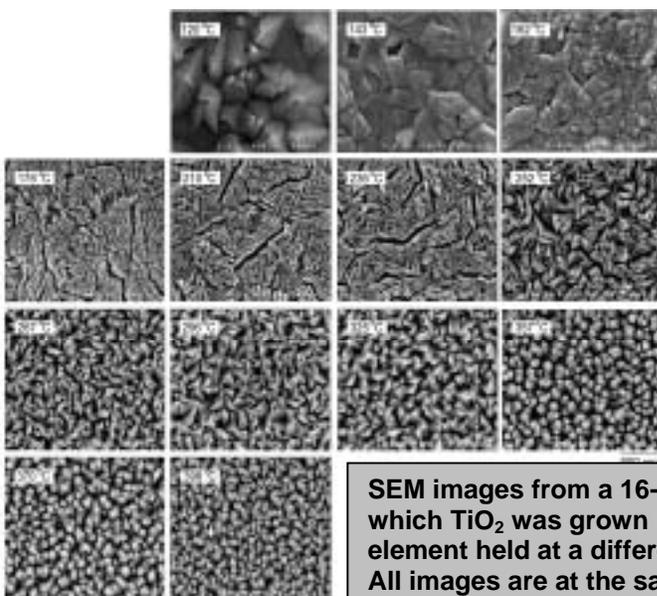
The argon spike systems will be used by USGS to measure the argon concentration of a new preparation of the monitor mineral MMhb-2. These data, when combined with high-precision ($\approx 0.2\%$) potassium concentration data from NIST, will enable the K/Ar age for MMhb-2 to be determined with a 10-fold improvement in accuracy.

The MMhb-2 will be certified by CSTL for potassium and argon concentrations and distributed as an SRM, which will be used by argon dating laboratories along with the spike systems to resolve longstanding differences. The spike systems will also enable USGS to re-measure the isotopic composition of atmospheric argon and calculate a new atomic weight for argon.

Efficient Development of Materials for Chemical Sensing Using MEMS-Based Microarrays
C.J. Taylor, D.C. Meier, and S. Semancik (836)

Special types of thin film materials are required to perform various functions within chemical microsensors and microanalytical systems. Sensing materials, for example, must be tailored to detect the specific gases encountered in chosen application areas with sufficient sensitivity, selectivity, and speed. Other examples include preconcentrator and separator materials where chemical and microstructural properties must allow preferential adsorption or reaction at large number of surface sites. A combinatorial approach using MEMS-based microarrays has been developed to efficiently deposit and screen thin film sensing materials for detection of chemical warfare agents and planetary gases.

The chemical sensing properties of metal oxide materials were explored using combinatorial methods for characterizing sensitivity, selectivity, and stability in sensing applications.



SEM images from a 16-element array in which TiO₂ was grown by CVD with each element held at a different temperature. All images are at the same magnification.

Films of gradually varied composition and microstructure are deposited on an array by holding each element of the microarray at a different temperature. Microarrays of 4, 16 and 36 thermally isolated and individually addressable microhotplate elements are used. Each $\approx 100 \mu\text{m} \times 100 \mu\text{m}$ element can rapidly measure and control temperature ($20 \text{ }^\circ\text{C}$ to $500 \text{ }^\circ\text{C}$) locally, and also monitor the electrical properties of deposited films using surface microelectrodes. The primary advantage of the approach is that a large number of different sensing films can be

fabricated and screened for sensing properties in one experiment and in a very small area. We have demonstrated this

approach for the CVD growth of TiO₂ (from titanium nitrate - see figure - and titanium isopropoxide) and SnO₂ (from tin nitrate) sensing films. The microarrays enable highly efficient



database development to correlate film processing and sensing performance. The multi-sample studies on TiO₂ and SnO₂ have proven extremely valuable in identifying deposition protocols for sensing films that perform well in a Defense Threat Reduction Agency (DTRA) -sponsored project for chemical warfare agent detection, and in a NASA-sponsored project for detecting trace gases in planetary environments. We have also examined the thermal processing of high-area porous SiO₂ structures from silsesquioxane-block copolymer blends for fabricating microscale preconcentrators.

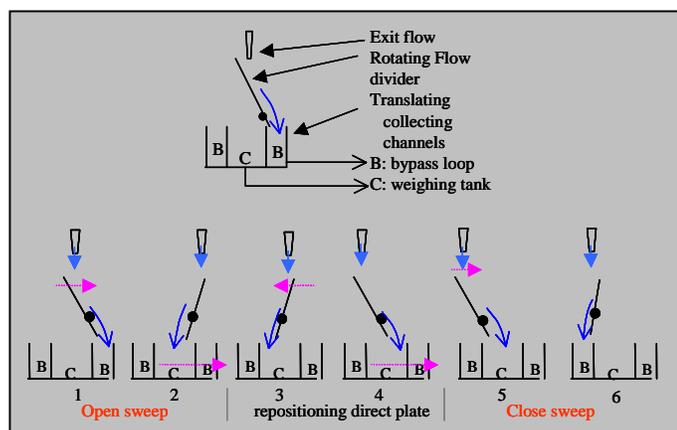
CSTL scientists have demonstrated that combinatorial microarray platforms can be used to accelerate the development of chemically functional materials for chemical sensing. The MEMS-based platforms are expected to be broadly applicable to other materials science research problems.

Error-Free Liquid Flow Diverters for Calibration Facilities

T.T. Yeh, P.I. Espina (836); and N.P. Yende (Council of Scientific and Industrial Research, South Africa)

As NIST has refurbished its water flow rate measurement facilities, research on methods to reduce this gravimetrically based system's measurement uncertainty has been performed. Diversion of the flow passing through metering devices into the system's collection tank is an operation critical in accurate measurement of the flow rate. Various techniques have been suggested to reduce diverter valve errors in commonly used valve structures. A new concept has been developed that makes use of a double-action flow diversion process whereby the components of the diverter valve error are self-canceling. This self-cancellation effect occurs when the flow accumulation for the first and second diverter valve sweeps are functional inversions, summing to the full calibration flow at corresponding times in their diversion period. Several variations in the general approach have been developed conceptually and one of these options has been chosen for testing and implementation. Initial testing indicates substantial reduction in diverter error component contribution. A complete evaluation of this approach in ≈ 50 mm pipe sizes to quantify uncertainty component contributions is planned. If reduction in component uncertainty is significant, this design will be incorporated into the refurbishment of the main water flow rate measurement facilities at NIST.

NIST is improving national liquid flow measurement standards and methods to support improved accuracy in their dissemination.



A rotating diverter valve with translating flow-directing channels

Initial testing indicates substantial reduction in diverter error component contribution. A complete evaluation of this approach in ≈ 50 mm pipe sizes to quantify uncertainty component contributions is planned. If reduction in component uncertainty is significant, this design will be incorporated into the refurbishment of the main water flow rate measurement facilities at NIST.

Reduced measurement uncertainty in liquid flows provides U.S. industry with improved accuracies and maintains U.S. leadership in flow rate metrology globally.

Hydrogen Profiling in Bulk Samples via Nuclear Analytical Techniques

H.H. Chen-Mayer, D.F.R. Mildner, G. Lamaze, and R.M. Lindstrom (839)

The ability to map hydrogen is a much sought-after goal in the field of non-destructive testing. Currently, many industrial and defense agencies, such as aircraft and auto industries, are highly active in imaging of components for inspection and reverse engineering purposes. Transmission neutron radiography has been the standard technique for imaging hydrogen in bulk samples, owing to the high penetration of neutrons in most materials. The Nuclear Methods Group is exploring various techniques for hydrogen profiling in bulk samples by combining prompt gamma activation analysis (PGAA) and neutron incoherent scattering (NIS) techniques, building on the established PGAA technique for

Hydrogen in metals presents a significant challenge both scientifically and economically. As the simplest impurity, even at $\mu\text{g/g}$ (ppm) levels it can cause structural degradation – a serious technical problem that still needs urgent solution after more than fifty years. On the other hand, for hydrogen storage, materials that allow highest hydrogen incorporation are desired.

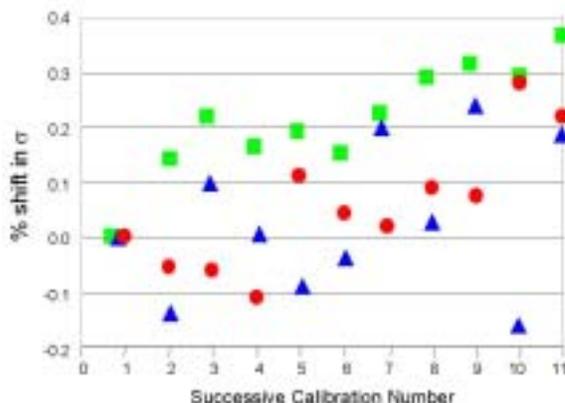
characterizing hydrogen standards prepared by volumetric hydrogen gas reaction with titanium metal.

As a proof of principle, we simulate varying concentration of hydrogen in the matrix by using polypropylene films of 7 μm thick sandwiched in a stack of 1-mm thick titanium plates. The scattered intensity from the concentrated hydrogen in the films is greater than that from the titanium plates, resulting in vertical ridges in an otherwise smooth image viewed through a 0.5 mm slit using a neutron-imaging detector. We can detect the change in the number of polypropylene films, and the change from a hydrogen-free titanium plate to one containing small amounts of hydrogen. We have established the relationship between the image intensity and the hydrogen concentration. Qualitatively these results demonstrate that the hydrogen-containing samples can be observed through a pinhole camera using NIS. Quantitative results have been obtained by image analysis that converts scattered intensity to the amount of hydrogen along the beam path, thereby achieving hydrogen profiling at a sub-mm scale and at 100 $\mu\text{g/g}$ (ppm) levels. PGAA has been used to verify the absolute concentration of H, whereas NIS provides rapid and position sensitive H detection.

The NIS technique provides hydrogen information that is difficult to obtain by other methods. Compared to transmission radiography, scattering imaging has the advantage of greater contrast and lower noise. In addition, traditional neutron radiography suffers from the limitation that the component must be inserted between the beam and the imaging detector. In an NIS imaging system the detector can be placed on any convenient location, offering maximum flexibility for fieldwork when used in conjunction with portable neutron sources.

Improved Vacuum Transfer Standards **R.F. Chang (836)**

CSTL's goal is to disseminate NIST's realization of the Pascal at high vacuum levels directly to its customers and into widely used commercial gauges. However, commercial vacuum transfer gauges are unstable and hamper efforts to effectively disseminate NIST's uncertainties to its customers. In FY02, we identified the causes of instabilities in spinning rotor gauges (SRGs), which are one of the more stable type of vacuum transfer gauges. We quantified the effect of several factors on the accommodation coefficient (σ) of SRGs, which is directly tied to gauge calibration. These factors were selected because they typify the treatment of gauges and rotors of many calibration customers after they leave NIST.



The spinning rotor gauge (SRG) has become the transfer standard of choice for vacuum calibrations from 10^{-4}Pa to 1 Pa due to good calibration stability (changes < 0.5%/year). While internal NIST SRGs exhibit even better long-term stability, some gauges of calibration customers have shown inexplicably large shifts.

Three SRGs show an average of $\approx 0.1\%$ (0.4% max.) shift in calibration stability between 11 successive mountings and de-mountings.

De-mounting and re-mounting of the suspension head caused a 0.1% shift in σ , which was comparable to the difference between baked and unbaked systems. Surprisingly, sudden loss of rotor suspension

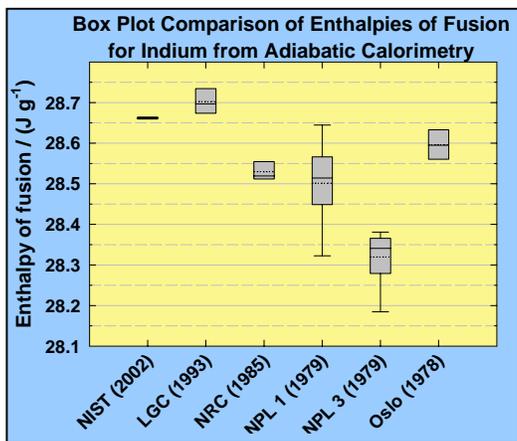
did not have any measurable effect on σ , contrary to long-held belief. This work quantified the effects of several factors for the first time, but even taken in total, they cannot explain some of the larger instabilities observed in customer gauges. Additional aspects to explore include effects of magnetic polarization and other surface treatments upon the calibration stability of SRGs. This information will likely explain the remaining large calibration shifts observed by some customers, and also provide more data to help investigate the feasibility of an on-demand SRG calibration service.

Quantitation of these effects on SRGs has not been documented before, and in fact, goes against commonly held beliefs about their performance in the mid-range vacuum community. The results can significantly affect the treatment and operation of all SRGs, and will provide the basis for developing an uncertainty budget for the proposed on-demand SRG rotor calibration service.

International Standards for Calorimetry and Thermodynamics
D.R. Kirklin and D.G. Archer (838)

Thermodynamic and calorimetric measurements have wide usage, ranging from product specification and quality control to research. Many calorimetric or thermodynamic instruments must be calibrated and methods validated for proper operation. A high-temperature adiabatic calorimeter has been made operational this year. This new instrument was used to measure enthalpies and temperatures of fusion of two certified reference materials (indium and tin) from the Physikalisch-Technische Bundesanstalt (PTB) for differential scanning calorimetry. The collaborative project with PTB was conducted under the Memorandum of Understanding signed between NIST, PTB, and Bundesanstalt für Materialforschung und -prüfung (BAM). The newly determined enthalpy of fusion value for indium has an uncertainty that is about

Use of calorimetric and thermodynamic measurements ranges from product specification and quality control of manufacturing to state-of-the-art research. In response to these needs, NIST provides at least 14 current Standard Reference Materials (SRMs) for calorimetry.



an order of magnitude better than that for the best previous measurements (see box plot). This new calorimeter will be used for certification of the temperatures and enthalpies of fusion of two more SRMs for calibration and validation of differential scanning calorimetry (DSC).



New and better measurements for the enthalpy of combustion of SRM 217d iso-octane were undertaken this year so as to provide reference data for precision and bias statements required by some organizations such as ASTM. Certifications of SRM 2233, cyclopentane for calibration of DSC, and SRM 2234, gallium for calibration of DSC, were completed this year. These two SRMs are particularly important because low-temperature cali-

These accomplishments will allow NIST customers to perform more accurate calibrations of instrumentation and perform validation determinations of measurement methods.



bration of DSC must be accomplished with organic materials, which are not generally available in sufficient purity to be used in concert with literature values of temperatures and enthalpies of phase changes.

Laying the Foundation of Tandem Mass Spectrometry (MS/MS) and Ion Trap Libraries
J.K. Klassen, Q.-L. Pu, and S.E. Stein (838)

A program has been initiated to extend the coverage of the widely used NIST Mass Spectral Library to MS/MS and ion trap mass spectra. This has involved the creation and evaluation of an MS/MS library as well as the first NIST measurements of these spectra for inclusion in the library. A diverse MS/MS collection of 1,000 spectra has been donated for use in our program by a major instrument vendor. One particular application area, pesticide analysis, has been targeted for initial development, and 600 spectra of 75 compounds have been examined on ion trap instruments over a range of collision energies. We are now actively engaged in a round-robin exercise involving these pesticides with a number of environmental laboratories. A variety of measurements on a newly acquired triple quadrupole instrument have been made for both common calibration and internal standards and simple peptides.

The existing NIST mass spectral library is composed entirely of 'classical' electron ionization mass spectra. Spectra generated by ion trap and MS/MS instruments, which are becoming widely used for chemical identification, may not match these classical spectra. Additions to the NIST library are being made to enable its use with these two varieties of instruments.

While ion trap and MS/MS spectra are routinely generated in laboratories in a wide range of application areas, reliable reference libraries are unavailable. With the addition of a limited number of carefully selected spectra to the current library, a major increase in the reliability of library searching is expected to result, especially for laboratories that are involved in the identification of small organic compounds.



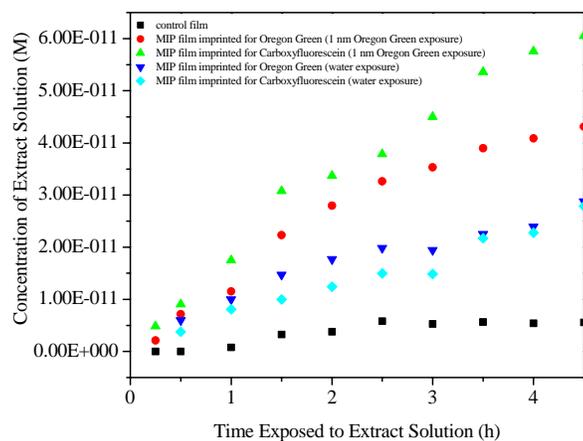
An important finding is that while the ion collision energy needs to be considered and controlled, variations in spectra between instruments are not too great to prohibit effective library searching. Also, a collection of ion trap spectra has been developed that includes specific varieties of compounds whose spectra are known to be different in ion traps than in conventional mass spectrometers. This will enable users of ion traps to be able to employ the NIST Library with fewer 'corrections' for ion trap behavior. Approximately 200 such spectra have been measured in two ion trap instruments. This work has been done in close cooperation with the two ion trap instrument manufacturers. The analyzed substances are primarily relatively small organic molecules with low ionization energies and/or high proton affinities. The work has also led to improvements in the automated extraction of mass spectra by the NIST AMDIS program.

Future Plans: The ion trap library will be tested with blind or unknown GC/MS data to ensure that the database is large enough to improve identification of ion trap spectra. An MS/MS round-robin study with selected pesticides and conditions will be undertaken to further establish limits of variability in these spectra. Available ion trap and MS/MS spectra will be made available to current NIST library users.

Molecularly Imprinted Stationary Phases for Microfluidic Chemical Separations **A.C. Henry and L.E. Locascio (839)**

The synthesis of molecular imprint polymer (MIP) stationary phases that are specific for one type of molecule is being studied as part of an effort to develop microfluidic separation techniques. Work has focused on the creation of open tubular (as opposed to packed column) stationary phases that are covalently tethered to plastic microfluidic channels. The synthesis of the MIP films incorporates surface-bound alkene groups into the polymerization of methacrylic acid (MAA) in the presence of ethylene glycol dimethacrylate (EGDMA). Prior to the polymerization, a template compound is added to the mixture. The functional groups on the template molecule can interact by hydrogen bonding, etc. with the functional groups on the MAA. After polymerization, the template molecules are extracted, leaving behind

Molecular imprinted polymer (MIP) stationary phases that are specific for a certain type of molecule may lead to separation techniques with enhanced selectivity. Potential applications include chiral molecules of interest to the pharmaceutical industry.



Extraction of Oregon Green from MIP matrix after exposure to 1 nmol/L Oregon Green in pH 7 phosphate buffer

slightly more specific for the Oregon Green molecule. This may be attributed to the structural similarities of the two molecules. When the films were exposed to Rhodamine B and then analyzed, no concentration trend was evident, implying only nonspecific surface interactions. The ultimate goal of this project is to synthesize MIP stationary phases tethered to the plastic microchannel that will allow for the separation of proteins on the basis of their quaternary and tertiary structures. In addition, this work may also lead to a benchtop separation system that will discriminate between racemates such as those regularly synthesized in the pharmaceutical industry.

CSTL researchers are developing MIP stationary phases that can be used in capillary electrochromatography separations of similar molecules. This work could significantly impact characterization of chiral pharmaceuticals because the tools being developed would be cost-effective, small, and require only minimal amounts of solvents, making them appropriate for use in high-throughput screening.

SRMs for Near Infrared (NIR) and Raman Spectroscopy

W.S. Hurst and J.E. Maslar (836); E.S. Etz (837); and S.J. Choquette (839)

Raman spectroscopy is now finding its place in the industrial environment for process measurements and quality control. The lack of accepted practices, standards and spectral libraries has been a main obstacle to the acceptance of Raman in industrial settings and is a barrier to its use in regulated industries. At the present time, Raman spectra are instrument dependent and a convolution of the spectrometer instrument response and scattering characteristics of the sample. The series of SRM 224x standards is intended to largely solve this problem. These are glasses that luminesce when irradiated with the spectrometers' laser. The shape of this luminescence is certified by NIST and is described by a simple polynomial expression. This polynomial, together with a measurement of the luminescence spectrum of the SRM, can be used to determine the spectral response correction that is unique to each instrument. This will enable the correction of a sample Raman spectrum so that it is system independent. SRM 2241 Relative Intensity Correction Standard for Raman Spectroscopy for systems operating with 785 nm excitation was certified this year. SRM 2036 is a newly introduced NIR diffuse reflectance wavelength standard. This standard provides the same level of calibration confidence as NIST's existing SRM 1920a but is easier to produce, utilizes the



same materials as SRM 2036/2065, and eliminates the strong dependence of band position on the resolution of the instrument that is inherent in the use of SRM 1920a. These new standards enable the calibration of these spectrometers at a fraction of the cost of current methods and resolve several technical issues with an existing SRM. The use of these standards will enable the development of standardized Raman spectral libraries, instrument-to-instrument comparisons, and transfer of calibration methods between labs and instruments.

Users of Raman and NIR instrumentation in the pharmaceutical community and other industries need standards that provide traceability. CSTL scientists are addressing this need through the introduction of two new SRMs.

The introduction of SRM 2241 and the next set of glasses for 532 nm, 488nm to 514 nm and 1064 nm Raman excitation sources will enable the use of this technique in industries that are regulated by the FDA. The lack of performance validation and system correction standards has inhibited this technique from being widely used in the pharmaceutical, chemical, and forensics community.

Critical Evaluation of XRF for Making Matrix-Independent Quantitative Chemical Measurements

J.R. Sieber and A.F. Marlow (839)

Research in matrix-independent X-ray fluorescence spectroscopy (XRF) methods has progressed apace of metal SRM development projects. Building on the foundation laid during Portland cement and zeolite SRM development work and the efforts of scientists at Corporation Scientifique Claisse, techniques were developed to prepare metals and alloys for borate fusion. Once the metal (chips

Matrix-independent methods of analysis, regardless of the instrumentation, offer direct traceability to the mole through calibration using ultrahigh purity materials and gravimetric preparation. This is critically important to the global metrology infrastructure. Such methods can provide the same benefits to industry labs thereby reducing the practice of calibrating with SRMs.

or powder) is oxidized, it can be fused and synthetic standards can be prepared for a matrix-independent calibration. Relative expanded uncertainty estimates as low as 0.2 % have been achieved for major and minor constituents of steel and aerospace alloys. Low uncertainty and calibration using high-purity compounds makes the results directly traceable to the mole.



New Version of the NIST Electron Elastic-Scattering Cross-Section Database

C.J. Powell (837); A. Jablonski (Polish Institute of Physical Chemistry); and F. Salvat (Univ. of Barcelona)

Cross sections for the elastic scattering of electrons by atoms are needed for modeling the transport of electrons in materials by Monte Carlo simulations and other means. Such transport calculations are needed to improve the accuracy of surface analyses, bulk analyses, and thin-film analyses of inhomogeneous samples by the techniques of Auger-electron spectroscopy (AES), X-

ray photoelectron spectroscopy (XPS), electron-probe microanalysis (EPMA), and analytical electron microscopy (AEM). The cross sections are also needed for similar transport calculations in other applications including radiation dosimetry, radiation therapy, radiation processing, radiation protection, and electron-beam lithography.

Reference data is needed to improve the accuracy of surface analyses by Auger-electron spectroscopy and X-ray photoelectron spectroscopy and for bulk and thin-film analyses by electron-probe microanalysis and analytical electron microscopy.

NIST Scientific and Technical Databases

A new version of the NIST Electron Elastic-Scattering Cross Section Database (SRD 64) was completed in FY2002 and is expected to be released in early FY2003. This version (Version 3.0) contains two major changes

from Version 2.0. First, the cross sections and related data were calculated from a relativistic Dirac partial-wave analysis in which the potentials were obtained from Dirac-Hartree-Fock electron densities computed self-consistently. This potential is believed to be more reliable than the Thomas-Fermi-Dirac potential used previously. Second, the upper energy limit for the cross-section data has been extended from 20 keV to 300 keV. As a result, the database should be useful for a wider range of materials-characterization applications.

This reference data should make it possible to make more accurate simulations of electron transport to provide more reliable analyses of inhomogeneous specimens by AES, XPS, EPMA, and AEM. There will be similar benefits for other applications such as radiation dosimetry and electron-beam lithography.

Odd/Even Chain Length Effects on Solute Retention in Reversed-Phase Liquid Chromatography

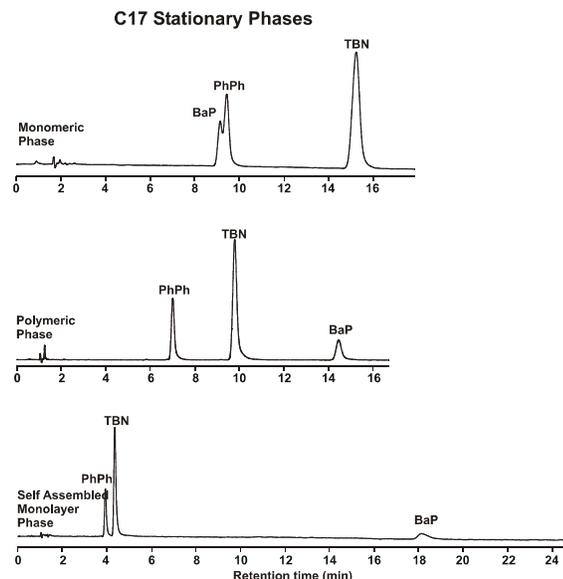
L.C. Sander and C.A. Rimmer (839)

Chromatographic separation of organic compounds in complex sample matrixes is often a requisite to the accurate and reproducible measurement of targeted species for applications such as healthcare, food and nutrition, and the environment. Although liquid chromatography (LC) is a mature analytical technique, separations are often developed from empirical observation rather than theory, and solute retention remains a topic of active consideration. As part of an ongoing effort to understand fundamental chromatographic processes in LC, a series of columns were prepared to probe relationships between solute retention and stationary phase properties.

CSTL scientists are studying fundamental processes in liquid chromatography (LC), with the goal of improved analytical methods for applications in healthcare, food and nutrition, and the environment.

Stationary phases were synthesized with alkyl chain lengths ranging from 13 to 18 carbons, by three different synthetic approaches (monomeric, polymeric, and self-assembled monolayer). In addition to the effect of chain length, the influence of temperature, bonding density, and bonding chemistry on chromatographic performance was evaluated by use of SRM 869a Column Selectivity Test Mixture for Liquid Chromatography.

The phases were characterized in terms of methylene selectivity, shape selectivity, and band broadening. No significant chromatographic differences were found to result from the synthetic routes, except that the different bonding chemistries provide a different range of bonding densities. The approach used in bonded phase synthesis limits the density of the alkyl chains that can be achieved. For bonding densities ranging from 2 $\mu\text{mol}/\text{m}^2$ to 8 $\mu\text{mol}/\text{m}^2$, a linear increase in methylene selectivity was observed with increasing bonding density. Longer chain length columns with higher bonding



This work has resulted in an improved understanding of solute retention mechanisms in LC which will facilitate the development of improved analytical methods based on knowledge of column properties (rather than a trial and error approach). Ultimately, measurement precision and accuracy may improve for relevant analytical problems.

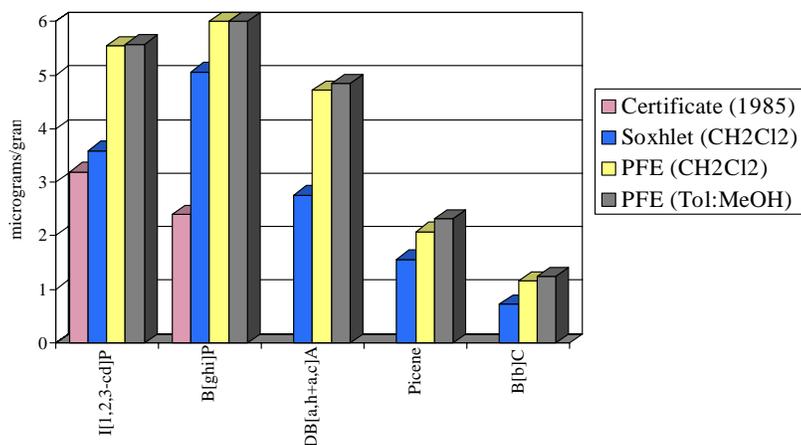
densities provided enhanced selectivity for shape-constrained solutes. These findings may facilitate the development of improved analytical methods for classes of analytes such as vitamins (carotenoids, retinoids, tocopherols), biomolecules (steroids) and environmental contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyl congeners).

Assessment of Pressurized Fluid Extraction for Enhanced Extraction of Environmental Samples

M.M. Schantz, B.A. Benner, Jr., D.L. Poster, and S.A. Wise (839)

Until recently, Soxhlet extraction with a variety of solvents was the only extraction method employed for certification measurements of organic constituents in natural environmental-matrix SRMs such as sediment, tissue, and particulate matter. The typical Soxhlet extraction requires 16 h to 20 h of extraction time using 200 mL to 300 mL of solvent. With the demand for reduced solvent usage and faster analyses, alternative extraction techniques have been investigated. Extraction methods such as supercritical fluid extraction (SFE) and pressurized fluid extraction (PFE) have been evaluated and compared to traditional Soxhlet extraction for the determination of organic contaminants such as polychlorinated biphenyls (PCBs), chlorinated pesticides, and polycyclic aromatic hydrocarbons (PAHs) in environmental-matrix SRMs. SFE typically uses carbon dioxide with the addition of more polar solvents such as methanol as modifiers; PFE uses conventional liquid solvents at elevated temperatures and pressures (100 °C to 200 °C and 7 MPa to 14 MPa). The purpose of these investigations was to demonstrate whether SFE and PFE were comparable to Soxhlet extraction for the determination of organic contaminants in natural environmental matrices and as such could they be used in the certification of such natural-matrix SRMs.

Traditional Soxhlet extraction is time-consuming and requires large volumes of solvents. CSTL scientists are investigating alternative extraction methods for natural-matrix SRMs.



Comparison of Soxhlet extraction and PFE for extraction of higher molecular weight PAHs in SRM 1650 Diesel Particulate Matter

matter. For these matrices, the comparability of PFE to Soxhlet extraction for the determination of PAHs, PCBs, and chlorinated pesticides was demonstrated. For the diesel materials, PFE showed greater extraction efficiencies by about 20% than Soxhlet extraction for the higher molecular weight (MW) PAHs, such as indeno[1,2,3-cd]pyrene (MW 276), benzo[ghi]perylene (MW 276), dibenz[a,h + a,c]anthracene (MW 278), picene (MW 278), and benzo[b]chrysene (MW 278), as shown in the figure for SRM 1650 (Diesel Particulate Matter).

PFE has now been incorporated into the analytical measurement scheme for the certification of environmental-matrix SRMs and has been used to assess the microhomogeneity of these SRMs. PFE is now being evaluated for use in the extraction and measurement of active and/or marker organic constituents in botanical dietary supplements.

PFE has also been demonstrated to be useful for the extraction of microsamples (as small as several mg), which allows for the determination of the microhomogeneity of these natural-matrix SRMs.

Ruggedness Testing of HP-ICP-OES for Single and Multielement Determinations ***M.L. Salit and G.C. Turk (839)***

A high-performance approach to the measurement of elemental composition using inductively coupled plasma optical emission spectroscopy was developed at NIST several years ago in order to make detailed comparisons of elemental calibration solutions. This technique uses an innovative experiment design coupled with a novel drift correction approach to minimize and quantify the major sources of uncertainty in this chemical analysis. The performance of this method, *High-Performance Inductively Coupled Plasma Optical Emission Spectrometry* (HP-ICP-OES) was recently documented for 64 elements, where the typical relative expanded uncertainty observed is 0.1% (*Analytical Chemistry* **2001**, 73, 4821-4829). This technique utilizes unmodified, commercially available equipment, is well suited to automation, and yields results comparable to the best attainable by so-called

CSTL researchers are expanding the applicability of HP-ICP-OES to measurement problems in optoelectronics and high-purity metals, and transferring this measurement capability to laboratories outside of NIST, including precious metal refiners and commercial standards producers.

“classical” methods, the craft- and labor-intensive benchmarks of best practice. HP-ICP-OES is now being expanded beyond the original intended application to multielement analyses and complex matrix samples. In the past year the technique has been successfully applied to the analysis of compound semiconductors for optoelectronics, high-purity metals, and platinum group metals both at NIST and at private-sector laboratories. We have worked closely with laboratories wishing to duplicate our capabilities, and have developed software tools to enable this technology transfer. Using their existing instrumentation, these laboratories have seen order-of-magnitude reductions in their measurement uncertainties, with dramatic impact on their laboratory operations.

The implementation of HP-ICP-OES is only in its infancy, but comments received from collaborators outside of NIST indicate that the implementation of HP-ICP-OES has had a very favorable impact on their operations.

METALOR[®]

According to Metalor “ the NIST-developed HP-ICPOES technique has revolutionized our Platinum Group Metal (PGM) assay lab. Assay completion times that ran 20 to 30 days now average 4 to 6 days. Assay precisions have improved by more than an order of magnitude. Accuracy has greatly improved...”

SpectroML—an Extensible Markup Language for Molecular Spectroscopy Data
A. Arslan, A.D. Nguyen, J.C. Travis, R. Schäfer, and G.W. Kramer (839)

The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. However, in the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange beyond the capabilities of these protocols.

Multiple, incompatible data formats have hindered the interchange and storage of analytical chemistry data. An XML-based markup language is being developed by NIST scientists to facilitate data exchange.

Today’s analytical chemists need not only to interchange data generated within a specific technique, they need to interchange, import, export, store, and combine all their data from multiple

sources at multiple sites. Users are now no longer content with just having “pictures” of their data (i.e., data plots). They need real access to their data from within a report itself—they want to be



able to expand plots, re-integrate peaks, overlay spectra, etc. without having to revert to the instrument or computer system that generated the original data. In industrial settings, experimental data belong to and must be accessible to the entire organization, not just the laboratory personnel who generate it. Proprietary data formats and existing interchange programs simply cannot fill such corporate needs for data accessibility.

We have created an XML-based markup language for UV/visible data called SpectroML and are applying it to the interchange of data from our spectrometers and our optical filters database. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectrometry and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers among ourselves, our colleagues at other national metrology institutes, and our NIST-Traceable Reference Material (NTRM) vendors. We have worked with the ASTM E13 Molecular Spectrometry and Chromatography Committee and representatives from a number of instrument companies to create a task group for developing a unified XML-based approach for interchanging molecular spectrometry and chromatography data. This year we completed the software to convert the data

files from our reference spectrometer (HAS-II) to SpectroML and are currently working to do the same for the Perkin Elmer Lambda 900 instrument. We have recently begun the task of interfacing SpectroML to our optical filters database. With SpectroML as the *lingua franca*, we will need only one application to interface all our instruments to the database. When this software is in place, the task of quality assurance monitoring for our NTRM optical filters program will be automatable.

The development of an XML-based universal molecular spectrometry and chromatography markup language could revolutionize data interchange, eliminate long standing barriers to data exchange, enable new data manipulation software, facilitate new web-based notions for data usage, and permit the development of realistic schemes for long-term data archival.

Transducer-Assisted Piston Gauge Calibrations

D.A. Olson (836) and T. Kobata (National Metrology Institute of Japan)

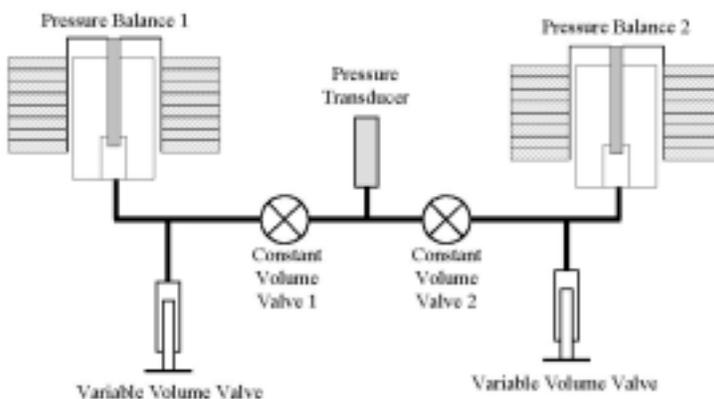
Existing piston gauge calibration methods require a great deal of operator skill and experience to find a pressure balance point and render a decision about when the equilibrium is “sufficient” so that the next datum can be set and acquired. Our objective is to improve piston gauge calibrations by

NIST is improving its calibration services through the development of a new system for pressure gauge calibration. This system reduces the dependence on operator judgment and provides more consistency through automation.

reducing or removing operator judgment and manual data entry, and introduce more consistency through automation, with no penalty in overall uncertainty. To meet this objective, a method to automate the data-gathering process in the calibration of one piston gauge against another has been developed. Rather than adjusting



small “trim” masses until the balances generate equal pressure, the two balances are brought into approximate pressure equilibrium and a pressure transducer measures the remaining pressure difference. The transducer-assisted traditional fall-rate methods have been demonstrated to agree with the combined standard uncertainty of the methods in nearly all cases, and to within the combined expanded uncertainty (k=2) for all cases. The combined standard uncertainties for this method are 53 Pa to 112 Pa, making them on the same order as the resolution of the pressure transducer (56 Pa). The relative combined standard uncertainties are 3.6×10^{-6} or less at 20 MPa, and 1.1×10^{-6} or less above 50 MPa, and compare quite favorably with the relative combined standard uncertainty of the NIST Transfer Standard at 280 MPa, which is 16×10^{-6} (k=1). To keep the uncertainty in the method comparable to the uncertainty of the fall rate method, the pressure difference between the two piston gauges, in this case, was $|\Delta P| \leq 10^5$ Pa, which keeps the transducer uncertainty equal to or less than other component uncertainties. An unexpected advantage of the transducer-assisted method is far greater sensitivity over extant methods, which also allows us to observe



Schematic of hardware set-up for Transducer Assisted Calibration

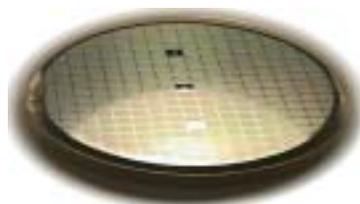
small “trim” masses until the balances generate equal pressure, the two balances are brought into approximate pressure equilibrium and a pressure transducer measures the remaining pressure difference. The transducer-assisted traditional fall-rate methods have been demonstrated to agree with the combined standard uncertainty of the methods in nearly all cases, and to within the combined expanded uncertainty (k=2) for all cases. The combined standard uncertainties for this method are 53 Pa to 112 Pa, making them on the same order as the resolution of the pressure transducer (56 Pa). The relative combined standard uncertainties are 3.6×10^{-6} or less at 20 MPa, and 1.1×10^{-6} or less above 50 MPa, and compare quite favorably with the relative combined standard uncertainty of the NIST Transfer Standard at 280 MPa, which is 16×10^{-6} (k=1). To keep the uncertainty in the method comparable to the uncertainty of the fall rate method, the pressure difference between the two piston gauges, in this case, was $|\Delta P| \leq 10^5$ Pa, which keeps the transducer uncertainty equal to or less than other component uncertainties. An unexpected advantage of the transducer-assisted method is far greater sensitivity over extant methods, which also allows us to observe

2nd-order effects such as effective area dependence on piston stroke height. Thus, we have achieved our objective to improve piston gauge calibrations and introduce more consistency through automation, with no penalty in overall uncertainty.



The transducer-assisted method has already impacted NIST calibration services and allowed us to discover systematic problems in a recently purchased piston gauge that could not have been noticed using traditional calibration methods. Widespread adoption of this straightforward method will provide piston gauge manufacturers a far more sensitive diagnostic tool than ever before available, and could culminate in substantive performance improvements for this mature field.

11. Microelectronics



CSTL supports the microelectronics industry by providing the necessary physical standards for process control of parameters such as temperature and humidity. CSTL also provides reference materials to facilitate quality control in manufacturing, as well as providing reliable kinetic and thermophysical property data to facilitate process modeling, to meet critical industry needs. CSTL research remains on the cutting edge in order to provide dramatically improved measurement tools to keep advanced U.S. microelectronics manufacturing globally competitive.

The U.S. electronic component industry has grown in recent years and shipments are expected to exceed \$300 billion by 2004.

Completion of Suite of Dopants in Silicon Standards

R.L. Paul, R.R. Greenberg, R.M. Lindstrom (839); and D.S. Simons (837)

The U.S. semiconductor industry relies heavily on secondary ion mass spectrometry (SIMS) for characterization of the depth distribution of critical dopants (boron, arsenic, and phosphorus) in silicon. To achieve high accuracy in the concentration determination by SIMS, standards of known dopant concentration, conveniently provided by ion implants of certified dose, are required. International SEMATECH (a consortium of semiconductor manufacturers) recently listed SRMs of implanted phosphorus, boron, and arsenic in silicon as a high-priority need. Standard Reference Materials of boron and arsenic implants in silicon (SRMs 2137 and 2134) have previously been developed by NIST for use as SIMS calibration standards. In pursuit of a phosphorus in silicon SRM, a radiochemical neutron activation analysis (RNAA) procedure was developed, critically evaluated, and shown to have the necessary sensitivity, chemical specificity, matrix independence, and precision to certify phosphorus at ion implantation levels in silicon. A phosphorus-in-silicon SRM (SRM 2133) was prepared by implantation of a 200 mm diameter silicon wafer. The wafer was cut into 1 cm² pieces for analysis by RNAA. All potential sources of uncertainty were evaluated in order to establish RNAA as a primary method for certification. The method was used to certify the phosphorus concentration of SRM 2133 Phosphorus Implanted Silicon as 9.55×10^{14} atoms·cm⁻² ± 0.12×10^{14} atoms·cm⁻².

Ion-implanted standards of known dose for B, As, and P are needed for accurate concentration-depth profiles of these dopants in silicon.

The three SRM implants in silicon will allow the semiconductor industry to achieve low systematic error for dopant concentration measurements as specified in the 2001 International Technology Roadmap for Semiconductors.

Development of Process Monitoring and Diagnostic Techniques

M.A. Sobolewski, K.L. Steffens (836); and E.C. Benck (PL)

Plasma processing reactors have historically been designed and operated using empirical methods alone, but continued evolution of these tools requires a much greater reliance on process and reactor modeling. Indeed, model-based process design and control is an important need identified in the *National Technology Roadmap for Semiconductors*. To obtain more reliable predictions of the spatial uniformity, chemistry, and electrical properties of processing plasmas, further progress in model development and validation is

Advanced measurement methods, data, and models are needed to characterize plasma etching and deposition processes important to the semiconductor industry, enabling continued progress in process optimization, process control, and model-based reactor design.

required. Also, to enable improvements in process control, a need exists to develop sensors that are compatible with the manufacturing environment.

Measurement techniques, data, and models provided by NIST assist industry in improving their plasma modeling and characterization efforts.

In dielectric etching plasmas, CF is an important species to understand because it is thought to participate in the formation of the fluorocarbon polymer layer which enables selective etching of SiO₂. Planar laser-induced fluorescence (PLIF) has been used to measure two-dimensional density maps of CF as a function of power and pressure in CF₄/O₂ and C₄F₈



Plasma processing reactor

plasmas and as a function of power in CF₄ with and without a Si wafer. Spatial variations in plasma temperature can cause spatial differences in gas density and reaction rates. Thus, understanding gas temperature in etching plasmas is also important. The technique of PLIF of the CF radical has been extended to enable two-dimensional temperature mapping in fluorocarbon plasmas. Temperature maps have been measured in CF₄ plasmas from 27 Pa (200 mTorr) to 107 Pa (800 mTorr). Temperature spatial variations of up to 150 K have been observed in these plasmas. Temperature increases with pressure and power is lowest near the cooled electrode surfaces.

Development of SiGe Compositional Standards for the Semiconductor Industry
R.B. Marinenko, J.T. Armstrong, S. Turner, E.B. Steel, and D.S. Simons (837)

SiGe technology is having a profound impact on the wireless and computer industries enabling the development of electronic components that are smaller, faster, less noisy, require less power, and are nearly as inexpensive to produce as silicon components with little retooling. With this new technology has come the need

Next-generation computer chips and most current cellular telephones are based on SiGe technology. Industry has requested a compositional standard to meet this growing industry usage.

CSTL scientists are providing the semiconductor industry with a needed SiGe bulk reference material that is the first step in providing the thin-film reference material it needs for the manufacture of SiGe wireless and next-generation microprocessor components.

to characterize the thin films used in the new devices.

We are working with the semiconductor industry to use the electron probe microanalyzer (EPMA), Auger analysis, analytical electron microscopy (AEM), and secondary ion mass spectrometry (SIMS) to develop bulk and thin-film materials that can be used as compositional standards for SiGe thin film manufacturing.

Bulk SiGe wafers cut from single-crystal boules were evaluated with the EPMA for micro- and macroheterogeneity for use as primary standards for future characterization of SiGe thin films on Si that are needed by the microelectronics industry as reference standards. Specimens with nominal atom fractions of 14 % Ge and 6.5 % Ge were rigorously tested with wavelength dispersive spectrometers (WDS) using multiple point, multiple sample, and duplicate data acquisitions. These data were compared to pure Si and Ge wafers tested under the same experimental

<i>Heterogeneity Test for Si, Ge, and SiGe Wafers</i>				
Expanded Uncertainty (99%) mass fraction (in %)				
Sample	SiK1	SiK5	GeL1	GeL5
StdWafers		0.94%	1.36%	0.60%
SiGe14	1.22%	1.29%	1.38%	1.55%
SiGe6.5	1.75%	1.55%	4.67%	4.64%

procedures. The SiGe14 had the least heterogeneity with an overall expanded uncertainty (99%) in the mass fraction of 1.6 % or less for both elements while the SiGe6.5 specimen had an expanded uncertainty in the mass fraction as large as 4.7 % for Ge and less than 1.8 % for Si.

Critical Evaluation of GDOES for Depth Profiling of Layered Materials

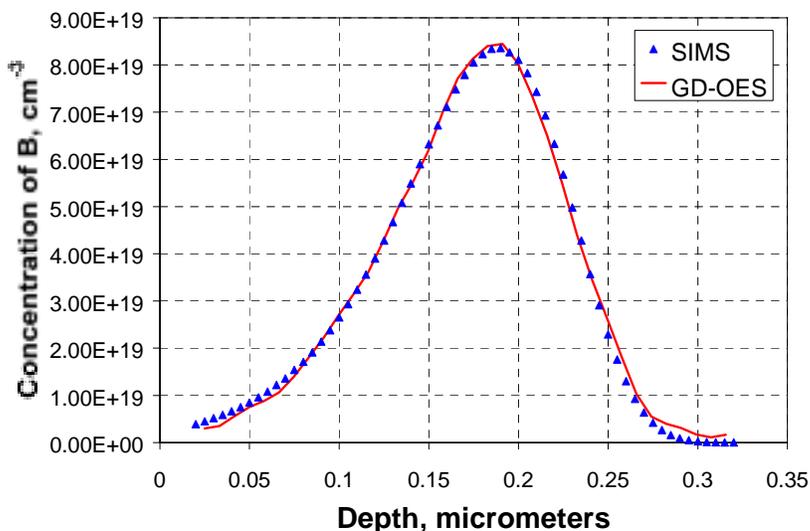
M.R. Winchester (839)

While the quantitative determination of elemental composition as a function of depth is likely to become even more important in the future, this remains a difficult, time consuming, and expensive metrological problem. Consequently, there is a need for better, faster, and more economical characterization methods. At NIST, glow discharge optical emission spectrometry (GDOES) is being developed for this purpose. GDOES is inherently much faster and much less expensive than most competing methods of surface analysis.

The quantitative determination of elemental composition as a function of depth on surfaces is an important analytical problem in a wide variety of modern technologies.

The capabilities of GDOES depth profiling are demonstrated through the development of a GDOES method for the characterization of SRM 2137 Boron Implant in Silicon Standard for Calibration of Concentration in a Depth Profile. This sample consists of a single crystal Si substrate into which ^{10}B has

been implanted at a nominal energy of 50 keV. The surface was rendered disordered by Si ion implantation prior to the implantation of ^{10}B . A GDOES depth profile obtained for SRM 2137 is shown in the figure. The corresponding Secondary Ion Mass Spectrometry (SIMS) depth profile is also shown for comparison. The GDOES data were acquired at glow discharge conditions of 1000 V / 30 mA using a sampling frequency of 5 Hz. Five individual depth profiles were coadded, in order to improve the signal-to-noise



ratio. A low-pass Fast Fourier Transform (FFT) was then applied to the coadded profile for smoothing. The GDOES concentration axis was not calibrated for this work, but was simply adjusted to allow the overall shape of the depth profile to be compared with the quantitative SIMS data. The GDOES depth axis was calibrated using independent measurements of the sputtering rate performed via profilometry. The results are encouraging, in that the GDOES depth profile matches very well previous depth profiles obtained using SIMS. This points out the potential usefulness of GDOES depth profiling. Once fully developed, GDOES should provide United States industry with a useful metrological tool, potentially affecting many industries that rely upon thin films, coatings, and other surface layers.

CSTL researchers are working to improve the analytical capabilities of GDOES, especially with regard to analytical sensitivity, depth resolution, and the accuracies of the concentration and depth axes.

Critical Evaluation of NDP and PGAA for Determination of Boron in Silicon
G. Lamaze, H.H. Chen-Mayer, R.M. Lindstrom, E.A. Mackey, and R.L. Paul (839)

A critical evaluation of Neutron Depth Profiling (NDP) and Prompt Gamma Activation Analysis (PGAA) has been undertaken to determine the state of uncertainties of these methods with the goal of identifying significant sources of error that can be reduced in the future. Additionally, new values of the minimum detectable amounts of boron in silicon have been established. In

Because nuclear methods are able to provide quantitation in a non-destructive measurement, they provide cornerstones for other measurement techniques.

The establishment of a complete uncertainty budget for both NDP and PGAA allows the method to be quantitatively compared with other analysis methods. Establishing minimum detection limits for boron in silicon allows these techniques to fit into the semiconductor roadmap.

NDP, experimental measurements of stopping powers have led to new values that are about 5% different from literature values, thereby reducing uncertainties from that source. The improved values of the stopping power lead to increased accuracy in the determination of film thickness and density by the NDP method. PGAA has established a new lower limit of detection for boron in silicon that is 3 ng/g. We reported complete uncertainty budgets at the 2002 Winter Meeting of the American Nuclear Society in Washington. The establishment of a complete uncertainty budget for both NDP and PGAA allows the method to be quantitatively compared with other analysis methods. Establishing

minimum detection limits for boron in silicon allows customers to know immediately whether our techniques are applicable for their needs. Future work includes a new cold neutron beam line for both PGAA and NDP to be installed in the near future that will lower backgrounds significantly and should lead to both lower detection limits and lower uncertainties. This will also lead to a new NDP lower detection limit of boron of 5×10^{15} at/cm³ (approximately 40 ng/g in bulk samples).



Far-Ultraviolet Spectra of Compounds Important in Next-Generation Lithography
V.L. Orkin (838)

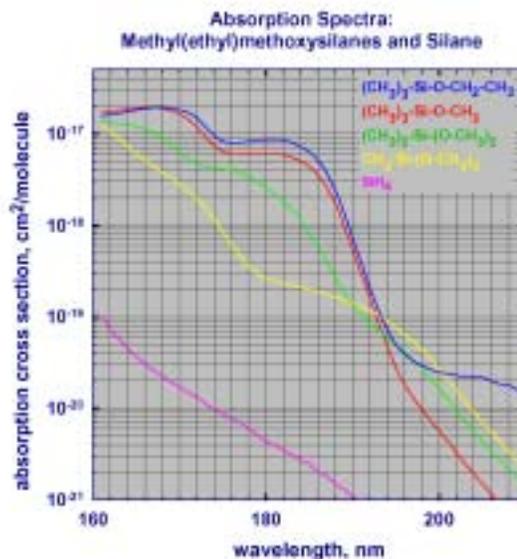
Atmospheric pressure deep UV lithography using fast chemically amplified photoresists will be the mainstay of semiconductor production into the foreseeable future. The development of materials and techniques for both 193 nm (based on ArF excimer lasers) and 157 nm (based on F₂ excimer lasers) exposure tools for UV lithography should soon become available. A challenge for the utilization of these shorter wavelengths is photodeposition on the surfaces of the many lenses required to focus and direct the light. These deposits cause light scattering and poorer imaging. Although the problem has been observed to some extent at longer wavelengths, it becomes very serious for the deep UV radiation. The control of this problem requires a quantitative understanding of the photochemical properties of the gases that are likely to be present in the lithography apparatus. To help address these problems, we have measured the deep UV spectra of a set of silicone-containing compounds likely to be used in next-generation lithography.

Important contamination sources in lithography were chosen for study based on input from the first Lithography Contamination Control and Monitoring Roadmap Workshop.

The absorption spectra of nine different silicone-containing compounds were measured down to 160 nm (the limit of our apparatus). With the exception of silane, all the Si-containing gases studied exhibit very strong absorption at shorter wavelengths. At 193 nm, the absorption cross sections are relatively small, except for hexamethyldisilazane. For hexamethyldisiloxane and the alkyl-methoxysilanes, the absorption cross section increases by more than two orders of magnitude between 193 nm and 160 nm.

The results of this work provide the necessary background information that will allow plans for the design of next-generation lithography equipment to proceed with quantitative information on the photochemical behavior of this compound set.

<http://www.nist.gov/kinetics/spectra>

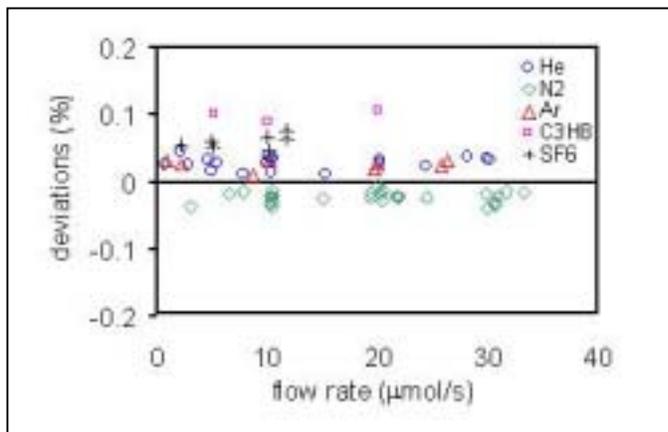


Measurements for Vacuum Process Control R.F. Berg (836)

The semiconductor electronics industry uses thermal mass flow controllers (MFCs) to control processes such as plasma etching. A wide variety of toxic, flammable, and corrosive gases are used at flow rates below 1000 $\mu\text{mol/s}$ (1 $\mu\text{mol/s} \approx 1.3$ standard cm^3/min .). This variety challenges the companies that manufacture and calibrate MFCs.

NIST is working with the semiconductor industry to address the need for more accurate MFC performance.

A few years ago, participants at an MFC workshop at NIST predicted that industry requirements for flow uncertainty will fall below 1%, and they called for corresponding improvements of national standards. In response, we are developing and improving standards for gas flow in the range from 0.01 $\mu\text{mol/s}$ to 1000 $\mu\text{mol/s}$. We have developed a flow metering approach based on capillaries as a transfer standard having improved reproducibility. The capillary flow meter (CFM) is a second-generation transfer standard whose flow elements consist of long, coiled, quartz capillaries. A hydrodynamic model of the CFM standard has been developed and validated against two primary flow standards. Tests with nitrogen agreed with both primary standards at flow rates from 0.1 to 1000 $\mu\text{mol/s}$. Tests with other gases verified that the model correctly handles gases with widely varying thermophysical properties. A comparison with Italy's metrology institute (IMGC) showed agreement to within 0.1% from 0.2 $\mu\text{mol/s}$ to 800 $\mu\text{mol/s}$. Flow comparisons with two U.S. manufacturers of gas flow



standards tested each company's primary standards to high accuracy and demonstrated NIST's emerging capability to measure small gas flow rates with excellent precision and accuracy.

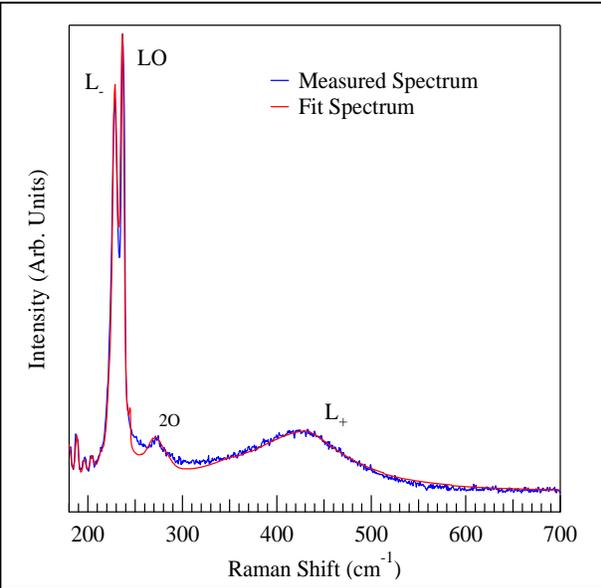
The improved model of the CFM extends the transfer standard's usefulness to a wide variety of gases. One of the domestic flow comparisons helped identify an important improvement for the company's flow standard.

Non-Contact Free Carrier Density Measurements for Compound Semiconductors **J.E. Maslar and W.S. Hurst (836)**

Transport of free carriers is central to the operation of all optoelectronic devices and reliable measurement of the carrier properties is critical. Hall or capacitance-voltage measurements are traditionally used to obtain this information, but require electrical contact. This precludes their use *in situ* during growth or processing and, typically, even on actual device layers. Raman spectroscopy, as an optical technique that can be used for transport property determination, does not suffer from these limitations. In addition, it is non-destructive, spatially resolved, and can be applied to a specific buried layer, sometimes a problem for traditional electrical measurements. A number of issues exist that are central to determining the accuracy and precision of this method, including the semiconductor under investigation, measurement system parameters, and Raman spectral model used to fit the measured spectra. NIST is systematically addressing such issues.

Raman spectroscopic techniques provide a nondestructive means of determining the carrier concentration of semiconductor materials.

Raman spectroscopic systems, optimized for narrow and wide band gap materials, were assembled and used to measure the Raman spectra of n-type doped GaN, GaAs, and GaSb films. An example of the measured Raman spectrum for n-type GaSb is given in the figure. Fitting the data with a Raman spectral model results in a determination of carrier concentration of $\approx 6 \times 10^{18} \text{ cm}^{-3}$. Relatively simple spectral models were developed and employed to determine free carrier concentrations from the experimental Raman spectra.



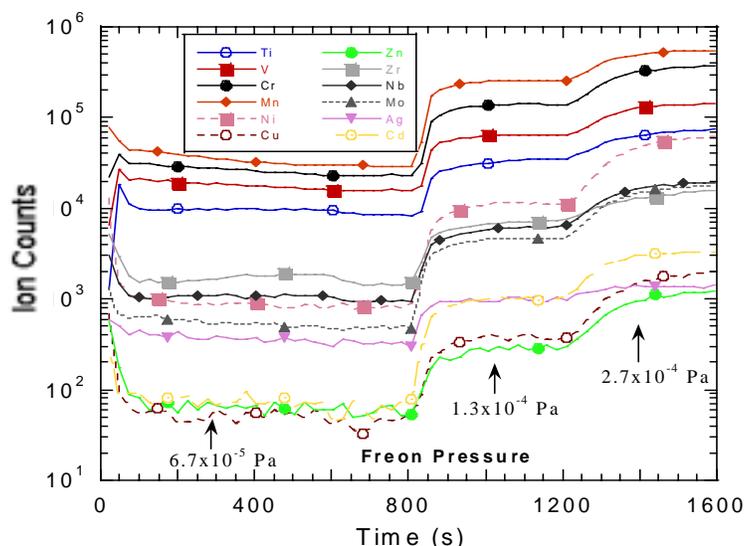
This project facilitates the utilization of Raman spectroscopy for spatially resolved off-line characterization, as well as process monitoring and control, during film growth and etch processing. A potential user should be able to determine the expected accuracy and precision for a given material, set of measurement conditions, and Raman spectral model.

CSTL scientists are examining the precision and accuracy of Raman spectroscopy to facilitate its use for semiconductor characterization.

Preliminary Evaluation of Positive Secondary Ion Yield Enhancements Using Chlorodifluoromethane and Tetrachloroethene Backfilling
P. Chi and J.G. Gillen (837)

Positive secondary ion yields in Secondary Ion Mass Spectrometry (SIMS) are strongly enhanced by the presence of reactive gas species. Oxygen primary ion bombardment or oxygen gas backfilling of the analytical chamber is commonly used for this purpose. However, for some metal elements such as Nb, Mo, Ag, and Cd, oxygen bombardment or gas backfilling does not enhance the ion yields. Recently, experiments using SF_5^+ primary ions suggested that a fluorinated sample surface might provide some level of secondary ion signal enhancement. However, SF_5^+ primary ion beams are not generally available on commercial SIMS instruments. This led us to explore alternative methods for enhancing ion yields by backfilling the sample surface with either chlorodifluoromethane (freon) or tetrachloroethene (C_2Cl_4) gas while depth profiling with an argon primary ion beam.

The figure shows a depth profile of 12 metal ion species at various freon backfill pressures. Most elemental secondary ion signals increased by one order of magnitude as the freon backfill pressure was increased from the 6.7×10^{-5} Pa to 2.7×10^{-4} Pa.



Element	Ion yields for Freon backfill norm. to O_2 backfill	Ion yields for Tetrachloroethene backfill norm. to O_2 backfill
Mg	52	25.8
Al	0.7	0.62
Si	1	0.58
Ti	2.5	0.97
V	3.4	1.72
Cr	2.8	1.31
Fe	0.8	0.53
Mn	2.7	1.40
Ni	1.1	0.41
Cu	0.4	0.35
Zn	4.9	1.51
Nb	8.9	1.50
Mo	N/A	3.22
Ag	5.0	4.30
Cd	10	7.70

Ion yield enhancements for 15 metal species under 2.7×10^{-4} Pa freon and tetrachloroethene backfilling are listed in the table. The yields for Al, Fe, and Cu are lower while Si and Ni are unchanged under freon backfill as compared with oxygen backfill. However, the yield for Mg, Ti, V, Cr, Mn, Zn, Nb, Mo, Ag, and Cd with freon backfill was several times higher than that of oxygen. The yield enhancement for Mg, V, Cr, Mn, Zn, Nb, Mo, Ag, and Cd were also higher with tetrachloroethene backfill, but the yields for Al, Si, Ti, Fe, Ni, and Cu were slightly lower as compared with freon backfilling. The sputtering rate under freon and tetrachloroethene flooding is about 2.5 and 1.6 times faster, respectively, than oxygen flooding under similar sputtering conditions.

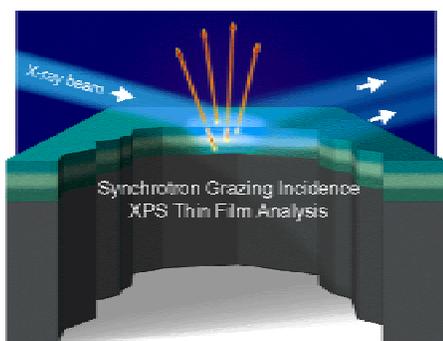
Use of either freon or tetrachloroethane gas backfilling was found to yield enhancements in secondary ion yields for many metallic elements, providing higher sensitivity for trace analysis by SIMS.

Results of SiO₂/Si Comparison between Neutron Scattering and grazing incidence x-ray photoelectron spectroscopy (GIXPS)

T. Jach and E.W. Landree (837)

The metrology of gate dielectric films, an important consideration in complementary metal oxide semiconductor (CMOS) semiconductor fabrication, crosses a new measurement threshold when film thicknesses are less than 10 nm. The current thickness of these layers (and new materials being investigated to replace them) is of the order of 3 nm. Recent comparisons using established techniques, or even different instruments of the same type, indicate that there are uncertainties in the measurements of simple SiO₂/Si films arising on this scale, which far

exceed the supposed precision of the individual methods. In addition to the inherent need to reconcile disparate measurements of SiO₂ films, the possibility for greater digressions arises as multilayer gate dielectrics are grown. Discrepancies between the results reported by different



techniques begin to creep into measurements at the level of 10 nm for three different reasons. Some may occur because different physical properties are actually measured and the “thickness” defined by these methods starts to differ as the scale decreases. Secondly, the assumed values of physical parameters determining a measurement may be inaccurate on a scale that was not previously of concern. Finally, some techniques employ processes that may change the sample on the scale of interest. We were able to test the first two possibilities with a comparison measurement between grazing incidence x-ray photoemission spectroscopy and neutron reflectivity.

During the past year we were able to complete measurements on a set of samples of SiO₂/Si in the thickness range of 3 nm to 7 nm. The methods employed, grazing incidence x-ray photoelectron spectroscopy and neutron reflectometry, are comparable techniques that rely on grazing angle beams scattering from thin surface layers. They differ, however, in relying on totally different physical

interactions that depend, in turn, on different physical properties of the layer materials. Both methods provide values for the thicknesses of the layers, while being able to distinguish between the oxide layers and layers of contamination that may reside on the surface. We observe consistent differences between measurements made by the two methods. This is attributed partly to the difference in physical properties measured, and partly to the uncertainties about the correct physical values to use in determining thickness.

The microelectronics industry is constrained to reduce the thickness of gate oxide layers as part of the overall scaling down of CMOS device dimensions. However, methods that would be used to measure the thicknesses on this scale are not in agreement.

Based on cited NIST results, GIXPS has been adopted as the method of choice for characterizing surface contamination on the Japanese research fabrication line dedicated to developing next-generation process technology (400 mm Si wafers).

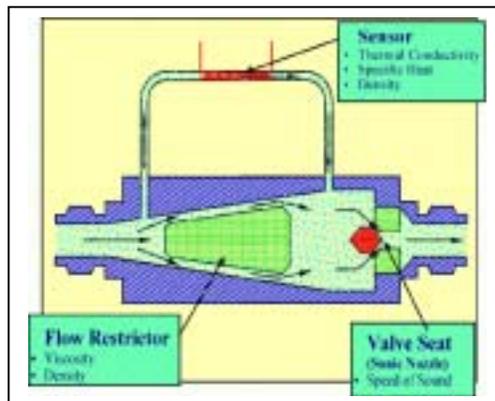
Thermophysical Properties of Gases used in Semiconductor Processing
J.J. Hurly, K.A. Gillis, and M.R. Moldover (836)

The semiconductor industry has identified a significant need for accurate thermophysical property data for semiconductor processing gases.

The semiconductor industry has identified process gases, “surrogate” gases, “carrier” gases, and their binary mixtures as the greatest area of need for accurate thermophysical property data. CSTL is measuring the thermophysical properties of gases widely used in semiconductor processing using novel, accurate, NIST-developed acoustic techniques.

The properties include the speed of sound, ideal-gas heat-capacity, density (equation of state), viscosity, and thermal conductivity. Accuracy targets for thermophysical property data have been established that meet the needs for modeling chemical vapor deposition (CVD) processes and calibration of mass flow controller (MFC) using surrogate gases.

In FY02, we completed speed of sound measurements in three surrogate gases and nine process gases. Typically, the standard relative uncertainty of the speed of sound was less than 0.01 %. The ideal-gas heat capacity was determined to within 0.1 % from the zero-pressure intercept of each isotherm. The slope and curvature of each isotherm provided information about each gas’s non-ideality from which we developed an equation of state to predict the gas’s density to within 0.1 %. The theory of the Greenspan acoustic viscometer was completed. Using this theory, the viscosity results for five test gases agreed within ± 0.5 % with reference data. An acoustic resonator optimized to measure the thermal conductivity of surrogate gases and process gases has been designed and fabricated and is currently being characterized with gases of well-known thermal conductivity. Measured data are disseminated as a database available via the internet. This database includes the heat capacity at constant pressure, thermal conductivity, viscosity, and a pressure-density-temperature relation for process gases as well as the diffusion coefficients for the gaseous mixtures.



Thermophysical properties required to model generic mass flow controller (MFC) device components.

Improved thermophysical property data developed at NIST gives the semiconductor industry the capability to achieve process control with improved accuracy and product reproducibility.

<http://properties.nist.gov/semiprop>

12. Measurement Standards

... supporting emerging technologies and the nation's measurement infrastructure

NIST provides a wide variety of measurement, standards, and data services and programs to help U.S. industry improve its international competitiveness, commercialize new technology, and achieve total quality in all facets of business operations. CSTL maintains and disseminates measurement standards for chemical, biomolecular, and chemical engineering measurements by developing Standard Reference Materials (SRM[®]s), Standard Reference Data (SRD), and by providing Calibration Services. CSTL partners with NIST Technology Services to help disseminate the standards to NIST customers.



SRMs provide scientists and engineers in industrial and academic research with internationally accepted standards for critical technical decision making. NIST pioneered, and continues to lead, in the development of certified reference materials used for quality assurance. Traditionally, SRMs have been the primary tools that NIST provides to the user community for achieving chemical measurement quality assurance and traceability to national standards. Currently, NIST catalogs nearly 1400 SRMs; in 2002 NIST sold approximately 31,000 SRM units to about 6,600 unique customers. Approximately 17,000 of the units sold were from the \approx 700 different types of materials that are certified for chemical composition. CSTL provides technical leadership for most of the chemical and compositional standards produced by NIST. SRMs are used for three main purposes: to help develop accurate methods of analysis (reference methods); to calibrate measurement systems; and to assure the long-term adequacy and integrity of measurement quality assurance programs. NIST SRMs also legally constitute part of the National Measurement System infrastructure of the United States and, as such, are essential transfer mechanisms for national as well as international measurement traceability.

Chemical Standards Development

Examples of new standards in production in CSTL in several key areas:

Health - Cardiac Troponin I

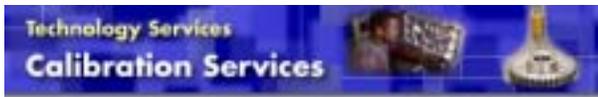
Food - Nutraceuticals/Herbal Supplements

Environmental - Low Sulfur and Mercury Coal

Commodities - Suite of Cements, Sulfur in Diesel fuels, metals/metal alloys, Electronic Scrap Artifact Material (first SRM for recycling)

Forensics - Gunshot and Explosive Residues, and Chemical and Biological Weapons Detection.

To meet the growing need for traceable standards, CSTL pioneered the NIST Traceable Reference Materials (NTRM) program. An NTRM is a commercially produced reference material with a well-defined traceability linkage to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. The NTRM concept was implemented initially in the gas standards area to allow NIST to respond to increasing demands for high-quality reference materials needed to implement the "Emissions Trading" provisions of the Clean Air Act of 1990 (while facing the reality of constant human and financial resources at NIST). The program has been highly successful. Since its inception, 12 specialty gas companies have worked with NIST to certify over 9000 NTRM cylinders of gas mixtures that have been used to produce more than 500,000 NIST-traceable gas standards. A recent study conducted by RTI International estimates that the "net benefits" of the NTRM program projected through 2007 will be between \$50M and \$63M with a social rate of return of about 225%. The NTRM concept has been expanded to our Optical Filter Standards Program. Currently, this program is just getting underway with three vendors.



In addition to SRMs and SRD (discussed in a separate section), calibrations are utilized to disseminate measurement standards. CSTL plays a significant role in NIST's Calibration

Services, providing calibrations for volumetric test measures, pressure and vacuum gauges, thermometers, hydrometers, humidity measuring instruments, fluid flow meters and air speed instruments and optical filters. These calibrations help customers achieve the highest measurement quality and productivity. In FY 2002, there were 1,687 test folders created, NIST-wide, for 685 unique customers. CSTL served 174 different companies and completed calibration services from 844 test folders. Instrumentation manufacturers and users rely on our measurement services to provide traceability to U.S. national measurement standards.

Physical Standards Development
Examples of New Physical Standards under development in CSTL in the following core areas:
Temperature - Primary Acoustic Thermometry
Pressure - Intrinsic Pressure Standards in the range 0.3 MPa to 5 Mpa
Flow - New Primary Gas Flow Standards decrease uncertainties more than a factor of 2
Humidity - Rapid, low-cost methods for calibration of permeation tube generators
Density - New Liquid Density Standards

Selected Chemical and Physical Standards Activities in FY 2002
(Described in more detail in elsewhere in this report)

Automotive and Aerospace

SRMs for Low Level Automotive Emissions

W.J. Thorn III (839)

SRMs for Hydrogen in Titanium

R.M. Lindstrom, R.L. Paul, and H.H. Chen-Mayer (839)

Chemical and Allied Products

Critical Benchmark Standards for the Cement Industry

J.R. Sieber and A.F. Marlow (839); B.S. MacDonald and C.N. Fales (TS); S.D. Leigh and J.H. Yen (ITL); D. Broton (Construction Technology Laboratories); and S. A. Wilson (USGS)

Critical Benchmark Standards for the Metals Industry

J.R. Sieber, A.F. Marlow, J.D. Fassett, G.C. Turk, R.M. Lindstrom, M.L. Salit, and M. Winchester (839); and J.R. Rumble (TS)

Environmental Technologies

SRMs for Contaminants on Air Particulate Matter

S.A. Wise, R. Oflaz, J.R. Kucklick, B.J. Porter, D.L. Poster, M.M. Schantz, R.L. Zeisler (839)

Gravimetric Results of a Fine Particulate Carbonaceous Reference Material on Quartz-Fiber Filter

G.A. Klouda (837) and J.J. Filliben (ITL)

Total Carbon Determination in Buffalo River Sediment Using Isotope Dilution Mass Spectrometry: Comparison of Techniques

D.B. Klinedinst, R.M. Verkouteren (837); and K. Revesz (U.S. Geological Survey)

SRMs for Contaminants in Soils, Sediments and Tissues

C. Beck II, T.A. Butler, R.R. Greenberg, M. Howard, W.R. Kelly, J.R. Kucklick, R.M. Lindstrom, S.E. Long, E.A. Mackey, J.L. Mann, A.F. Marlow, K.E. Murphy, R. Oflaz, R.L. Paul, B.J. Porter, D.L. Poster, M.M. Schantz, J.R. Sieber, G.C. Turk, R.D. Vocke, S.A. Wise, L.J. Wood, L.L. Yu, R.L. Zeisler (839); and S. Tutschku (Universitat Leipzig, Germany)

Standards to Support Stack Emissions Testing

W.D. Dorko (839)

Development and Critical Evaluation of a New ID/ICP-MS Method for Mercury in Sediments and Fossil Fuels

S.E. Long and W.R. Kelly (839)

Development and Critical Evaluation of New Methods for Determination of Methylmercury and Butyltin Species in Marine Tissues and Sediment

M.M. Schantz, S.A. Wise (839); and S. Tutschku (Universitat Leipzig, Germany)

Developing an Advanced Primary Ozone Photometer - A Collaboration with BIPM

P.M. Chu, J.E. Norris, and F.R. Guenther (839)

Food and Nutrition

Completion of Suite of SRMs to Support Nutritional Labeling Issues

K.E. Sharpless, J.M. Brown Thomas, S.A. Margolis, B.C. Nelson, C.S. Phinney, J.R. Sieber, and L.J. Wood (839)

Reference Methods and Materials for the Chemical Characterization of Dietary Supplements

S.A. Wise, K.W. Phinney, L.C. Sander, and K.E. Sharpless (839)

Reference Methods and Standards for Biotech Foods

D.M. Bunk (839)

Forensics and Homeland Security

Y Chromosome Assays and Standards Support Commercial Technologies

J.M. Butler, R. Schoske, P.M. Vallone, J.W. Redman, and M.C. Kline (831)

Standardization of Sample Preparation Techniques used in Analyzing Microorganisms by MALDI-TOF Mass Spectrometry

C. Nelson, B.J. Porter, M.J. Welch, B.C. Nelson, E. White V (839); and W.G. Mallard (838)

Verification of Calibration Procedure for Testing Military Gas Masks

R.A. Fletcher, L.R. King, J.A. Small (837); M.R. Winchester (839); J.C. Yang and G.W. Mulholland (BFRL)

New Measurement Technologies and Standards to Aid in Crime Scene Investigations

W.A. MacCrehan (839); M. Layman, J. Secl, and A. Dorsey (George Washington Univ.)

Reference Methods and Standards for Alcohol and Drugs of Abuse Testing
M.M. Schantz, J.V. Goodpaster, L.T. Sniegowski, S. Tai, and M.J. Welch (839)

Health and Medical Technologies

The Complete Mitochondrial DNA (mtDNA) Genome Sequence of Human Cell Line HL-60 and Its Inclusion in NIST's mtDNA SRM

B.C. Levin, D.K. Hancock, K.L. Richie (831); K.A. Holland (Gettysburg College); M. Coble and T. J. Parsons (Armed Forces DNA Identification Laboratory); L. J. Kienker (FBI Laboratory); and D.W. Williams (Georgia Bureau of Investigation)

Critical Evaluation of ID ICP-MS vs. TIMS as New National Reference Method for Electrolytes in Blood Serum

K.E. Murphy, S.E. Long, J.D. Fassett, and M.S. Rearick (839)

Newly Certified Trace Elements in Urine and Blood SRMs

R.D. Vocke, C. Beck II, O. Ertas, W.R. Kelly, S.E. Long, and M.S. Rearick (839)

Standards for in vitro Diagnostics and International Trade

W.E. May, D.M. Bunk, J.D. Fassett, W.R. Kelly, S.E. Long, K.E. Murphy, B.C. Nelson, M.B. Satterfield, L.T. Sniegowski, S. Tai, R.D. Vocke, and M.J. Welch (839)

Toward Comparable Cardiac Troponin I Measurements: Selection of a Primary Reference Material

D.M. Bunk and M.J. Welch (839)

Industrial and Analytical Instruments and Services

Atomic Standard of Pressure

M.R. Moldover, J.W. Schmidt (836) and K. Szalewicz (University of Delaware)

Development of High-Precision Isotope Reference Standards

A.P. Miiller (836)

Improved Vacuum Transfer Standards

R.F. Chang (836)

International Standards for Calorimetry and Thermodynamics

D.R. Kirklin and D.G. Archer (838)

New SRMS for Near Infrared and Raman Spectroscopy

W.S. Hurst, J.E. Maslar (836); E.S. Etz (837); and S.J. Choquette (839)

Transducer-Assisted Piston Gauge Calibrations

D.A. Olson (836) and T. Kobata (NMIJ, Japan)

Microelectronics

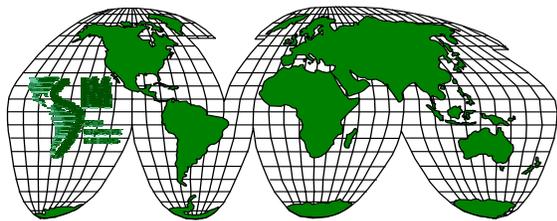
Completion of Suite of Dopants in Silicon Standards

R.L. Paul, R.R. Greenberg, R.M. Lindstrom (839); and D.S. Simons (837)

Development of SiGe Compositional Standards for the Semiconductor Industry

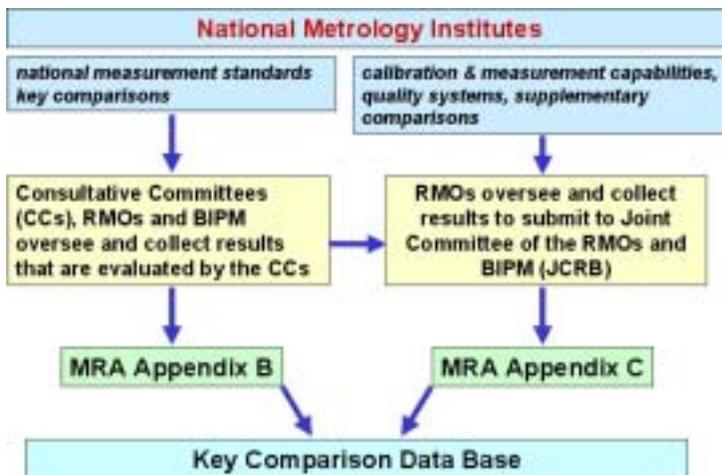
R.B. Marinenko, J.T. Armstrong, S. Turner, E. B. Steel, and D.S. Simons (837)

International Measurement Standards



Increased requirements for quality systems documentation for trade and effective decision-making regarding the health and safety of the U.S. population have increased the need for demonstrating “traceability to NIST” and establishing a more formal means for documenting measurement comparability with standards laboratories of other nations and/or regions.

NIST is the U.S. National Metrology Institute (NMI) and the agency of the U.S. Government responsible for U.S. efforts under the Treaty of the Metre. The Committee International des Poids et Mesures (CIPM), and its various consultative committees, organizes comparisons of national measurement standards. After review, the results of these comparisons are recorded in the BIPM database. In addition, coordination of similar efforts with Regional Metrology Organizations (RMOs) extend the comparison efforts to as many participants as practicable.



<http://kcdb.bipm.org/BIPM-KCDB/>



In October 1999, NIST signed the CIPM Mutual Recognition Arrangement (MRA).

The objectives of the MRA are:

- To establish the degree of equivalence of national measurement standards maintained by NMIs;
- To provide for the mutual recognition of calibration and measurement certificates (CMCs) issued by NMIs; and
- Thereby to provide governments and other parties with a secure technical foundation for the wider agreements related to international trade, commerce, and regulatory affairs.

Since 1999 CSTL has systematically compared U.S. national measurement standards to establish degrees of equivalence of U.S. national measurement standard with those of other NMIs. These international activities add value to NIST standards and services, particularly for our customers involved in international trade. These MRA-related activities guarantee recognition of U.S. standards by U.S. trading partners.

Regional Metrology Organizations (RMOs) play an important role in the MRA. They have the responsibility for carrying out key comparisons within their regions. They also carry out supplementary comparisons and other actions to support mutual confidence in the validity of calibration and measurement certificates through the Joint Committee of the RMOs and the BIPM (JCRB). They are also responsible for the entries into the BIPM key comparisons database for the calibration and measurement capabilities of their member NMIs.



Systema Interamericano de Metrologia (SIM) is the RMO that includes the United States. The metrology organizations that make up SIM are known as: NORAMET (North America), CAMET (Central America), CARIMET (Caribbean), ANDIMET (Andes Region), and SURAMET (South America). Regional cooperation leads to a wider harmonization of measurements and standards, facilitates free flow of trade, and is a necessary first step to globalization. NIST and National Research Council

(NRC) Canada link SIM countries to the BIPM, and as such, provide greater opportunities for international trade and commerce. CSTL staff provides leadership for SIM by chairing the Chemical Metrology Working Group of SIM, and by serving as the U.S. representative to the JCRB, in order to assure the effective, fair, and metrologically sound implementation of the MRA.

CSTL's Analytical Chemistry Division has led the critical review of CMC data for Appendix C for the BIPM Database. SIM has approximately 1140 CMC entries scheduled for inclusion in the BIPM database. By the beginning of 2003, the BIPM database will include approximately 3000 CMCs for chemistry. About 1000 will be from NIST.

CIPM Consultative Committees

The CIPM has set up a number of Consultative Committees, which bring together the world's experts in their specified fields as advisers on scientific and technical matters. Among the tasks of these Committees are the detailed consideration of advances in physics that directly influence metrology, the preparation of Recommendations for discussion at the CIPM, the identification, planning and execution of key comparisons of national measurement standards, and the provision of advice to the CIPM on the scientific work in the laboratories of the BIPM.

CIPM Consultative Committees for Temperature (CCT) and Mass (CCM)

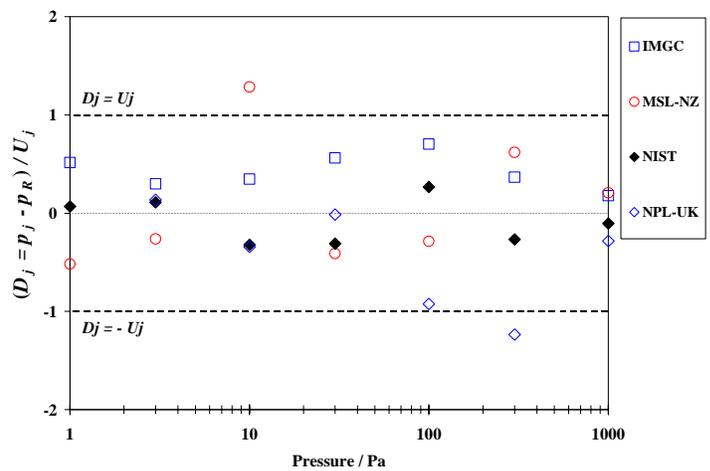
Researchers in CSTL's Process Measurements Division have significant participation in the CC's for Temperature (CCT) and Mass (CCM). CSTL has lead or participated in many Key Comparisons (KCs) in the past several years. In thermometry, these efforts have resulted in establishing equivalence levels over the ITS-90 range from 14 K to 1235 K. KCs organized by the CCM include Division activities in both the pressure and vacuum and the flow project areas. In the pressure and vacuum project the Division leads or participates in KCs that cover the pressure range 3×10^{-6} Pa to 500 MPa. NIST has piloted three CCM Key Comparisons in the last several years, completing two in FY02 that demonstrated general equivalence among the participants, revealed no systematic bias between alternative realizations of the Pascal, were the only CCM KCs completed on schedule, and set the standard for the manner in which KCs should be conducted. In addition, CSTL researcher G. E. Mattingly chairs the CCM's Working Group for Fluid Flow (WGFF). The WGFF has formed its strategy, structure, and schedule for comparisons in six areas: water; hydrocarbon liquids; low-pressure air and high-pressure; high-flow natural gas; air speed; and liquid volume. The WGFF chair, working closely with the NIST Statistical Engineering Division, has developed a sound, statistically based experimental design and supporting analysis method applicable to all KCs. The design of these protocols and transfer standards will set precedents that should advance the state-of-the-art in conducting all future flow laboratory comparisons. This metrological plan was presented to the CCM in May 2002. The BIPM Director noted that this plan should result in a unique comparability of the flow standards in the participating NMIs and fulfill "the ideal of the MRA". NIST will pilot the KC for the low-pressure gas flow. Recent advances in U.S. standards and procedures (described below) are expected to materially improve the performance-level of this KC.

International Comparisons of Pressure Standards (CCM Key Comparisons)
A.P. Miiller, P.J. Abbott, and A. Lee (836)

The status of NIST participation in eight international comparisons of pressure and vacuum standards, at the highest metrological level, is presented. These comparisons help establish degrees of equivalence of national standards from 3×10^{-6} Pa to 500 MPa. NIST has piloted three CCM Key Comparisons (two to completion in FY02). The completed comparisons were the first successful international comparisons in this pressure range (1 Pa to 1000 Pa, absolute and differential modes), and demonstrated general equivalence among the participants, revealed no systematic bias between alternative realizations of the Pascal, and were the only CCM pressure comparisons completed on time. The measurement phase of the third comparison (3×10^{-6} Pa to 9×10^{-4} Pa) has been completed and is in the analysis and reporting phase.

To help alleviate technical trade barriers, the relative agreement of national pressure and vacuum measurement standards needs to be assessed, established, formally recognized, and maintained.

CCM.P-K4 and -K5 have formally established the degree of equivalence between several NMIs in an industrially important pressure range. Despite over a decade of attempts, -K4 and -K5 are the first successful comparisons in this range, have unambiguously established degrees of equivalence between NMIs, and revealed no significant relative bias among the different techniques to realize the Pascal from 1 Pa to 1000 Pa. *The innovative transfer package developed for this comparison has formed the basis for many variants in regional comparisons throughout the world.*



Results for CCM.P-K4: shows the deviation of measurements at NMIs from the reference value divided by the uncertainty (95% confidence level).

Comparisons CCM.P-K4 and CCM.P-K5 were formally approved by the CCM in FY02. In recognition for overcoming major technical and engineering barriers in developing robust, state-of-the-art low-pressure transfer standards that enabled the successful completion of these two precedent-setting comparisons, A. P. Miiller was awarded the 2002 CSTL Technical Achievement Award. He was also appointed to the Chair of the Low-Pressure Working Group of the CCM. Comparison CCM.P-K3 is also piloted by NIST. The transfer-standard package that was developed at NIST consists of two spinning rotor gauges, three Bayard-Alpert ionization gauges, and began circulation in FY99. The comparison is behind due to delays between participants, but measurements were completed in FY02, although some participants have not yet submitted their results.

The purpose of intercomparisons is to establish degrees of equivalence between national pressure and vacuum standards for pressures from 3×10^{-6} Pa to 500 MPa. International trade is, in part, based upon the equivalence of measurements. Some of the largest segments of industrial measurements are in the areas of pressure and vacuum.

A Summary of CCM Key Comparisons in Pressure

Comparison Number	Pressure Range	Transfer Standard	Status	Pilot NMI*	Participants*
CCM.P-K1.a, b	0.05 - 1 MPa (gauge)	Piston Gauge	Approved	a-6 b-3	1ab, 2a, 3ab, 5ab, 6ab
CCM.P-K1.c	0.08 - 7 MPa (gauge)	Piston Gauge	Approved	5	1, 2, 3, 4, 5, 6
CCM.P-K2	10 – 120 kPa (absolute)	Piston Gauge	Analysis & Reporting	2	1, 2, 5, 6, 10, 12, 13, 14, 15, 16, 17, 18
CCM.P-K3	3×10^{-6} - 9×10^{-4} Pa (absolute)	Spinning Rotor and Ion Gauges	Analysis & Reporting	1	1, 2, 5, 7, 8, 9
CCM.P-K4	1 - 1000 Pa (absolute)	Low Pressure Transducers	Approved	1	1, 2, 5, 7, 8, 9, 10
CCM.P-K5	1 – 1000 Pa (differential)	Low Pressure Transducers	Approved	1	1, 2, 5, 10, 11
CCM.P-K6	10 – 120 kPa (differential)	Piston Gauge	Analysis & Reporting	2	1, 2, 5, 6, 10, 13, 14, 15, 16, 17, 18

*

List of NMIs:

1. National Institute of Standards and Technology (NIST) – U.S
2. National Physical Laboratory (NPL) – United Kingdom
3. Laboratoire National D'Essais (LNE) – France
4. National Research Laboratory for Metrology (NRLM) – Japan
5. Istituto di Metrologia "G Colonnetti" (IMGC) – Italy
6. Physikalisch-Technische Bundesanstalt (PTB) – Braunschweig, Germany
7. Physikalisch-Technische Bundesanstalt (PTB) – Berlin, Germany
8. Korean Institute of Standards and Science (KRISS) – S. Korea
9. National Physical Laboratory (NPLI) – India
10. Commonwealth Scientific and Industrial Research Organization (CSIRO-NML) – Australia
11. Measurement Standards Laboratory (MSL) – New Zealand
12. Bureau International des Poids et Mesures (BIPM)
13. Institut National de Metrologie (INM) – France
14. National Research Council (NRC) – Canada
15. Nederlands Meetinstituut (NMI) – Netherlands
16. Office Fédéral de Métrologie (OFMET) – Switzerland
17. National Institute of Metrology (NIM) – China
18. D.I. Mendeleev Institute for Metrology (VNIIM) – Russian Federation

Consultative Committee for Amount of Substance (CCQM)

NIST has taken a leadership role in the International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM) and the Chemical Metrology Working Group of the Interamerican System for Metrology (SIM) in order to assure the effective, fair and metrologically sound implementation of this MRA. The CCQM has formed seven working groups: (1) gas analysis, (2) organic analysis, (3) inorganic analysis, (4) electrochemistry, (5) biometrology, (6) surface analysis and (7) key comparisons. These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. CSTL's Analytical Chemistry Division is leading various activities within five of the seven working groups and chairing the Organic Analysis Working Group. During the past five years, CSTL has participated in 46 of the 53 comparison studies conducted under the auspices of the CCQM, and served as Pilot Laboratory in 18. An additional 36 studies are planned for the next three years, and NIST has committed to pilot 9 of these. A full listing and status report for recent KCs are provided below.

CSTL's ACD is also working with CITAC (Cooperation in International Traceability in Analytical Chemistry) to establish practical, yet metrologically sound, vertical traceability links between the NMIs and chemical testing labs in various countries and regions around the world.

CCQM Key Comparisons

Completed

- CCQM K-1, CO, CO₂, NO, and SO₂ in N₂, and natural gas
- CCQM K-2, Cd, Pb in natural water
- CCQM K-3, CO, CO₂, propane in N₂
- CCQM K-4, Ethanol in air
- CCQM K-5, DDE in Fish Oil
- CCQM K-6, Cholesterol in Serum
- CCQM K-7, BTX in N₂/air
- CCQM K-8, Elemental solutions standards
- CCQM K-9, pH 7 (phosphate)
- CCQM K-10, BTX in air (ng/g level)
- CCQM K-13, Pb, Cd in sediments
- CCQM K-29, Zn in Rice

Reports Drafted

- CCQM K-11, Glucose in Serum, Draft B report
- CCQM K-12, Creatinine in Serum, Draft B report
- CCQM K-16, Natural gas, Draft A report
- CCQM K-17, pH 4.1 (phthalate), Draft A report
- CCQM K-21, DDT in Fish Oil, Draft B report
- CCQM K-24, Cd in Rice, Draft B report
- CCQM K-25, PCBs in Sediments, Draft A report

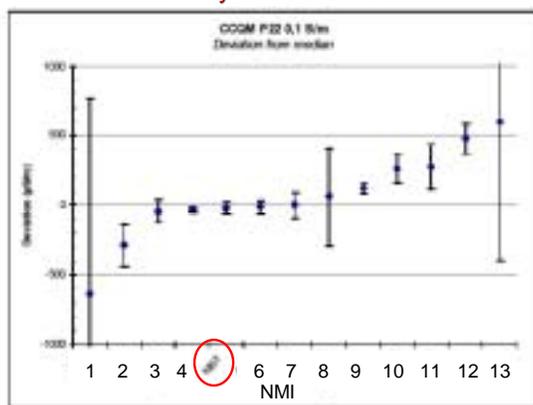
Selected CCQM KCs Completed in FY 2002

International Benchmarking of NIST's Classical and Electroanalytical Measurement Capabilities

K.W. Pratt (839)

In FY 2002, NIST participated in 4 CCQM pilot studies connected with international benchmarking of classical (assay) techniques, pH metrology, and anion solutions. Each study was designed to assess the factors that govern the agreement attained among the participants [national metrology institutes (NMIs)] for the given measurand. The results of each study indicated that the NIST measurements are at the state of the art for the respective measurement. Results for the pH and assay studies also revealed sources of systematic bias that are difficult to detect using results obtained at a single NMI.

CCQM-P22: Conductivity (primary and secondary measurements) Study Period: 2001



Nominal 0.1 S/m (1000 µS/cm)

NMIs. This second justification utilizes the information obtained from different NMIs performing a given primary measurement using their own realization to obtain information not attainable by a single NMI performing its primary measurement alone.

The pH pilot study (CCQM-P37) was performed in an effort to elucidate systematic effects that contributed to unexpectedly large deviations of certain NMIs (not including NIST) in the previous pH KC, CCQM-K17.

The HCl assay pilot study (CCQM-P19.1) was undertaken to minimize the effects of packaging and shipping that were observed in the HCl solution distributed to the participants in CCQM-P19, the previous study. CCQM-P19.1 also had a wider range of NMIs participating and therefore included a better evaluation of H⁺ and Cl⁻ assay methods. NIST was the coordinating laboratory.

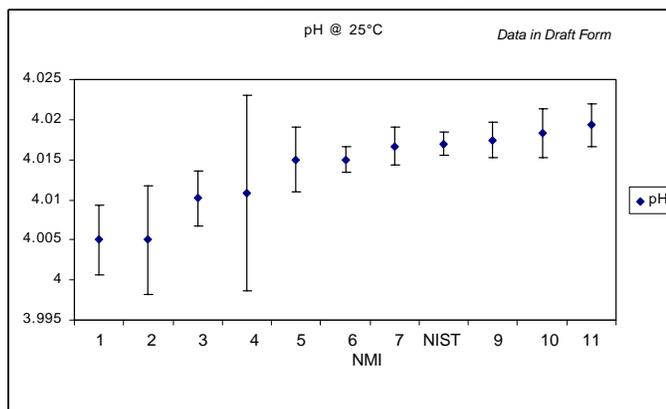
CCQM-P36 [potassium acid phthalate (KHP) assay] provided information on the importance of CO₂ interference in assay of this widely used acidimetric standard and exposed a bias of approximately +0.04 % at NIST that had arisen since the latest KHP SRM certification. CCQM-P19.1 (HCl assay) vindicated the use of improved packaging to eliminate the influence of packaging on participants' results and provided the first statistically useful comparison of H⁺ and Cl⁻ assays. CCQM-P37 (fundamental pH study) revealed obscure factors connected with primary pH (Harned) cell design and usage that should result in improved uncertainties for future NIST pH

The results of each study indicated that the NIST measurements are at the state of the art for the respective measurement. Results for the pH and assay studies also revealed sources of systematic bias that are difficult to detect using results obtained at a single NMI.

Each pilot study evaluated the degree of agreement obtained for the given measurand for each NMI. The pilot study in most cases served as a preliminary trial to a future key comparison (KC), the quantitative results of which will fix the international acceptance of measurements by each NMI (including NIST). Also, the pilot studies investigated systematic, uncontrolled sources of bias that limit the agreement obtained between

CCQM-K17: pH of Phthalate Buffer Solutions

pH nominal value: 6.9 at 25 °C Dates of Study: 2001
[15 °C, 25 °C, 37 °C]



SRMs. The CCQM-P32 results (PO_4^{3-} and Cl^- anion solutions) supported the NIST claims for measurement capability for the respective measurements.

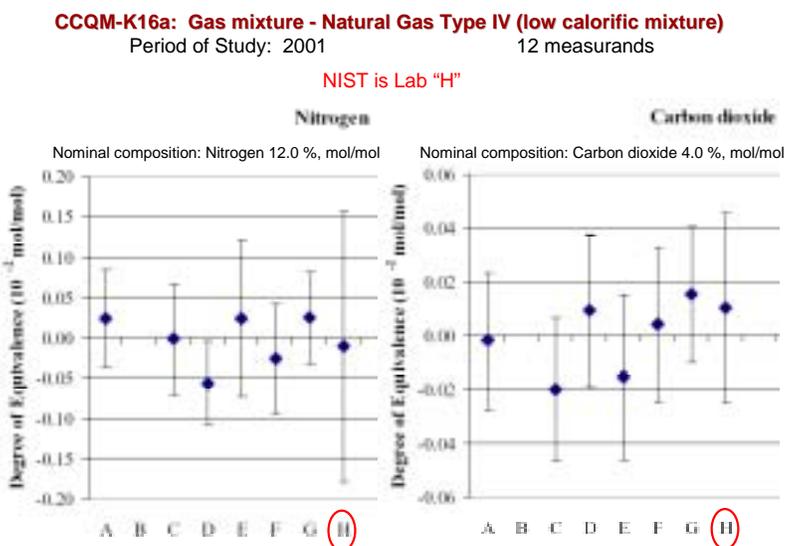
The results of the pilot studies in KHP assay and anion solutions provided the first estimate under CCQM auspices of the international agreement that is attainable for the respective measurands. These pilot studies are likely to result in corresponding future KCs that underpin the claims of measurement capability for the participating NMIs (including NIST).

The results of the HCl assay and fundamental pH studies provided essential information regarding primary pH measurements and systematic sources of uncertainty that impact all primary pH measurements. These results directly impact details of the methodology used by the NMIs participating in already-planned future pH KCs.

International Benchmarking of CSTL Capabilities in Gas Metrology **F.R. Guenther (839)**

Over the past year, NIST participated in CCQM-P23 Preparation of Gravimetric Gas Standards Pilot Study and CCQM-K16 Natural Gas Key Comparison. As the year ends we are also participating in CCQM-P41 Greenhouse Gases Pilot Study, and working with NMIJ (Japan) on the prototype for the CCQM-K22 volatile organic chemicals (VOCs) Key Comparison. Each study was designed to assess the comparability attained among the participants [national metrology institutes (NMIs)] for the given measurand. The results of each study indicated that the NIST measurements are at the state of the art for the respective measurement. Results for CCQM-P23 revealed a bias among the NMIs which was traced back to the isotopic abundance of ^{13}C in the pure carbon monoxide that used to prepare the gas standards. Once this difference was factored into the results, all the NMIs were in good agreement.

Each study evaluated the degree of agreement obtained for the given measurand for each NMI. The pilot studies in most cases serve as a preliminary trial to a future key comparison (KC), the quantitative results of which will fix the international acceptance of measurements by each NMI (including NIST). Also, the pilot studies investigated systematic, uncontrolled sources of bias that limit the agreement obtained between NMIs. This second justification utilizes the information obtained from different NMIs performing a given primary measurement using their own realization to obtain information not attainable by a single NMI performing its primary measurement alone



The CCQM-P23 Preparation of Gravimetric Gas Standard Pilot Study was intended to study the source of uncertainty in the preparation of primary gas standards. Carbon monoxide was chosen because it was thought that this measurand was not difficult to handle and analyze. Thus it should be relatively straightforward to determine small uncertainty sources. The results showed major biases between the NMIs, especially NIST and KRIS (Korea). After much work at NIST and several other NMIs, the source of the bias was traced to differences in the ^{13}C abundance of

the pure carbon monoxide (CO). It turns out that gas suppliers strip out the ^{13}C CO from the pure gas to sell to producers of isotopically pure organic compounds. The result is that primary standards produced with the depleted ^{13}C CO will show a bias on certain non-dispersive infrared analyzers (NDIR). The bias is not seen in gas chromatographic methods. This result has major implications in the automotive industry, where measurements are needed to within 1 % of the true value to meet regulations.

The CCQM-K16 Natural Gas Key Comparison was intended to measure the comparability of Natural gas measurements at the participating NMIs. Unlike past comparisons, this natural gas included the heavy hydrocarbons. Due to shipping problems, NIST was able to analyze only one of the two cylinders sent to participants. NIST's results show excellent agreement with the majority of the NMIs. A similar Pilot Study was organized by NIST for the SIM countries. This study indicated that there remains much work to be done in SIM to bring the comparability among SIM NMIs in line with the CCQM. NIST is working with NMIJ in Japan on the proposed CCQM-K22 Volatile Organic Compounds (VOC) in Air.

Presently the CCQM-P41 Greenhouse Gases Pilot Study is proceeding, and will be completed by early 2003. This study will demonstrate the comparability of the NMIs for methane and carbon dioxide. This will set the stage for future comparisons in global warming gases, and should enable efforts to mitigate and control emission of these gases internationally. The CCQM is working closely with the World Meteorological Organization to link the measurement of these gases in the atmosphere to the SI.

International Benchmarking of CSTL Inorganic Analytical Measurement Capabilities
R.R. Greenberg, J.D. Fassett, K.S. Murphy, R. Zeisler, E.A. Mackey, L.L. Yu, L.J. Wood, J.R. Sieber, and A.F. Marlow (839)

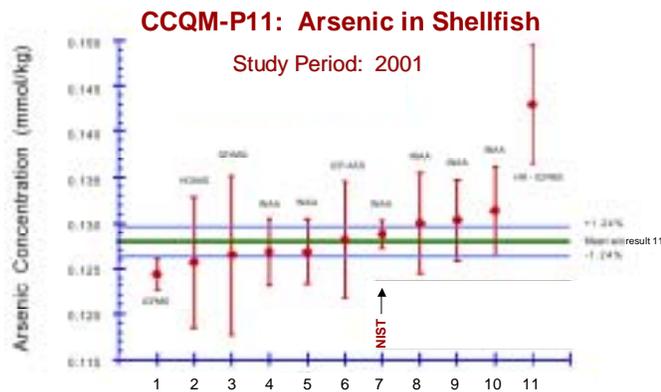
Key comparisons are an important method of demonstrating the claimed measurement capabilities of national metrology institutes (NMIs), as well as establishing the equivalence of measurements made by different NMIs. To serve US interests, it is important that NIST successfully participates in relevant measurement comparisons under the CIPM Mutual Recognition Arrangement.

NIST/CSTL successfully participated in five pilot and key comparisons of the inorganic working group of the CCQM this year: CCQM-P11 Arsenic in Shellfish, CCQM-K24 Cadmium in Rice, CCQM-P29 Zinc in Rice, CCQM-P13 Synthetic Food Digest, and CCQM-P25 Minor Elements in Steel.

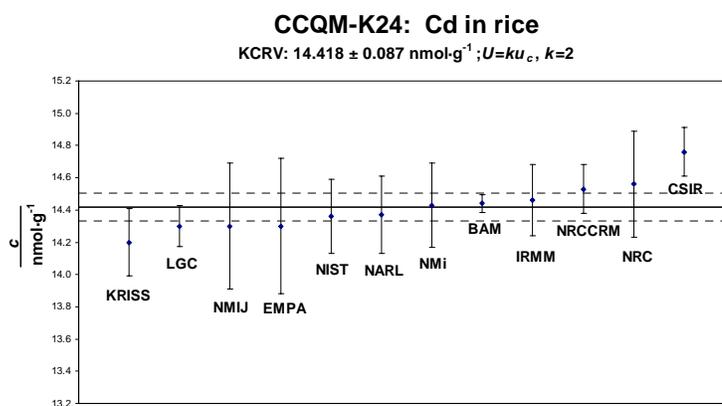
NIST led the pilot study on Arsenic in Shellfish, which was based on an oyster tissue similar to SRM 1566b. Arsenic is a toxic element and, thus, its determination in oyster tissue, a food matrix, is of general concern. Results were received from eleven

laboratories from nine countries (see figure). The NIST results of $(0.1287 \pm 0.0015) \mu\text{mol/g}$ were in excellent agreement with the mean of $(0.1279 \pm 0.0016) \mu\text{mol/g}$ determined from all participants except one, who apparently had problems with their analysis. After the results became available, the CCQM decided to continue on to a key comparison for Arsenic in Shellfish (CCQM-K31).

The rice comparison was split into both a key and pilot comparison. Thus, for those participants who felt comfortable with their Cd measurement capabilities, they could register for the key comparison CCQM-K24.



The same material was used for a pilot comparison CCQM-P29 Zn and Cd in rice. We chose to participate in the key comparison; the NIST ID-ICP-MS and INAA determinations were combined and reported. This result was in excellent agreement with the 10 other laboratories [NIST: (14.36 ± 0.23) nmol/g; CCQM: (14.40 ± 0.09) nmol/g (see figure). For the pilot comparison, the two NIST Zn results (ID-ICP-MS and INAA) were reported separately.



Likewise, the Zn results were in excellent agreement [NIST ID-ICP-MS: (0.3554 ± 0.0067) μ mol/g; NIST INAA: (0.3559 ± 0.0095) μ mol/g; CCQM: (0.3527 ± 0.0024) μ mol/g]. The major source of uncertainty for all NIST measurements in both K24 and P29 was in achieving a dry-mass basis weight. It was recognized that the CCQM-P29 Zn data was of “key comparison” quality as well. Rather than continue on to a key comparison, it was decided to accept that “the light of K24 shines at least as far as Zn.” Cadmium in rice represents a toxic element in food; zinc is a nutritional element.

The Pilot Study CCQM-P13 Metals in Artificial Food Digest was proposed by LGC to benchmark measurement of nutritional, essential, and toxic elements in foods. They proposed to avoid the difficulty in assigning a consensus key comparison reference value by spiking a synthetic matrix solution with gravimetrically known amounts of the target analytes: Ca (nutritional), Cu (essential) and Cd (toxic). Thus, issues associated with both dissolution and homogeneity were avoided. We decided to determine only the elements Ca and Cu; we felt that our capabilities for Cd had been demonstrated adequately in previous studies. We have been applying ICP-OES methodology with standard additions calibration in our recent certification measurements of food. (This year we made certification measurements for SRM 2387 Peanut Butter, SRM 2385 Spinach, and Milk Powder.) Thus, we felt it was most appropriate that this method be used in the Pilot Study. The results for Ca and Cu were +1.3 % and -0.8 % relative to the reference values, respectively. The average deviations from the reference values were -0.5 % and +3.5 % respectively. The observation was made that the laboratories using IDMS had larger deviations, -2.1 % and +3.8 %, illustrating again that it is not the technique that endows accuracy but the skill of the laboratories that apply the technique.

The first metal and metal alloy study evolved from proposals by NMIJ (Japan), BAM (Germany), and NIST. Designated CCQM-P25, it is for minor elements (Cr, Mo, Ni, and Mn) in a low-alloy steel. These elements control specific steel properties. ACD determined all elements in the steel using its recently developed matrix independent XRF borate fusion method, and three elements (Cr, Mn, and Mo) were determined by INAA. We also coordinated the submission of results from 4 U.S. industry laboratories. The end-of-year deadline for this study meant that comparative results have not yet been formally revealed. However, results were shown graphically at the Fall 2002 CCQM Inorganic Working Group meeting, and the NIST results were consistent with those submitted by other NMIs.

The metal and metal alloy industries are huge and the economic impact of chemical measurements is very large. The range of commercial metal and alloy materials is also very large and, thus, it has been a challenge to identify the appropriate measurement activity for CCQM in this area.

Strategic Bilateral Collaborations and Intercomparisons with NMIs
W.E. May, F.R. Guenther, S.A. Wise, and R.R. Greenberg (839)

In addition to these global and regional activities described in this report, we are also establishing a limited number of strategic bilateral collaborations and intercomparisons with NMIs around the world. For example, our collaboration with the Netherlands Measurement Institute (NMI) for determining the equivalence of primary gas standards has resulted in a formal “Declaration of Equivalence” that is recognized by the U.S. EPA and European environmental regulatory bodies as documenting the equivalence between seven NIST and NMI primary gas mixture suites. A bilateral comparison of optical transmittance at five visible wavelengths and for transmittance levels between 1.0 and 10^{-3} is currently underway between NIST and the National Physical Laboratory (NPL) of the UK. This is the first international comparison for the second-generation CSTL



reference spectrophotometer and is a precursor to an anticipated Memorandum of Cooperation and Declaration of Equivalence between NIST/CSTL and NPL with respect to optical transmittance. Equivalence between the NIST and NPL transmittance scales is expected to ease the regulatory traceability burden for end users, especially

multinational pharmaceutical companies. Our formal agreement with NRC-Canada (via the NAFTA Treaty) for cooperation in marine environmental studies has fostered collaborations in the certification of a several certified reference materials important for trade and environmental decision-making between the U.S. and Canada. We have a Cooperative Arrangement with NIMC

CSTL continues to work with CITAC (Cooperation in International Traceability in Analytical Chemistry) to establish practical, yet metrologically sound, vertical traceability links between the NMIs and chemical testing labs in the various countries and regions around the world.

(Japan) for collaborative efforts in the area of pure volatile organic compound standards. These highly pure reference compounds will serve as primary references for many of our gas mixture SRMs and our quantitative reference database (SRD-79). Several additional strategic bilateral arrangements with other National Metrology Institutes and standards laboratories are being discussed.



13. Data and Informatics

... assuring that U.S. Industry has access to accurate and reliable data, and predictive models

NIST DATA One of CSTL's goals is to assure that U.S. industry has access to accurate and reliable data and predictive models to determine the chemical and physical properties of materials and processes. CSTL's data and informatics activities impact all industry sectors from biotechnology and microelectronics to energy and instrument manufacturers. The NIST **Standard Reference Data (SRDs)** series has grown to over 50 electronic databases in chemistry, physics, materials, building and fire research, software recognition, and electronics. Versatile interactive databases provide easy access to high quality NIST data. Many databases are now available via the World Wide Web. Through this program CSTL provides SRDs for analytical chemistry, atomic and molecular physics, biotechnology, chemical and crystal structure, chemical kinetics, industrial fluids and chemical engineering, materials properties, surface data, and thermodynamics and thermochemistry. A few of the highlights in the area of Data and Informatics are described in the sections below, and a full listing of activities, with references to the appropriate program section, is also provided.

During 2002, a new version of the **NIST Chemistry WebBook** was released. The WebBook remains one of the most widely used resources for chemical and physical property data, and usage is expected to grow at about 20% per year. During the past year, over 500,000 distinct IP addresses accessed the available information. The wide variety of users in industry, academia, and government, and the high rate of return users attest to the value of this resource. The WebBook will also be linked to NIST SRDs such as the **NDRL/NIST Solution Kinetics Database** that are now accessible on the Internet.

Another significant achievement in 2002 was the completion of a new version of the **NIST Mass Spectral Library**. The library now includes over one-third more spectra, and some of the spectra formerly in the library were replaced with higher-quality replicates or newly acquired spectra. These improvements will enhance the reliability of the library for identifying unknown, volatile chemical compounds by GC-MS. Approximately 2,500 new libraries are installed on instruments each year.

An updated version of the **Reference Fluid Thermodynamic and Transport Properties (REFPROP)** database was also recently released. This database provides industrial users with the most accurate properties available for pure fluids and fluid mixtures used in refrigeration and air conditioning systems. The new version includes additional fluids and mixtures, improved property models, increased calculational speed, and an enhanced user interface.

The **Protein Data Bank (PDB)** is a unique worldwide repository for three-dimensional structural data for biological macromolecules. The program was initiated in 1971 at Brookhaven National Laboratory and is now managed by the Research Collaboratory for Structural Bioinformatics (RCSB), whose three members include: Rutgers, The State University of New Jersey; the San Diego Supercomputer Center at the University of California; and the National Institute of Standards and Technology. Currently, there are nearly 20,000 entries in the archive, and many secondary sources of information are derived from PDB data. Uniformity of structural data is crucial for meaningful application of these data in the pharmaceutical and biotechnology industries, and NIST has played an important role in developing data standards that ensure global uniformity and facilitate data exchange.

Summary of Data and Informatics Activities for FY 2002
(Described in more detail in elsewhere in this report)

Pharmaceuticals and Biomanufacturing

The Protein Data Bank

T.N. Bhat, G.L. Gilliland (831); and P. Fagan (TS)

Target Site of Intron Gain Inferred by a System for Phyloinformatic Analysis (SPAN)

A.B. Stoltzfus (831/CARB), and W-G. Qiu (CARB/UMBI); and N. Schisler (Pomona College),

Theoretical Studies of Enzyme Mechanisms

M. Krauss (831)

Chemical and Allied Products

Notre Dame Radiation Laboratory (NDRL)/NIST Solution Kinetics Database on the Web

T.C. Allison, R.E. Huie, and P. Neta (838)

Photochemical Reduction of CO₂ Catalyzed by Metalloporphyrins

P. Neta (838) and J. Grodkowski (Institute of Nuclear Chemistry and Technology, Warsaw)

An Efficient Model to Compute the Exact Exchange Potential in Density Functional Theory

C.A. Gonzalez and T.C. Allison (838); E. Ludena, V. Karasiev, and I. Gonzalez (Instituto Venezolano de Investigaciones Cientificas); and Y. Simón-Manso (Universidad de Chile)

Environmental Control of α -Cr₂O₃ (0001) Surface Chemistry

A.M. Chaka (838), N. Harrison (Imperial College and Daresbury Laboratory),
A. Wander (Daresbury Laboratory), and X.-G. Wang (GM Research Laboratories)

Cylinder Filling Ratios for Compressed Liquids

M.L. Huber and D.G. Friend (838)

A Standard Chemical Identifier

D. Tchekhovskoi and S.E. Stein (838)

The NIST WebBook - NIST Chemical Reference Data for Industry

P.J. Linstrom and W.G. Mallard (838)

Thermodynamics Research Center (TRC) Comprehensive Program on Critical Data Evaluation

M.L. Frenkel, R.D. Chirico, Q. Dong, G.R. Hardin, and R.A. Stevenson (838);
X. Yan and X. Hong (Contractors); and V.V. Diky (Belarusian State U., Belarus)

Measurements, Modeling and Data for Pressure-Driven Membrane Separations

C.D. Muzny, K. Benko, and J.L. Scott (838); M. Chapman-Wilbert and K. Price (U.S. Bureau of Reclamation); and J. Ranville (Colorado School of Mines)

Gas Chromatography Retention Index Library: A Major New Resource Nears Completion

J.K. Klassen, P.J. Linstrom, and S.E. Stein (838)

The NIST Mass Spectral Library: A Major Update

A. Mikaya, S.E. Stein, D.V. Tchekhovskoi, C.L. Clifton, and Q.-L. Pu (838)

Industrial Fluid Properties Simulation Challenge: Benchmark Standards

D.G. Friend, J.W. Magee, A.M. Chaka, R.D. Mountain, R.D. Johnson III, L.A. Watts, E.W. Lemmon, R.D. Chirico, M.O. McLinden, A. Laesecke, and T.J. Bruno (838)

Properties and Processes for Cryogenic Refrigeration

R. Radebaugh, P.E. Bradley, and M.A. Lewis (838); R. Gates (Univ. of Colorado); L. Reynolds (Univ. of Colorado); J. Gary and A. O'Gallagher (ITL); and J. Pfotenhauer (Univ. of Wisconsin)

Energy Systems

Properties of Refrigerant Mixtures Near the Critical Point

M.O. McLinden, R.A. Perkins, E.W. Lemmon, A. Laesecke, and J.W. Magee (838)

Release of Version 7.0 of the NIST REFPROP Database

E.W. Lemmon, M.O. McLinden, and M.L. Huber (838)

Viscosity Measurements and Model Comparisons for Second-Generation Alternative Refrigerant Blends

A. Laesecke (838)

Thermal Conductivity of Natural Gas Constituents

R.A. Perkins (838)

Chemistry and Properties of Trace Components of Fuel Gas

T.J. Bruno, W.C. Andersen (838); and A. Abdulagatov (Dagestan Scientific Center of Russian Academy of Sciences)

Stability of Potential High-Temperature Heat Transfer Fluids

D.G. Friend, T.J. Bruno, and W.C. Andersen (838)

Environmental Technologies

Kinetics and Thermophysical Properties of Ionic Liquid Systems

P. Neta, J.W. Magee, M.L. Frenkel, A. Laesecke, M.O. McLinden, R.A. Perkins (838); J. Grodkowski (Institute of Nuclear Chemistry and Technology, Warsaw); and G. Kabo (Belarusian State Univ.)

Molecular Simulation of Solute Diffusion (Sodium Chloride) in Supercritical Steam

A.H. Harvey and R.D. Mountain (838); and J.C. Bellows (Siemens Westinghouse)

Industrial and Analytical Instruments and Services

SpectroML—an Extensible Markup Language for Molecular Spectroscopy Data

A. Arslan, A.D. Nguyen, J.C. Travis, R. Schäfer, and G.W. Kramer (839)

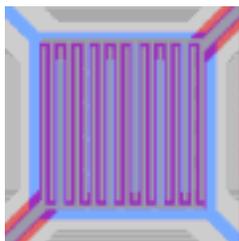
Microelectronics

Thermophysical Properties of Gases Used in Semiconductor Processing

J.J. Hurly, K.A. Gillis, and M.R. Moldover (836)

14. Technologies for Future Measurements and Standards

... anticipating and addressing next generation measurement needs of the nation



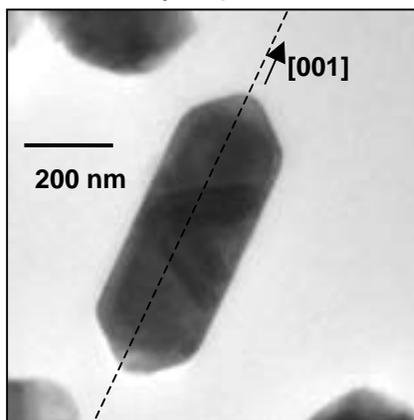
CSTL maintains a strong basic research program in broad aspects of measurement science that positions us with the capability to provide the required measurements, standards, and data to support future technology development. New measurement techniques are important for a host of industries including biotechnology, healthcare, environmental technologies, and the chemical process industry. Many of our activities, such as protein crystallography and materials microanalysis, are at the leading edge of science and technology.

Characterization of the Morphology of Faceted Nanoparticles by Transmission Electron Microscopy

S. Turner and D.S. Bright (837)

Characterization of the morphology of faceted nanoparticles is important in a variety of applications. The morphology controls the abundance, chemical composition and atomic arrangement of the particle surface and therefore controls the availability and activity of specific chemical sites on nanoparticles. For example, different crystallographic facets in catalysts have different efficiencies for promoting reactions. In pigment particles, certain morphologies are more desirable for the durability of paint. In the astrobiological field, considerable

Detailed knowledge of particle morphology can be critical to the chemical analysis of nanoparticles and to the increasing range of nanoparticle industry applications.



Faceted rutile (TiO_2) pigment particle oriented so that c axis (arrow) and projection of holder rod axis (dashed line) are aligned. Tilting around axis allows for improved morphology determination.

effort has gone into the characterization of faceted magnetite particles formed by some types of bacteria as markers for extraterrestrial life. Characterization of faceting by transmission electron microscopy (TEM) is challenging because a TEM image is a two-dimensional projection of material. In this work, the application of a double-tilt rotation holder to improved manipulation of particles was evaluated and a method for morphology modeling developed. By using this holder it is possible to orient a crystallographic direction of interest in a particle along a sample holder axis. A series of images is then collected and measurements of the dimensions made by using tools in a NIST-developed Lispix program. Possible models for the particle faceting are developed using commercially available SHAPE software. Comparisons of the experimental measurements to dimensions determined for different models of the morphology are then made to determine the best model for the particle morphology.

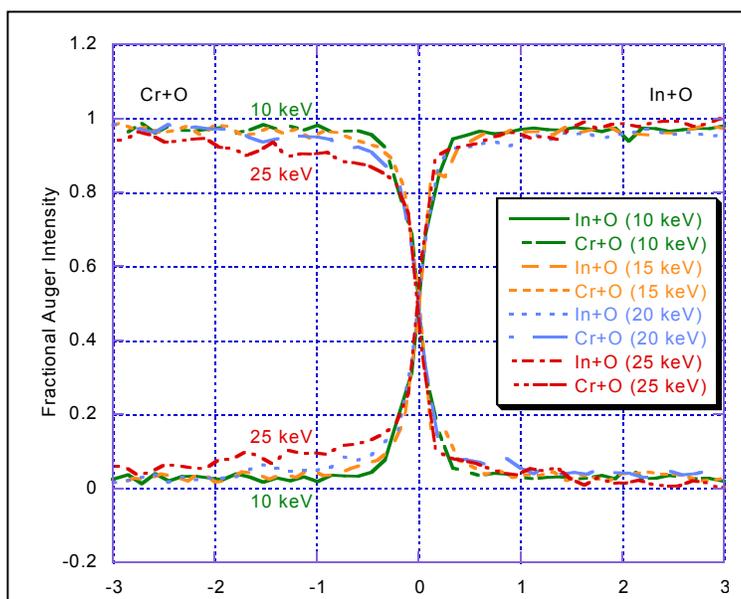
This technique should allow chemical characterization of faceted particles to be more accurate because the x-ray and absorption path lengths can be directly measured and modeled.

Effects of Backscattered Electrons on the Lateral Resolution and Analysis Area of a Scanning Auger Microscope

C.J. Powell, S.A. Wight, and J.T. Armstrong (837)

The lateral resolution, δr , is a critical parameter in a scanning Auger microscope (SAM) because it is a measure of the distinguishability of small objects. For quantitative interpretations of Auger-electron signals, however, it is also necessary to consider the analysis area (the area from which all or a defined percentage of the detected signal originates). We have determined δr from linescans across a chemical edge for our newly acquired SAM at beam energies of 10 keV, 15 keV, 20 keV, and 25 keV. The magnification standard consisted of lines of chromium and chromium oxide of varying widths and separations on an indium-tin-oxide coating that was deposited on a fused silica substrate. The figure shows the fractional intensities of the In+O and Cr+O component Auger signals as the beam was scanned across an edge for each of the four beam energies.

The analysis area needs to be carefully considered in quantitative Auger microscopy, particularly for "spot" analyses of particles or small inclusions.



At each energy level, there is a rapid change in the Auger intensities in the vicinity of the edge and slow changes ("tails") away from the edge. While these tails do not significantly affect the lateral resolution, they considerably increase the analysis area over simple expectations (e.g., that this area should be a small multiple of $\pi(\delta r)^2$). This increase of the analysis area will significantly influence Auger analyses of particles and inclusions.

Auger linescans showing the relative fractions of the In+O and Cr+O Auger signals as a function of scan distance across a line edge of a magnification standard for beam energies of 10 keV, 15 keV, 20 keV, and 25 keV.

These experiments and associated model calculations give guidance on the analysis area relevant to Auger analyses of particles and small inclusions.

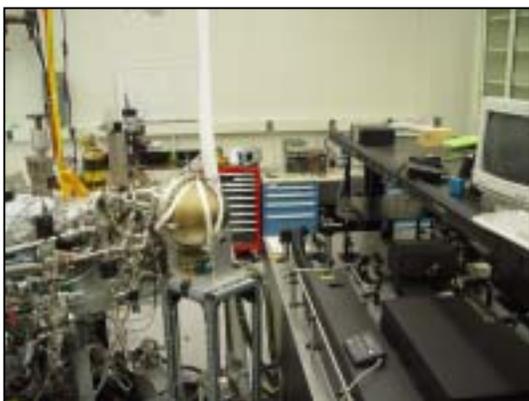
Electronic Structure and Transport in Model Molecular Electronic Systems

J.D. Batteas and S.W. Robey (837); R.D. van Zee and C.D. Zangmeister (836)

The drive to increase electronic device performance, with the associated push to ever-smaller device dimensions, has lead industry observers to conclude that silicon-based technology will reach a point of diminishing gains in the near future. This, in turn, has generated interest in alternative technologies based, for instance, on single-electron devices and molecular components. It is hoped that the tremendous flexibility available with organic synthetic chemistry and self-assembly techniques can be harnessed to produce non-linear devices

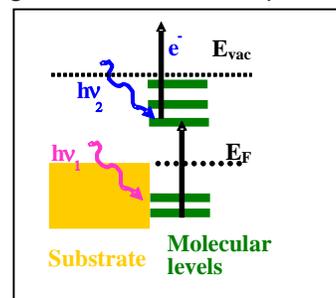
CSTL scientists are developing the measurement techniques and expertise necessary to understand electronic structure and dynamics in mesoscopic systems comprised of organic thin films and small molecular aggregates.

analogous to silicon-based diodes and transistors, but comprised of single or small numbers of molecules.

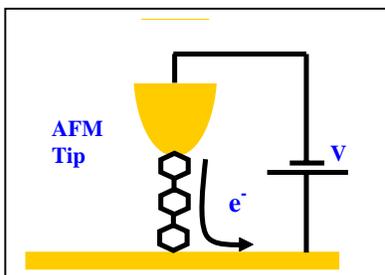


The CSTL effort in molecular electronics includes developing a battery of techniques that will provide necessary information on electronic structure and electron transport in candidate molecular electronic systems. The methods being used include two-photon photoemission, which accesses unoccupied electronic levels and tracks electron relaxation effects, and scanned probe techniques, which can characterize electron

transport down to the single molecule level. Validation of the performance of laboratory instrumentation against well-characterized systems is underway.



Two-Photon Photoemission



Scanned Probe Transport

An ultrahigh vacuum analysis system was assembled and coupled to a femtosecond Ti:sapphire laser for studies of electronic structure using two-photon photoemission. A scanned-probe system was purchased and preliminary studies of d-c transport through small numbers of conjugated molecules were also begun.

This work will provide information that, when coupled with theoretical input, will help to illuminate the physical mechanisms that produce the useful, non-linear effects observed in molecular electronics device-prototypes. This information is also required to develop measurement standards and test protocols for such devices.

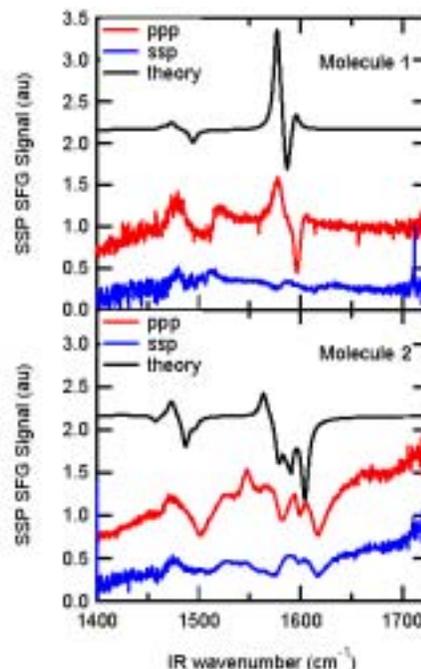
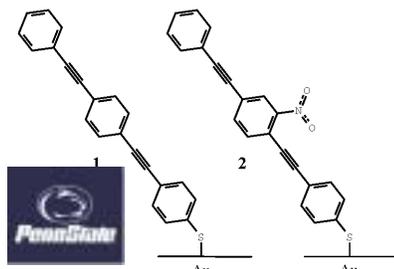
Spectroscopic Study of the Structure of Oligo-Phenylene-Ethynylene Monolayers with Molecular Electronics Implications

C.S.-C. Yang, P.T. Wilson, and L.J. Richter (837); R. D. Van Zee (836); and J. J. Stapleton and D. L. Allara (Pennsylvania State University)

Intense interest has recently been focused on the possibility of using chemical self assembly of appropriately chosen molecules to build electronic devices in a bottom-up fashion. This could lead to improvements in cost and device density over traditional semiconductor fabrication techniques. Simple electrical behavior such as conductance, rectification, and negative differential resistance (NDR) has been demonstrated in devices with a single monolayer of active molecules. Insights into the mechanisms underlying the observed molecular electronics phenomena have been limited due to the complete absence of spectroscopic tools appropriate to the study of the small molecular contacts.

The possibility of using electrically active molecules to build electronic devices has generated tremendous interest.

CSTL, in collaboration with PSU, is exploring the potential of using advanced nonlinear optical probes to characterize the structure of electrically active molecular films. High quality monolayers were (molecule 1 and 2) formed on Au substrates at PSU. Vibrationally resolved sum-frequency generation (VR-SFG) spectra were recorded over the spectral range 1000 cm^{-1} to 3200 cm^{-1} on both molecules at NIST. Shown in the figure are spectra recorded for two combinations of the polarizations of the three optical beams: ppp and ssp. The features at $\approx 1500\text{ cm}^{-1}$ and 1600 cm^{-1} for both molecules are due to vibrations of the 3 phenyl rings. The feature at $\approx 1540\text{ cm}^{-1}$ in molecule 2 is due to the NO_2 group. Also shown are predicted spectra based on *ab initio* calculations of the nonlinear line strengths of the two molecules. The *ab initio* spectra are in good agreement with the experimental spectra. The ratios of the resonance features for the two conditions can be used to determine the orientation of the molecule.



The tools used here have adequate spatial and temporal resolution to enable characterization of electronically perturbed states of the monolayer films.

VR-SFG spectra of oligo-phenyl-ethynylene monolayers

Negative Differential Resistance Explained: Guidelines and a Model to Design New Molecules

C.A. Gonzalez (838); Y. Simón-Manso (Universidad de Chile); and Y. Aray (Instituto Venezolano de Investigaciones Científicas)

The observation of abrupt changes in the slope in the current-voltage (I-V) characteristics of molecular devices formed by molecule-metal interfaces is one of the most promising phenomena in the field of molecular electronics. This phenomena could potentially lead to the manufacture of molecular electronic devices at the nanoscale such as diodes, gates, etc. Although there has been significant progress in the synthesis of such systems, the mechanisms leading to this behavior are not well understood. In this work a simple quantum-chemical model that provides insight into the understanding of the observed negative

CSTL is developing theoretical tools that can complement experimental efforts leading to the rational design of molecular electronics systems.

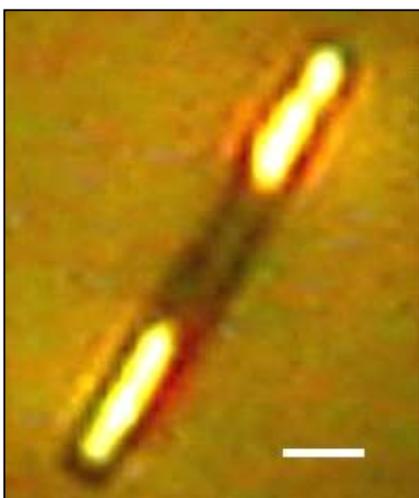
Scientists from different microelectronics companies have indicated their interest in simple models like the one presented in this work, because it would help them “screen” a potentially large number of candidate molecules.

differential resistance (NDR) in current-voltage characteristics of molecule-metal tunneling junctions was developed and validated. The model is based in quantum chemical calculations of the electronic structure of bridge molecules in a capacitor-like electric field that mimics the potential spatial profile of the junction.

The results show that a plausible mechanism for NDR behavior in planar molecular bridges, like the prototype molecule π -conjugated phenyl-ethylene oligomer 2'-amino-4,4'-di(ethynylphenyl)-5'-nitro-1-benzenethiolate (PEO) involves a substantial rearrangement of the charge density of the neutral bridge at a threshold voltage. These changes are highly affected by the presence of the nitro group, which acts as an electron basin of attraction that forces the system to behave as a quantum well trapped between two barriers. Predictions based on the model indicate that substituting the nitro group for electron withdrawing groups such as fluorine atoms will also lead to NDR behavior. Currently, these predictions are being validated by measurements performed on the recently synthesized π -conjugated phenyl-ethylene oligomer 2'-amino-4,4'-di(ethynylphenyl)-5'-fluoro-1-benzenethiolate. The model developed in this work provides a simple and powerful tool for the rational design of other molecular systems that could exhibit similar NDR behavior.

Nanowires with integrated chemical and biochemical species have been fabricated with the goal of having these structures serve as the building blocks in multifunctional nanosensors.

Fabrication of the Building Blocks of Multifunctional Nanoscale Sensors ***R.M. Hernández and S.J. Stranick (837)***



Optical micrograph of a 300 nm diameter nanowire comprised of Au outer segments and a polypyrrole inner segment. Scale bar is 1 μ m.

Nanowire systems can provide multiple options for sensor design with additional sampling and measurement capabilities made possible by the miniaturization and integration of these components. Because any biological elements can theoretically interface with any of a wide variety of signal transducers (e.g., optical, electrochemical, thermal, or acoustic), the potential range of devices and techniques can be substantial. Nanowires composed of metal and

Nanoparticle and nanowire technologies are potential building blocks for next-generation analytical sensors.

conducting polymer segments were successfully fabricated using chemical and electrochemical synthesis inside the preformed nanoscale pores of anodic alumina membranes. The figure shows a 300 nm diameter, 5 μ m long nanowire composed of alternating gold (bright) and polypyrrole (PPY) (dark) segments. Biochemical selectivity is imparted to the PPY segment by physical entrapment of antibodies, enzymes, and other receptors during electrochemical synthesis. In this work, the strong binding affinity between avidin and

A clear advantage of this entrapment process is that direct coupling or binding of the chemical/biological species is not required for immobilization/incorporation, i.e. a diverse array of chemical and biological analytes can be incorporated based on their ability to sense and rather than on their ability to bind.

biotin was used to demonstrate biochemical selectivity as well as to access the impact of synthesis conditions on biological activity. In this experiment, the presence of entrapped, fluorescently labeled avidin in PPY was verified by fluorescence microscopy, and it was found that a PPY matrix can support electrical transduction without causing a denaturing of avidin or a loss of bioactivity in avidin: a necessary condition for the generation of conductance-based sensors.

The Microanalytical Laboratory

L.E. Locascio, E.A. Waddell, T. Johnson, A.C. Henry, and P. Howell (839); D.J. Ross (836); and M. Gaitan (EEEL)

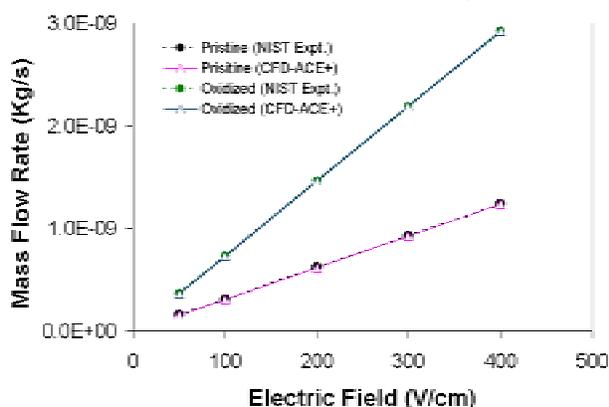
Industries involved in the development and commercialization of microfluidic technologies are interested in polymer substrates to reduce microfabrication and materials costs. An important

NIST scientists are targeting improved device performance as well as enhanced quality and reliability of chemical measurements performed in lab-on-a-chip devices.

part of the NIST program is to disseminate microfluidic measurement methods, fundamental flow and surface characterization data, and surface modification protocols to facilitate the commercial development of polymer microfluidic devices. The goals of the Microanalytical Laboratory project are three-fold: (1) to establish a fundamental understanding of microfluid flow

and develop models and methods to measure flow; (2) to devise methods for achieving chemical

selectivity; and (3) to develop novel approaches for device integration. We have continued to focus a great deal of effort on understanding and controlling electroosmotic flow in polymer microfluidic systems. As a part of this work, we have studied the effect of polymer surface modification on microflow; developed methods for accurately measuring microflow; and developed a series of highly efficient passive micromixers and flow splitters that are easily integrated into polymer microchannels. We have recently forged a collaboration with CFD Research Corporation, an established fluid modeling company, to develop models of microflow in polymer microchannels that rely on NIST data. This year, we have also been successful in developing several novel methods for improved chemical separations in polymer microfluidic systems including temperature gradient focusing, and electro-chromatography with molecularly imprinted stationary phases. The advantages associated with both techniques are that they afford greater selectivity than other methods that have previously been implemented in a microfluidic format. This work continues to involve



Experimental and simulated data on mass flow rate versus electric field strength for non-modified polystyrene channel (pristine PS) and chemically modified channel (COOH-PS).

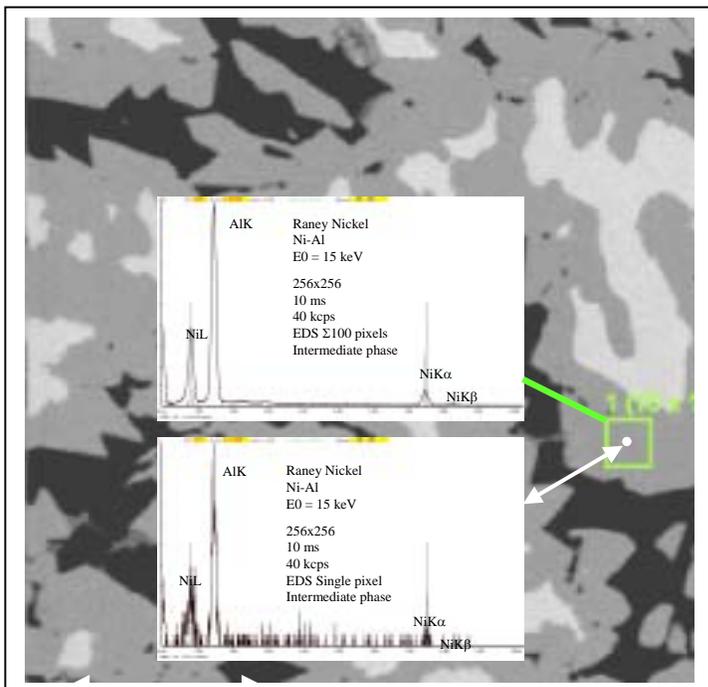
several active collaborations both internally (EEEL, PL, CSTL, and MSEL) and externally (Virginia Polytechnic and State University, Cornell, Stanford, Clemson, CFD Research Corporation).

NIST scientists have provided methods and data to support the growing microfluidics industry. A number of key collaborations have been established for the propagation of fundamental data and measurement methods.

High Speed Elemental Mapping with the Silicon Drift X-Ray Detector (SDD) on an SEM
D.E. Newbury, J.A. Small, and D.S. Bright (837)

The silicon drift detector (SDD) is a new type of semiconductor energy dispersive x-ray spectrometer (EDS) capable of operating at much higher count rates than the conventional monolithic Si-EDS (at least 250 kHz instead of 25 kHz). Spectrum imaging (SI), a powerful advance on conventional x-ray mapping, has been successfully demonstrated with the high-speed data stream of the SDD. In the SI mode, the beam is addressed to dwell for a specified time at a

Most mixtures of two or more elemental species tend to separate into two or more compositionally distinct phases at the micrometer to nanometer spatial scale. Existing x-ray mapping techniques are severely constrained by the count rate limitations of conventional Si-EDS.

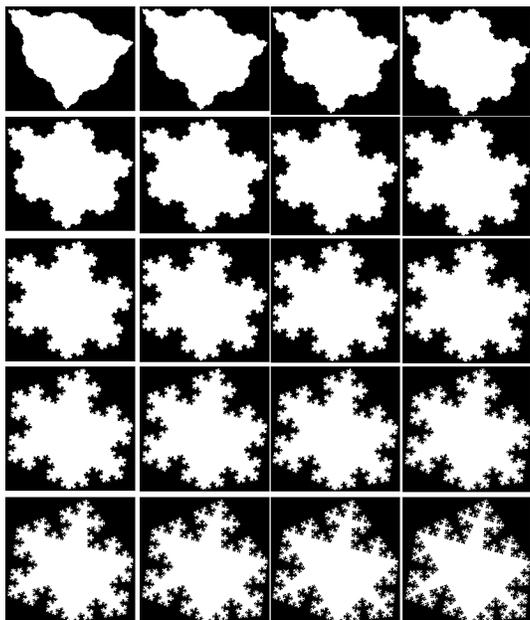


picture element (pixel) and a complete 2048 channel EDS spectrum is recorded, thus creating a database array [m x n x I(E)] of m rows by n columns by intensity vs. photon energy [I(E), 2048 channels]. The intensity per channel I(E) of this spectrum can be recorded to a depth of 16 bits. Because of the increased SDD photon counting speed, rapid mapping can proceed with a pixel dwell time as short as 10 ms, giving a total mapping time of approximately 650 s for a 256x256 map. The SI mode permits the analyst to efficiently collect all the available spectral data with no loss and to subsequently interrogate the resulting data cube concerning any possible constituent of interest, even if questions arise long after the specimen has been removed from the SEM. Rigorous quantitative mapping procedures can be applied that make

accurate background and peak deconvolution corrections to provide robust measures of minor and trace constituents, which are often misleading with conventional mapping procedures that rely only on "spectrum windows." The figure shows an example of a compositional map of the microstructure Raney nickel, a methanation catalyst, with the recorded database spectra obtained from a single pixel (pixel dwell time 10 ms) and from a 10x10 square of pixels. Major constituents are readily visible in the single pixel spectrum, while the 10x10 square of pixels has sufficient counting statistics for minor and trace constituents.

Real time x-ray mapping will significantly improve this critical characterization technique with broad applications in materials science.

Images of Particle-Like Fractals with Differing Shapes and Boundary Fractal Dimensions for Testing Algorithms that Measure Fractal Dimension
D.S. Bright (837)



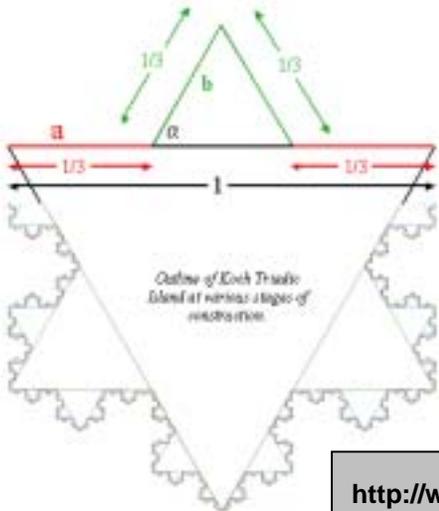
CSTL scientist provides a set of standard reference images and algorithms for testing and developing fractal dimension analysis for imaging systems.

The shape of a single particle may indicate its treatment, chemical composition, or place of origin. Microscopists continually seek numerical parameters that quantify shape in order to characterize and classify particles. The boundary fractal dimension, bFd, is a parameter that describes the roughness of the outline of the silhouette of an object. Investigators have started to use the bFd in geology, tribology, pharmacology, medical research, aerosol science, and food science. However, the bFd values from objects in digital images differ, depending on the measurement method, and hinder broader acceptance of the bFd.

Image series, bFd = 1.0-1.6, 10% actual size.

Investigators have tested their methods only on a few well known fractals, and these fractals sometimes even have dimensions outside the range of the dimensions measured for their samples. Sets of images were made available covering a wide range of bFd values that were generated from a family of fractals related to the Von Koch snowflake. This family covers the full range of bFd, 1.0 to 2.0, and has a range of shapes for each bFd. Not all of the fractals give suitable particle-like fractal images. Suitable images, their bFd values, and the software used to make any fractals from the family and measure bFd from their images are available on the web.

The images and bFd values will help investigators to characterize the accuracy of their fractal dimension measurement methods so that results can be compared and reliably used. This should promote use of and confidence in bFd measurements to characterize particles.



<http://www.nist.gov/lispix/Lx04Doc/img-sets/Koch-fractal/koch-dnld.html>

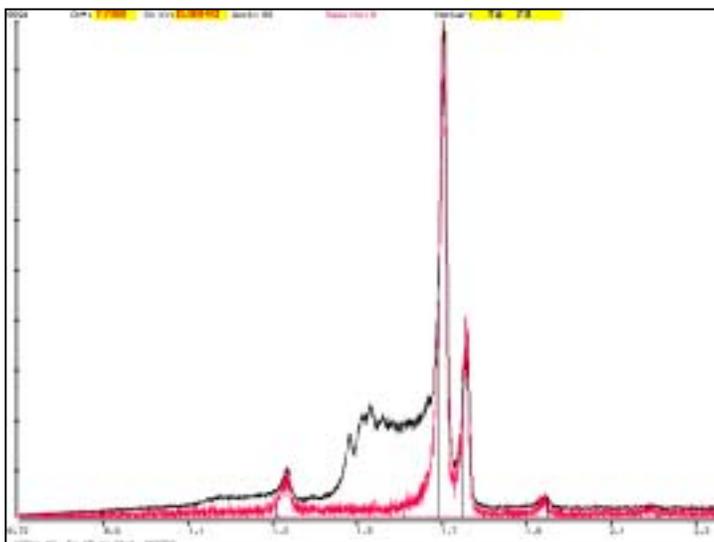
NIST Microcalorimeter Detector for High-Resolution X-Ray Detection

J.A. Small, D.E. Newbury, R.L. King, and T. Jach (837); S.W. Nam, K.D. Irwin, and S. Deiker (EEEL)

A NIST microcalorimeter energy dispersive x-ray spectrometer (μ cal EDS) has been successfully transferred from NIST Boulder to an analytical microscopy environment at NIST Gaithersburg, along with routine operation and maintenance of the cryogenic and cryoelectronic systems. Initial measurements demonstrated resolution of 9 eV or better (at $MnK\alpha$ and 40% deadtime) for short duration accumulations (100 s), suitable for measurements of major (concentration, $C > 0.1$ mass fraction) and minor ($0.01 \leq C \leq 0.1$ mass fraction) constituents. Application of the μ cal-EDS to resolving analytical situations with severe peak overlap in conventional EDS spectrometry (e.g., Cu-L and Zn-L) was demonstrated. Long duration (minimum 1000 s) measurements, necessary to accurately measure trace constituents ($C \leq 0.01$ mass fraction), were found to be subject to peak position and gain function instability. The instability results in degraded resolution by a factor of two or more. Researchers in Gaithersburg are currently working with NIST Boulder personnel to understand the cryoelectronic circuitry to diagnose and attain more stable energy calibration.

NIST researchers are utilizing a micro-calorimeter energy dispersive x-ray spectrometer (μ cal EDS) to support critical chemical measurement efforts for microanalysis research.

Future plans for the μ cal-EDS x-ray spectrometer include an extensive program of fundamental measurements in support of microanalysis needs, such as the application of low-voltage microanalysis to nano-scale characterization, accurate values of the relative peak heights within x-ray families, peak shifts due to chemical bonding, as well as direct application to materials chemistry problems.



The two overlapped spectra above demonstrate the stable (red spectrum) and unstable (black spectrum) regions on the same tantalum elemental standard.

C. Exploratory Research

Since 1999, the CSTL laboratory director has invited all technical staff to submit Exploratory Research Proposals. The insights gained from these proposals have become an integral part of our strategic planning, and a mechanism to engage all staff scientists in this important process. Scientists are encouraged to begin to discuss ideas among themselves, and where appropriate, combine the ideas into a single proposal. Over the past four years 52 exploratory research projects have been funded, and CSTL has invested close to \$4M. More importantly, we have been able to provide seed money for critical areas of research such as new approaches to modeling, measurement methods, or data development and dissemination, as well as novel mechanisms to provide reliable and timely measurement traceability to our customers. The work described in the following pages is a result of the research from FY 2002 funded projects.

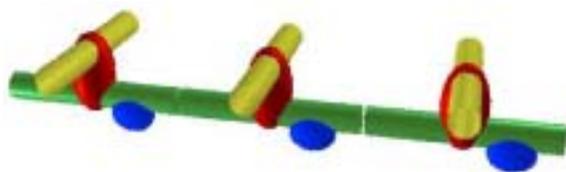
Development of Synthetic Protein-DNA Nanostructures

D. T. Gallagher (831)

CSTL Program Area: *Biomaterials*

The design and construction of a molecular lattice is an active goal in many laboratories, with a variety of different motivating applications. Most potential uses for such lattices are as scaffolds for attaching functional molecules such as enzymes (to make bioreactors), electrical/optical components (to make information storage and signal processing devices), or proteins (to make crystals for diffraction-based structure determination). Although many advances in control of molecular assemblies have been reported in recent years, no one has yet produced a designed, regular molecular lattice.

Some see the creation of a molecular lattice as a goal in itself, since it is consistent with the general nanotechnology trend toward finer structural control. At NIST, such lattices, by virtue of their precisely known geometry, will enable the development of powerful new measurement methods for biomolecular materials.



The difficulty is that lattice subunits must be capable of assembling in only one way. This implies that the subunits must be rigid and homogeneous, and that their geometry dictates specific controlled polymerization (lattice assembly) in three dimensions. Traditional organic polymers are either too flexible, too difficult to produce as homogeneous species, or lack the geometric properties that could support 3-D lattice assembly. Inorganic approaches such as metal coordination may produce crystals, but with spacings too small to support guest functionalities.

Choice of Materials: We have chosen to use protein and DNA to build the lattice. DNA is useful because it has known and predictable structural properties, forming (as duplex) long straight helical rods. Protein is necessary because DNA alone does not branch or crosslink; proteins that are already known to bind DNA will serve as lattice nodes. Of the approximately 200 DNA-binding proteins of known structure, several have geometry that is potentially suitable for lattice assembly. We selected the protein represented by Protein Data Bank structure 1AN2 for initial studies. This is a human transcription factor of the leucine zipper type, which binds DNA as a symmetric dimer. It was necessary to truncate the protein in order to be compatible with the lattice, so we will use only the first 51 amino acids, which includes both the DNA-binding and dimerization functionalities.

Design: A detailed (atomic) structural model has been developed for one design of a protein-DNA nanolattice. This lattice design has I4122 symmetry and about 15 nm repeat spacings. The

protein component is a disulfide doubled form of the dimeric (and truncated) 1AN2 protein. The added cysteine residues are at the N-terminus, a position that gives the resulting dimer-of-dimers the required 90-degree dihedral angle between the two bound DNA duplexes. The duplexes will then be given appropriate length (odd number of turns, divided by four) and termini (two-base sticky end overhangs) to enable assembly with other subunits.

CSTL researchers are aiming to produce a bivalent DNA-binding protein module that can serve in the assembly of specific biomolecular nanostructures. With appropriately designed DNA components, various constructs of increasing complexity (1-D, 2-D, 3-D, single subunit, two subunits, etc.) can be produced by small modifications in the design, providing a testable route to the higher complexity of the extended 3-D lattice.

PCR (polymerase chain reaction): We are currently using PCR methods to assemble a synthetic gene for the engineered protein, so that protein expression and purification can lead to assembly (with separately synthesized DNA components) of the lattice. Initially we will produce the protein without the terminal cysteine, so that it will bind only one DNA duplex. This can be easily verified by polyacrylamide gel electrophoresis methods. Once the DNA binding property is confirmed, then the cysteine form will be produced, and its disulfide dimerization assayed.

Next Steps: The cysteine-free engineered protein is predicted to retain both its dimerization and DNA-binding properties, but this must be verified. Following that, the cysteine form will be expressed and tested for formation of the dimer-of-dimers. The dimer of dimers will then serve as the bivalent module as it will bind two different DNA duplex molecules.

Production of Soluble and Enzymatically Active Gene Products (Proteins) in Escherichia Coli

P. Reddy (831)

CSTL Program Area: *Pharmaceuticals and Biomanufacturing*

In the protein expression endeavors, we frequently experience the production of inactive and insoluble protein aggregates called "inclusion bodies" or no protein expression at all due to proteolytic degradation. The framework of this project is directed towards engineering a universal protein expression plasmid vector that should provide a solution to these frequently encountered problem in protein expression.

Protein expression/gene expression is of vital importance to the biotechnology/ pharmaceutical industry to overproduce proteins in order to design small molecular drugs and protein pharmaceuticals and to fully understand cellular function.



We used the recombinant expression plasmid (pRE) vector with tightly controlled transcription/translation signals, which was originally developed for cloning and overproduction of lethal proteins. We then fused a segment of the gene for a chaperone protein GroEL, which is a universal protein folding machinery in the cell. A short DNA molecule that codes for a peptide linker with a specific protease (enterokinase) recognition sequence for subsequent release of the protein and a unique restriction endonuclease recognition sequence (CATATG) were incorporated downstream of the GroEL gene in the vector. All the fusions are in-frame with initiator ATG. The strategy is that the improved plasmid vector could then be used for cloning and overproduction of any gene.

One test gene (*Mycobacterium smegmatis* calmodulin) was chosen for making fusion to GroEL in the newly constructed plasmid vector. The test gene product was not expressed at all by itself perhaps due to proteolytic cleavage in the cell. Upon expression of the GroEL:calmodulin gene fusion in *E. coli*, we observed that calmodulin gene was successfully expressed as a fusion protein. We subsequently purified the fusion protein, cleaved the fusion protein with enterokinase and released calmodulin from GroEL. Hence, in principle we have constructed a plasmid vector for expression of soluble proteins.

Genome sequencing of several microbes and eukaryotes has revealed a wealth of knowledge on the gene segments that code for proteins. Nearly 50% of the proteins have no known function and hence are termed "hypothetical proteins". The challenges in the post-genomic era are: cloning of hypothetical genes, expression of the corresponding gene product (protein), and characterization of the protein for its function.

Next Steps: The vector will be tested with a variety of genes that are known to produce inclusion bodies.

Rapid Analysis of the Kinetics of Enzymatic Reactions by a Novel Stopped-Flow Microcalorimetry Method

F. P. Schwarz (831/CARB); and M. Stodeman (CARB/UMBI)

CSTL Program Area: *Pharmaceuticals and Biomanufacturing*

Research in microcalorimetry has led to the development of computer-controlled, high-throughput, rapid time-response and highly sensitive differential stopped-flow microcalorimeters (DSFM) that are amenable to monitoring the progress of an enzyme-substrate reaction calorimetrically. DSFM data for two types of enzyme-substrate reactions were analyzed in terms of the integrated form of the Michaelis-Menten rate equation to yield values for K_m , the substrate concentration required for the reaction to proceed at half its maximum velocity, and k_{cat} , the reaction turnover number. Analysis of the isomerization of fructose-6-phosphate to glucose-6-phosphate by phosphoglucose isomerase yielded values for K_m and k_{cat} from 293.4 K to 311.5 K and values for the product-substrate equilibrium constant that agreed with literature values. Analysis of the hydrolysis reaction of four N-acetyl amino acid derivatives by acylase at 298.2 K yielded values for K_m and k_{cat} , that were in fair agreement with values obtained by other methods. The exothermic heats of reaction for each substrate-enzyme reaction were also obtained at each temperature and exhibited an increase with temperature.

Since virtually all of the enzyme-substrate reactions involve the exchange of heat with the surroundings, the progress of any enzyme-substrate reaction can be universally and efficiently monitored by a DSFM. Rapid evaluation of enzyme kinetic parameters and their dependence on temperature and pH are needed by the biocatalyst industry and are needed to identify the function of unknown proteins.

The isomerization of fructose-6-phosphate to glucose-6-phosphate by phosphoglucose isomerase exhibits significant substrate-product equilibration. This exothermic reaction was monitored at 293.4 K, 298.4 K, 303.4 K, and 311.4 K and reversibly (endothermically) at 293.4 K and 298.4 K. Values of the equilibrium constant determined from the reverse and forward reactions were in agreement with literature values. The hydrolysis of N-acetyl derivatives of L-methionine, glycine, phenylalanine, and alanine at 293.2 K by acylase is exothermic and exhibits significant acetate product inhibition.

Major Accomplishments:
More than 80% of the progress of the hydrolysis and isomerization reactions monitored by DSFM agreed with the analytical expressions derived from the integrated form of the Michaelis-Menten rate equation.

The hydrolysis of N-acetyl-L-methionine was also investigated at 288.4 K, 308.5 K, and 318.5 K. Values for K_m and k_{cat} at 293.2 K were within the wide range of literature values determined by the more conventional reciprocal velocity versus substrate concentration plots. The results of the acylase investigation were presented at the 57th Calorimetry Conference and in a publication that is in press in *Analytical Biochemistry*.

Next Steps: A paper on the acylase reaction was submitted to WERB and a third one on the isomerization reaction is in preparation. Plans are underway to examine other types of enzyme reactions by DSFM.

Localization of Boron Neutron Capture Chemotherapeutic Agents by Secondary Ion Mass Spectrometry

S.V. Roberson and G.J. Gillen (837); and S. Chandra (Cornell Univ.)

CSTL Program Area: Health and Medical Technologies

This project evaluated the use of secondary ion mass spectrometry (SIMS) to image the location of isotopically tagged boronated compounds in T98G human glioblastoma cell cultures and to evaluate cell viability by means of the Na/K ratios. We have mapped the distributions of ^{10}B , Na, Mg, K, and Ca in freeze-fractured cell cultures. This work has the potential to provide valuable information regarding the optimization of therapeutically useful dosages for ^{10}B -containing compounds. Future plans include localizing the chemotherapeutic agent by means of molecular secondary ion signals and exploring means to enhance the signals obtained.

Glioblastoma multiforme is the most malignant form of brain cancer. Boron Neutron Capture Therapy (BNCT) has proven to be an effective treatment option for this form of cancer and provides a mechanism to destroy cancerous cells without injuring neighboring normal cells. The success of BNCT relies on the selective loading of boron in the tumor cells.

The purpose of this project was to use the high spatial resolution isotopic and molecular imaging capabilities of secondary ion mass spectrometry (SIMS) for the localization of isotopically tagged boronated compounds in tumor cell tissue cultures. There is significant interest in utilizing boron neutron capture therapy (BNCT) as a form of cancer radiation treatment that destroys cancer cells without injury to adjacent healthy tissue. BNCT has demonstrated effectiveness for treatment of several forms of cancer including glioblastoma and melanoma. Due to a lack of analytical techniques capable of measuring *in-vivo* ^{10}B concentrations (typically less than 100 $\mu\text{g/g}$ by mass) in tumors at the time of treatment, optimizing the therapeutic regimes has proven difficult. SIMS imaging is an ideal candidate for evaluating the distribution of BNCT drugs in tumor cell cultures and was used in this work to map Na, K, Mg, and Ca distributions as well as the ^{10}B and ^{11}B isotopes at sub-cellular spatial resolution.

One of the primary issues addressed in this work was the viability of the cell freeze-fracture preparation process and development of a method to identify undamaged cells. Cell cultures on silicon are rapidly frozen in liquid nitrogen to maintain the *in-vivo* distribution of diffusible elements and the chemotherapeutic agent. If the cell is injured in this process, the plasma membrane becomes permeable to ion gradients. Cells will consequently lose intracellular potassium and gain extra-cellular sodium. Cell damage occurs frequently during the freezing process. This damage can invalidate the SIMS imaging experiments due to possible migration of the analyte molecules. It is critical that regions of undamaged cells be localized for analysis. We use the Na and K secondary ion image intensities as indicators of cell viability. The image shows secondary ion images from a freeze-fractured specimen of a T98G human glioblastoma cell culture, containing ^{10}B enriched boronophenylalanine (BPA). A 25 keV Ga^+ primary ion was rastered over a 111 μm x 111 μm area to acquire the images. Damaged cells show high levels of sodium and

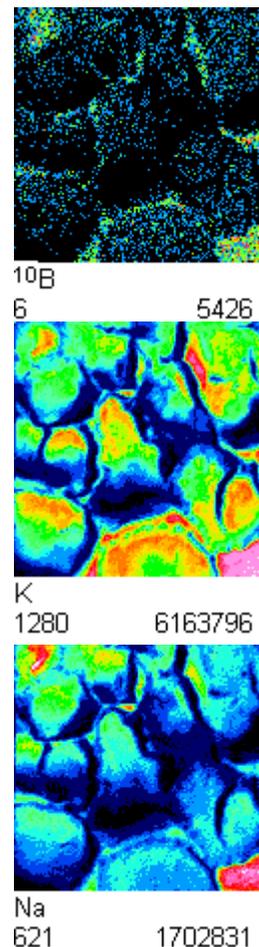
low levels potassium. The images show expected ratios for a region containing healthy cells. A second major issue was to determine if sub-micrometer localization of the ^{10}B were possible at physiologically relevant concentration levels.

The figure also shows the ^{10}B distribution in the cells. Sufficient sensitivity is available to determine the distribution of the ^{10}B . ^{10}B appears to be distributed nearly homogeneously throughout the cells. We deduce that the more intense signals (brighter areas) that are seen on the periphery of the cells are due to an artifact of the SIMS experiment and do not reflect an actual concentration gradient. The origin of this artifact will be addressed in future work. Because of the low concentration of the boronophenylalanine, secondary ion counts for the ^{10}B are one to two orders of magnitude lower than for the matrix elements, Na and K. In order to increase the sensitivity of the analysis, further analyses are being conducted using an O_2^+ microbeam. A collaboration has also been initiated to develop a new high-brightness ion source for this application.

The success of BNCT relies on the selective loading of boron in the tumor cells. The results indicate that SIMS is ideally suited for assessing cell health and for the evaluation of BNCT drugs. The technique could be used to provide initial screening and evaluation of the efficacy of new boron therapeutic agents.

Next Steps: This preliminary work provided promising data on the feasibility of utilizing SIMS to determine the localization of boronated cancer chemotherapy drugs. In the future, we will concentrate our efforts on increasing elemental secondary ion signals. We are collaborating with a small company on the design of a high-brightness O_2^+ source for microbeam

imaging on our magnetic sector instruments. Additional plans include mapping molecular secondary ion signals, locating sub-cellular structures, and quantifying the chemotherapeutic agents.



Development of Methods for the Speciation of Selenium in Health Markers
S.E. Long, D.M. Bunk, M.A. Arce-Osuna, and S. Christopher (839)
CSTL Program Area: Health and Medical Technologies

Selenium deficiency is correlated with damage to cell membranes due to accumulation of free radicals, while it also appears to be a pathogenic factor in the onset of osteoarthropathy, cardiomyopathy, and muscular disorders. Prior studies have been limited by large variability in the reported analytical data and difficulties associated with the absolute identification of the selenium species. Conclusive information on selenium content and metabolism in the human body has therefore been limited. The purpose of this work has been to develop an analytical measurement system for the separation and positive identification of relevant seleno-proteins and to accurately quantify them using inductively coupled plasma-mass spectrometry (ICP-MS). A new patentable hydride generation cell design together with a flow control system have been developed, which may be used to accurately quantify extremely low levels (pg/g) of selenium by ICP-MS.

The role of selenium metallo-proteins in the body is a subject of increasing interest to the clinical community. The development of a more accurate measurement system within CSTL may be used to provide a traceability link that would benefit the clinical community.

The goal of this research is the development and implementation of new analytical procedures for the positive identification and accurate quantitation of selenium species in targeted clinical systems. Previous studies have often been limited by poor analytical sensitivity, species specificity, contamination and losses that have led to conflicting information on reference intervals and estimates of toxicity and deficiency status. The new device developed by these researchers is based on a permeable membrane separation system, which can be mounted directly onto the ICP torch assembly. The design can be used to measure extremely low concentrations of selenium. The accurate determination of selected selenium species in clinical systems and the provision of reference systems will provide traceability for measurements by the health-care industry and for the in-vitro diagnostic (IVD) market.

Major Accomplishments:

- 1. Definition of critical measurement needs of the clinical community. Target clinical markers have been identified as total selenium, as well as selenoprotein P, albumin, and the seleno-amino acids in blood and urine.**
- 2. Development of a new hydride-generation interface and flow control system for the sensitive determination of selenium by ICP-MS.**

The new device developed by these researchers is based on a permeable membrane separation system, which can be mounted directly onto the ICP torch assembly. The design can be used to measure extremely low concentrations of selenium. The accurate determination of selected selenium species in clinical systems and the provision of reference systems will provide traceability for measurements by the health-care industry and for the in-vitro diagnostic (IVD) market.

Next Steps: This work will be assumed for a longer term by a recently arrived Ph.D. student. The isolation of seleno-proteins from serum will be accomplished using a multidimensional chromatographic approach, using metal affinity chromatography to selectively extract selenium-bound proteins from an abundance of serum proteins. Additional chromatographic steps will be used to further separate the seleno-proteins from each other before characterization by mass spectrometry of the intact protein and after proteolytic fragmentation. Amino acid analysis will be used to determine the presence of selenomethionine, selenocysteine, or selenocystine amino acid residues. Once the serum seleno-proteins have been identified, quantification of their selenium content can be made by LC/ICP-MS.

XML for Microanalysis

J. H.J. Scott (837)

CSTL Program Area: *Industrial and Analytical Instruments and Services*

XML development efforts on this project focused carefully on the original scope of the project (creating a spectrum file format), but the eventual XML application was designed to integrate with future components such as sample information file formats and instrument information file formats without sacrificing its performance as a spectral exchange tool. After testing several variations of the markup language and exploring design tradeoffs, a working prototype was implemented to store test data.

Although the original goal of this work was to provide a standard file format for the exchange of spectral data between microanalysis laboratories, it quickly became clear that this technology could provide a framework for broader information sharing in the microanalysis community. A well-designed Microanalysis Markup Language (MML) can capture information about all aspects of a microanalysis experiment (not just spectral results).

This has led to a natural convergence of this project and the SpectroML project led by Gary Kramer (additional information provided in the Industrial and Analytical Instruments and Services section). Although the two formats remain distinct because of the specific information being represented, the inherent flexibility of XML ensures compatibility and interoperability between the two applications. Because XML-formatted data exchange in the x-ray spectrometry industry is still in its infancy, an important opportunity has arisen to work on an open standard

The purpose of this project is to enable the free exchange of spectral information among microanalytical instrument users by developing an XML-compliant markup language for spectroscopy data and to provide an information framework for storing and sharing microanalysis data community-wide, allowing more sophisticated theoretical model building and model assessment to take place.

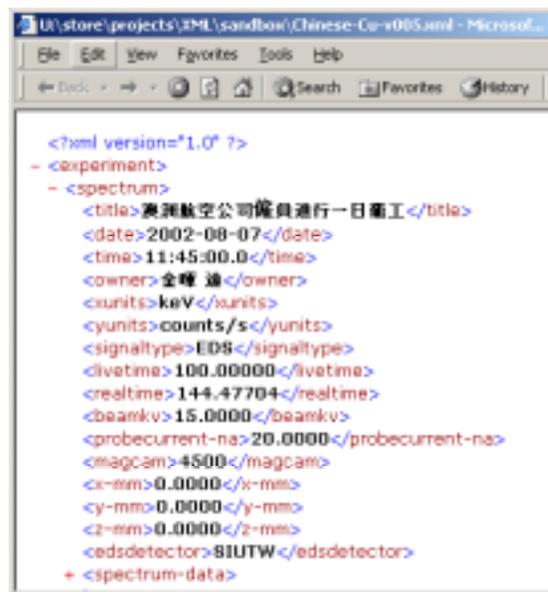
format with all the manufacturers before a proprietary approach has taken hold. One express purpose of this work is to encourage consensus among the end users and x-ray analysis system vendors. Buy-in from both the analytical instrument users and the system vendors is critical to the success of this project.

After testing several variations of the markup language and exploring design tradeoffs, a working prototype of the Microanalysis Markup Language (MML) was implemented to store test data. Variations using XML attributes in place of some elements were considered, but these were abandoned in favor of a more normalized approach.

Next Steps: Future work on the markup language will attempt to refine and improve the implementation, taking account of the suggestions from the user/vendor task group after circulation of a draft standard. A small group of beta test sites and working microanalysis laboratories will be used to ensure the XML application is practical and not burdensome. If sufficient industry interest is shown, NIST will aid industry in developing a national and/or international standard through ASTM E42 or ISO/TC202.

In addition to sparking immediate interest among end users and industry representatives at Microscopy & Microanalysis 2002, this work led to the reactivation of the Microbeam Analysis Society's computer activities subcommittee headed by Nestor Zaluzec at Argonne National Laboratory. This same committee helped develop the EMMFF file format, a simpler ASCII-based spectrum exchange format currently in use. XEDS manufacturers offered non-financial support and collaboration opportunities along with implementation suggestions and requests for features.

```
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<experiment>
  <spectrum>
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    <time>11:45:00.0</time>
    <owner>John Henry Scott</owner>
    <xunits>keV</xunits>
    <yunits>counts/s</yunits>
    |
    <x-mm>0.0000</x-mm>
    <y-mm>0.0000</y-mm>
    <z-mm>0.0000</z-mm>
    <edsdetector>SIUTW</edsdetector>
  <spectrum-data>
    <frame npoints="2048">
      0.000      0.0
      0.010      3.0
      0.020      1.0
      0.030      6.0
      0.040      5.0
      0.050      8.0
      0.060      20.0
```



Example Microanalysis Markup Language (MML) excerpts showing element structure (left) and default browsing display in Internet Explorer (right).

Fluorescence Thermometry for Temperature Measurements of Microscopic Surfaces
C. W. Meyer, K. G. Kreider, S. Semancik (836), and E. S. Etz (837)
CSTL Program Area: *Microelectronics*

The objective of this research is to make use of the temperature dependence of the fluorescence lifetime of certain phosphors for temperature measurement on a microscopic scale. The purpose of this research is to determine the viability of fluorescence lifetime microscopy (FLM) for measuring temperatures of surface areas with length scales 1 μm to 200 μm . FLM has become a highly developed technology over the past decade and it has been used extensively for biomedical research. The temperature-dependence of the fluorescence lifetime of certain phosphors gives FLM the potential to be a powerful tool for temperature measurement on a microscopic scale, but up to this point it has never been used for this purpose.

This work is expected to fulfill a need in microelectronics applications for accurate surface temperature measurements to monitor heat dissipation in power devices, such as in bipolar junction transistors and MOSFETs. It may also help improve the performance of small chemical sensors and biosensors, where the signal outputs are often temperature dependent.

The phosphor to be used for the fluorescence thermometry ($\text{Mg}_4\text{FGeO}_6:\text{Mn}$) has been obtained and fabricated into targets for sputtering onto the substrates of interest. Films of 1 μm have been sputtered onto the calibration block for preliminary studies and calibration. The assembly of the fluorescence microscope has been completed by mounting on the microscope a pulsed blue LED ($\lambda = 450 \text{ nm}$) for the excitation of fluorescence along with a photomultiplier tube (PMT) for detection of the fluorescence. An oscilloscope is currently being used to monitor the output of the PMT, and soon an analog-to-digital (A/D) converter and computer will be used to analyze the output of the PMT to determine the fluorescence lifetime.

Next Steps: Once the preliminary tests of the apparatus are done and it is shown to work properly, we will calibrate the fluorescence lifetime of the phosphor against a calibrated contact thermometer. The results of microscopic measurements will be compared to those performed for macroscopic surface areas. Measurement reproducibility using different samples of the phosphor

The objective of this project is to use FLM for measuring temperatures and temperature gradients on microhotplates, satisfying the need for measurement of these quantities on microhotplates and also demonstrating the potential of this approach for all applications in need of such measurements. Potential beneficiaries include those who work with microelectronics, microscopic chemical sensors and biosensors, and MEMS.

and the overall temperature measurement uncertainty will be determined. The spatial scales of the phosphor will be changed by changing microscope magnifications, and we will determine if FLM performs successfully and consistently for surface-temperature measurements at all available spatial scales. Assuming the FLM performs successfully, we will use it on microhotplates coated with a thin film of $\text{Mg}_4\text{FGeO}_6:\text{Mn}$ for measuring temperature gradients and performing thermal imaging.

The Initiation Step in Pyrophoric Combustion of Silane

K.K. Irikura (838) and M.S. Gordon, I. Adamovic, and H.M. Netzloff (Iowa State Univ.)

CSTL Program Area: *Data and Informatics*

In the semiconductor industry, combustion of pyrophoric gases such as silane (SiH_4) and phosphine (PH_3) is an important safety hazard. Although the combustion can have explosive force, it is initiated by relatively slow reactions. After an induction period, the primary products react rapidly to produce the characteristic flame or explosion. Silane combustion has been the subject of significant kinetics modeling efforts at NIST and elsewhere, but the initial step remains conjectural. We have suggested some new mechanisms for this reaction and are evaluating them using advanced methods of quantum chemistry. In addition to its importance for pyrophoric combustion, similar chemistry may be responsible for initiating the autoxidation of organic compounds, including plastics, elastomers, lubricants, and edible fats and oils. An understanding of how these reactions occur may lead to new ways of suppressing undesirable oxidative reactions.

Oxidative degradation of hydrocarbon derivatives is the organic equivalent of rusting. Unwanted oxidation of plastics, of which the U.S. produces 100 billion pounds annually, presents an important economic burden. Likewise, lubricating fluids no longer function properly if oxidized. Edible fats and oils become rancid and unfit for consumption. These chemical processes are all characterized by slow initiation followed by a faster chain reaction. Despite the practical interest, most purported progress has been merely speculative.

Superficially, the pyrophoric combustion of semiconductor-processing gases might not appear to be related to autoxidation. Both processes, however, involve oxidation by atmospheric oxygen and chain reactions that follow a much slower, unknown initiation step. In this project, we chose silane combustion as a model system for autoxidation due to the following advantages: (1) silane is a well-defined compound, (2) experimental data on ignition delays are available for comparison with models, (3) kinetic models have already been developed, (4) it is a small molecule amenable to high-level theory, and (5) it is of practical interest in its own right. We consider initiation reactions involving singlet and triplet oxygen, and also the “two-state” mechanism discovered recently in the gas-phase oxidation of saturated hydrocarbons by FeO^+ .

Next Steps: This project is in its early stages. We intend to evaluate the alternative mechanisms over the coming year, which will include kinetics modeling elsewhere in CSTL. If this project is successful, future work will address the major, long-standing problem of hydrocarbon autoxidation.

A contract was awarded to Iowa State University (ISU) in August. Nonetheless, we have already obtained a preliminary transition structure for the singlet reaction. The preferred theoretical method has also been identified. Although this capability is unavailable in standard software packages, it has been programmed into the developmental version of the ISU software.

Bimolecular Reaction Rates and Mechanisms Relevant to Nano-structure Formation

J.W. Hudgens (838)

CSTL Program Area: *Data and Informatics*

Gaseous organometallic compounds are used during manufacturing processes to deposit metals in semiconductor, optical, fuel cell, MEMS, and NEMS devices. The metal deposition rates and film quality are governed by the chemical environment above substrate surfaces formed by precursor compounds, by their decomposition products, and by the secondary products formed through bimolecular reactions with free radicals. Chemical modeling of any metal organic chemical vapor deposition (MOCVD) process is difficult due to the absence of reliable kinetic rate equations for organometallic species. Moreover, useful reference mass spectra are unavailable. This project comprises two laboratory activities. The first uses a novel "beam-swallowing" mass spectrometer to measure the first reference mass spectra of organometallic compounds. During FY2002 this apparatus was designed and its components were procured. The apparatus uses a pulsed molecular beam valve to introduce organometallic molecules for analysis. The design uses a series of apertures to shape the molecular beam so that thermally labile species never contact a hot ionizer surface. Because this "beam-swallowing" geometry inhibits the formation of pyrolysis products, the apparatus can measure high-quality reference mass spectra of radicals and unstable chemical species. By mounting a small pulse-heated, high-pressure flow reactor to the molecular beam valve, fundamental bimolecular mechanisms and reaction rates will also be measured.

This project will provide reference mass spectra and fundamental chemical kinetic properties of organometallic compounds that will facilitate reactor design, improve process monitoring accuracy, and enhance the safety of processes used to fabricate semiconductor, optical, fuel cell, MEMS, and NEMS devices.



These data will become available through the NIST Mass Spectral Database web site. The second uses mass spectrometry to measure bimolecular reactions between organometallic precursors and free radicals. The fundamental rate data will become available through the NIST Chemical Kinetics Database web site.

Next Steps: Future plans include measurements of reference mass spectra and bimolecular reaction rates. Reference mass spectra of organometallic compounds will become available through the NIST Mass Spectral Database web site. Bimolecular reaction rate coefficients, initially for $M(\text{CH}_3)_n$ ($M = \text{Sn}, \text{Cu}, \text{Ga}, \text{Zn}$) species, will become available through the NIST Chemical Kinetics Database web site.

Development of Accurate Quantitative Analysis Procedures for High-Angle X-Ray Emission Spectroscopy

J. T. Armstrong and J. A. Small (837)

CSTL Program Area: *Technologies for Future Measurements and Standards*

Electron backscatter diffraction (EBSD) is an important new analytical technique for micro- and nano-scale characterization of crystalline materials in scanning electron microscopes and similar instruments. There are several commercial manufacturers of EBSD detectors and this technique is being actively used for crystalline phase identification and phase orientation mapping of advanced materials with important commercial and defense applications. To properly characterize unknown materials, it is often critical to combine EBSD with quantitative x-ray emission (EDS/WDS) analysis; in most cases, the practical requirement is that these measurements be done simultaneously. However, EBSD analysis needs to be performed with the sample inclined so that the electron beam strikes it at a shallow angle (typically 20° above the surface plane); at such tilt angles, conventional correction procedures for x-ray analysis are not accurate and there are no generally agreed-upon alternatives. We have initiated work to determine the best analytical methodology, quality assurance testing, and data correction procedures to employ for high-tilt-angle EDS/WDS.

A practical series of analytical and quality control procedures were developed and an evaluation of microprobe correction algorithms was made to provide a robust method to enable quantitative x-ray microanalysis to be performed at large, variable specimen tilt angles relative to the electron beam and x-ray detectors.

The procedures and corrections explored were designed specifically to enable electron backscattered diffraction analysis (EBSD) and quantitative x-ray emission analysis (EDS/WDS) to be performed simultaneously on commercial scanning electron microscopes. They also enable integration of quantitative EDS/WDS with a variety of other surface and microanalysis techniques, such as Auger electron spectroscopy, x-ray photoelectron spectroscopy, and scanned probe microscopies. For the first time these procedures enable analysts to perform, simultaneously, accurate elemental analysis along with the best of the modern surface analysis methods. The beneficiaries of these procedures are microanalysts in a wide variety of commercial and homeland security applications from pharmaceuticals and microelectronics to explosives and forensic analyses.

We have performed extensive Monte Carlo calculations of electron trajectories in solid targets inclined at various angles with respect to the electron beam. We have tested these with experimental measurements made on our analytical scanning Auger microprobe, which is specially configured for doing x-ray emission analysis of inclined specimens. Preliminary

An easy-to-use internal standard quality assurance check was developed to ensure reproducible positioning. A polished specimen of SRM 482 AuCu is mounted with the sample. Using the AuCu standard, the ratio of the Au-L to Au-M and Au-M to Cu-K are measured. These ratios are very sensitive to beam, specimen, and detector geometry. Reproducing these ratios to a set value assures a consistent geometry, which enables Monte Carlo based corrections to be used to process analytical data.

measurements were also made on our field-emission scanning electron microscope with EBSD. These two instruments, because of the different azimuthal angles at which the x-ray detectors are mounted, represent the extreme conditions under which EBSD measurements are made. The experimental results are in good agreement with the Monte Carlo calculations, but show relatively poor agreement to several empirical corrections that had been adapted for shallower beam tilt angles. The peak-to-background ratio for x-ray lines with large overvoltage ($U = E_0/E_c > \approx 3$ to 4) at high tilt were found to be within 10% of the values measured at

normal incidence, suggesting that the correction method for particles and rough surfaces proposed by Small could be employed for tilted samples as well under these conditions.

However, the measured peak-to-background ratio for higher energy lines ($U < \approx 3$), at high specimen tilts, differs significantly from the values measured at normal incidence. The Monte Carlo simulation results indicate that polynomial “a-factors” (such as developed by Armstrong for normal incidence thick specimens and microparticles) could be tabulated and employed in a simple-to-use correction procedure. Demonstration matrices were calculated and found to produce accurate results. In performing the experimental measurements, we found that great care was needed to properly reproduce the correct sample and detector positions. The x-ray path length (and thus the amount of x-ray absorption vary significantly with detector azimuthal position, sample tilt (in both x and y directions) and sample z-positioning (height). The analytical results are highly sensitive to these factors.

Development of analysis and correction procedures enabling quantitative EDS/WDS analysis at high specimen tilt angles answers several of the most critical current microanalysis needs of industry. It enables simultaneous quantitative x-ray emission and EBSD analysis and phase orientation mapping. The quality assurance approach allows microanalysts to consistently operate at defined analytical geometries. The results of this project also enable x-ray emission analysis to be performed simultaneously with other complementary techniques that typically have tilted samples such as Auger electron spectrometry, dual beam FIB (focused ion beam), x-ray photoelectron spectroscopy, and the various ion, laser, and scanned probe techniques. By not requiring the electron beam to be perpendicular to the sample surface, these techniques enable development of hybrid and miniaturized instruments that would be still capable of high-accuracy x-ray analysis. Application of this approach can aid industry in many areas including improved quality control, failure analysis, process control, device fabrication, polymer and biomaterials characterization, etc.

Next Steps: This work completed a one-year feasibility study conducted as an exploratory research project. The results are promising enough to warrant future work. We plan to distribute polynomial a-factor correction matrices to the microanalysis community from our best Monte Carlo calculations of x-ray depth distributions for systematic binary element compositions at specific high specimen tilt angles. We plan to continue to test and refine these corrections and our analysis and quality assurance procedures by standards testing with our field emission scanning Auger and scanning electron microscopes. We plan to publish these methods once they have been refined and demonstrated to be robust.

Accurate, Traceable Measurement of Samples of Low Electrolytic Conductivity
K. W. Pratt and R. Shreiner (839)

CSTL Program Area: *Technologies for Future Measurements and Standards*

This work was designed to obtain traceable measurements of samples with values of low electrolytic conductivity (κ) for κ values approaching that for pure water. Traceability for conductimetric measurements rests on the calibration of the given cell, i.e., the determination of its cell constant, K_{cell} . The original plan was to verify two independent methods for determining K_{cell} : (a) via a separate *capacitance* measurement in the same cell, and (b) via point-of-use generation of a chain of CO_2 -free transfer standards for electrolytic conductivity. The actual course taken is outlined below.

CSTL researchers work to provide traceability for measurements of samples with values of low electrolytic conductivity (κ) for κ values approaching that for pure water. For these measurements, traceability rests on the calibration of the given cell, i.e., the determination of its cell constant, K_{cell} .

The first hurdle, production of a temperature-controlled, CO₂-free source of high-purity water, was overcome using an innovative coaxial coil. High-purity, CO₂-free water is fed through PTFE tubing inserted inside metal tubing and through the annulus between these two tubes. The outside metal jacket serves as a CO₂ barrier over the 10 m length of the coil. The PTFE tubing serves as a second barrier to exclude diffusion of ionic contamination (e.g., corrosion products from the metal tubing) into the cell feed water. At the downstream end of this coil, the Teflon tubing outlet feeds the conductivity cell. The annulus water goes to waste.

Using this approach and a flow-through conductivity cell, values for pure water were obtained that agreed to within 3% of literature values. Further measurements were deferred, pending construction of a working cell with K_{cell} optimized for pure water ($\kappa \approx 0.055 \mu\text{S cm}^{-1}$). It was further decided to construct an absolute cell, in which K_{cell} may be determined directly from physical measurements alone. Such a cell requires no calibration and yields independent verification of the two calibration methods noted above.

The approach decided on was based on a historical design for an absolute capacitor [1] using guard cylinders to eliminate end effects (fringing). The outer electrode, with inside diameter d_i , is an unsegmented cylinder. The inner electrode, with outer diameter d_o , is segmented into three sections with narrow gaps perpendicular to the axis of the cell. The middle section, of length l , is electrically isolated from the two end (guard) sections. An AC potential is applied to the outer cylinder. The end sections of the inner electrode are grounded. The middle section is held at virtual ground. The current flowing through the middle section is measured to obtain the resistance, R . Requirements for inert electrodes, cost, total mass, and dimensional stability of the cell led to Ti as the material of construction. Measurements using this cell are expected in FY 2003.

$$\kappa = \frac{\ln \frac{d_o}{d_i}}{2\pi l} \bullet \frac{1}{R}$$

In developing the model for the absolute cell, it was essential to estimate the effect of the attainable dimensional tolerances on the overall uncertainty of the measurement of κ for pure water. To this end, a cylindrical capacitor was constructed in which the offset from coaxial geometry ("centering") could be accurately varied, to obtain the resulting effect on K_{cell} . Using a capacitor for such studies (in place of a conductivity cell) avoided practical issues associated with the use of liquid electrolytes. The results agreed well with new theoretical calculations. They also exposed shortcomings in the uncertainty analysis of the landmark classical study of an absolute coaxial capacitor, performed from 1904 to 1906 to verify Maxwell's Laws experimentally.

Next Steps: A final milestone of this research was the realization that the dualism between the electrostatic field equations in a given capacitor and the corresponding equations governing an electrolytic conductivity cell with identical geometry applies to all geometries, provided that any nonideal field distribution (fringing) is the same in both applications. This principle, first discussed by Kohlrausch in 1906 but rarely exploited, subsequently, led to a fruitful series of discussions with Mike Moldover (CSTL's Process Measurements Division) and may lead to future tests of his cross-capacitor geometries in a novel form of absolute electrolytic conductivity cell.

This experiment had a spin-off result for pH SRM work: it demonstrated that alkaline pH buffers could be prepared using water taken directly from a point-of-use deionizing system without introducing a bias from CO₂. SRM certificates for these buffers have recommended preparing the SRMs using source water that is boiled to expel dissolved CO₂. This recommendation dates from the time when distilled (not deionized) water was used in laboratories. Since distilled water is CO₂-saturated, it must be boiled to expel the CO₂ before use, whereas deionized water collected at a point-of-use system is CO₂-free if adequately shielded from contact with the atmosphere.

Nanowire Chemical Sensors Fabricated by Dip-Pen Nanolithography

C.Taylor, M.J. Tarlov, and S. Semancik (836)

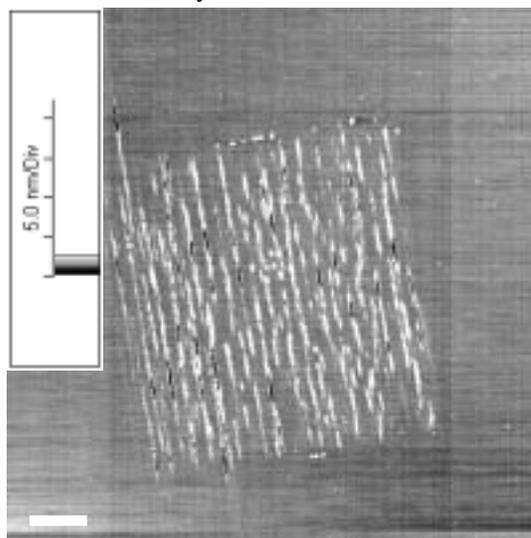
CSTL Program Area: *Technologies for Future Measurements and Standards*

Dip-Pen Nanolithography DPN is a promising new technology, however, the selection of precursors and solvents is still a trial and error process and the present study was no exception. DPN of copper was first attempted because it was thought that copper lines would have a high enough conductivity to measure easily and would also be reasonably inert to atmospheric oxidation. The strategy to form copper lines was to first write features of a copper salt and then reduce the copper salt in a hydrogen atmosphere to produce metallic copper. A CuCl_2 /n-propanol “ink” was found to possess several of the necessary solution properties to support DPN. First, the viscosity range was found to enable the writing of reasonably continuous lines. More viscous solutions were found to lead to splotchy, discontinuous lines, while less viscous solutions were found to yield exaggerated feature widths. Second, the n-propanol solution seemed to wet the surface of the cantilever well. Other solvents, such as water, did not wet the cantilever well, and the solution mixture did not adhere to the tip. Third, n-propanol acted as a good solvent allowing a high dissolved Cu(II) concentration (0.01mol solid/ 10 mL solvent mixture) and no flocculation was observed. A high CuCl_2 concentration was required to write appreciable features. Later trials showed some promising preliminary results using Gum Arabic, but more studies are required to verify these results.

CSTL researchers aimed to fabricate metallic nanowires and investigate the conductometric gas sensing behavior of the nanowires using Dip-Pen Nanolithography (DPN), a new nanolithography method.

The DPN experiments were performed in collaboration with Dr. Paul Sheehan of the Surface Chemistry Branch of the Naval Research Laboratory (NRL). NRL has Atomic Force Microscopy (AFM) capabilities that are ideally suited for the proposed DPN studies. The cantilevers were prepared by allowing them to soak in the solution for ~5 minutes. They were removed from the solution and blown dry using an air gun outfitted with a particle filter. The cantilever was then placed in the scanner head. The writing instrument was a Nanoscope 2 AFM. A rinsed and dried $\approx 1 \text{ cm} \times 1 \text{ cm}$ piece of oxidized silicon wafer was used as the substrate for the DPN experiments. The cantilever was then scanned across the surface in a square-shaped pattern using a visual basic routine written at NRL. Following DPN writing, the samples were transferred to another AFM (ThermoMicroscopes model CP) for imaging. The use of a separate AFM for imaging the DPN features was advantageous because potential imaging artifacts from carryover of the CuCl_2 /n-propanol “ink” during the imaging could be ruled out. Both DPN and imaging were performed in air.

A typical AFM image of a DPN pattern performed with CuCl_2 /n-propanol “ink” is displayed in the figure. The cantilever was scanned linearly across the surface in a square-shaped pattern approximately $5.5 \mu\text{m}$ on a side at a scan rate of 1 Hz. The scale-bar in the image is $1 \mu\text{m}$. The light, line-like features are approximately 100 nm or less in width and 5 nm or less in height. The longest continuous line is approximately $2 \mu\text{m}$. We presume that these features are hydrated CuCl_2 created by the DPN process.



DPN of CuCl_2 on a native oxide silicon surface.

D. Staff Recognition

Our staff is CSTL's greatest resource. We are proud of all of them and their accomplishments, both at work and in their communities. In FY 2002, the following were formally recognized for their achievements.



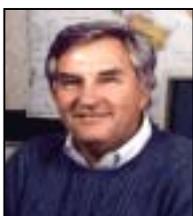
John Butler (831) was awarded the **Presidential Early Career Award for Scientists and Engineers** for advances in the fields of forensics and human identification. He also received the **U.S. Department of Commerce Silver Medal** for his contributions to the field of rapid and accurate DNA typing, particularly in support of human identification efforts in the wake of the terrorist attacks on the World Trade Center and the Pentagon.

Lloyd Currie, CSTL Scientist Emeritus, was invited to become a **Fellow of the International Union of Pure and Applied Chemistry (IUPAC)**. He was also awarded the **Ioannes Marcus Marci Medal**, which is the highest award of the Czech Spectroscopic Society of the Czech Academy of Sciences.



David Duewer (839) received the **U.S. Department of Commerce Bronze Medal** for his innovative use of visualization techniques to summarize and communicate complex statistical information.

Gary Gilliland (831) was awarded the **U.S. Department of Commerce Silver Medal** for establishing the Protein Data Bank as a unique international repository for structural data on biological macromolecules.



William (Mickey) Haynes (838) was awarded the **U.S. Department of Commerce Silver Medal** for his leadership of the Physical and Chemical Properties Division of CSTL.

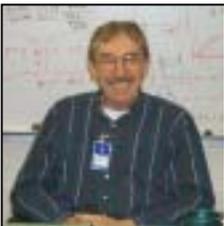
Aaron Johnson (836) was recognized as a "Modern Day Technology Leader" for the **2002 Black Engineer of the Year Award**. He was one of 50 young engineers to receive a Certificate of Merit.





William Koch, Deputy Director of CSTL, received the **U.S. Department of Commerce Silver Medal** for establishing CSTL as a world-class laboratory within NIST.

Chris Michaels, Lee Richter, and Stephan Stranick (837) received the **Samuel Wesley Stratton Award** for scientific advances in the areas of visible near-field scanning optical microscopy (NSOM) metrology, Raman NSOM, and infrared (IR) NSOM.



Archie Miller (836) and his coworkers G. Cignolo (IMGC-CNR, Italy), M.P. Fitzgerald (MSL-NZ), and M.P. Perkin (NPL-UK), received the **2002 CSTL Technical Achievement Award** for their paper "Final Report on Key Comparison CCM.P-K5 in Differential Pressure from 1 Pa to 1000 Pa" published in Metrologia 2002, 39, Tech. Suppl. 07002.

Laurell Phillips (838) was selected as the first recipient of the **CSTL Secretarial Achievement Award** for his outstanding skills and providing extraordinary service to the staff and programs of CSTL.



Ray Radebaugh (838) received the Federal Laboratory Consortium (FLC) Technology Transfer Award for his activities in collaborating with a small medical device company to develop cryogenic catheters that can freeze spots on the heart that cause heart arrhythmia.

Stephan Stranick (837) received the **U.S. Department of Commerce Bronze Medal** for his research on developing near-field scanning optical microscopies (NSOM) as a nanometer-scale chemical probe of catalytic materials.



Gregory Strouse (836) received the **U.S. Department of Commerce Bronze Medal** for his leadership in the dissemination of temperature measurements and standards and for establishing levels of comparability between U.S. national measurement standards and those of U.S. trading partners.

Gloria Thomas (836) and **Rose Hernandez (837)** attended the **52nd Annual Meeting of Nobel Laureates** in Lindau, Germany. The meeting provides an opportunity for students and young researchers to meet with Nobel Laureates.



The CSTL Divisions

CSTL's laboratory activities are primarily located at the NIST headquarters site in Gaithersburg, MD. We also have research activities in NIST laboratories in Boulder, CO, and at the Center for Advanced Research in Biotechnology (CARB) in Rockville, MD. CARB is a collaborative effort of NIST, the University of Maryland, and Montgomery County, MD.

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- Protein structure determination, properties, and modeling
- Biomaterials, biosensors, and bioelectronics
- Biocatalysis, bioprocessing measurements, and separations technologies

Process Measurements Division

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- Sensor research:
 - ◆ Micro-machined gas sensor arrays
 - ◆ Sensing applications of self-assembled monolayers
 - ◆ Thin-film thermometry devices
 - ◆ Pressure, vacuum, and flow sensors
- Measurements, models, and data for design and control of semiconductor and materials processing
- Engineering models and measurements for control of thermal systems and industrial processes
- Multiphase chemical reaction engineering

Surface and Microanalysis Science Division

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- Nanoscale chemical characterization
- Particle characterization and standards
- Electronic and advanced materials characterization
- Chemical measurement process
- Surface and interface chemistry
- Advanced isotope metrology
- Molecular scale chemical characterization of organic and biomolecules

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- Basic reference data
- Data for process and product design
- Properties of energy-related fluids
- Environmental fates of industrial chemicals
- Tools for chemical analysis
- Fundamental studies of fluids
- Cryogenic technologies
- Computational chemistry
- Basic measurements and standards

Analytical Chemistry Division

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- Chemical measurements research and services in:
 - ◆ Analytical sensing technologies
 - ◆ Classical analytical methods
 - ◆ Gas metrology
 - ◆ Laboratory automation technology
 - ◆ Nuclear analytical methods
 - ◆ Organic analytical methods
 - ◆ Spectrochemical measurement methods
- Tools for achieving international comparability of chemical measurements
 - ◆ NIST Primary Reference Materials (SRMs)
 - ◆ NIST Traceable Reference Materials (NTRMs)
 - ◆ Interlaboratory proficiency testing programs
 - ◆ International intercomparisons of measurement methods and standards
- Critical analytical data and specialty analyses
 - ◆ Quantitative FTIR database for open path sensing applications
 - ◆ Aqueous solubility and Henry's Law constants database for environmentally relevant compounds
 - ◆ Analysis/Chemical characterization of high priority samples

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