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## Analytical Chemistry Division (839)

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Analytical Chemistry Division

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

### Division Overview

#### I. Introduction

The Analytical Chemistry Division is one of five Divisions in the Chemical Science and Technology Laboratory, National Institute of Standards and Technology. The Division has approximately 90 scientists, technicians, and administrative/clerical support staff and an annual budget of about \$15M of which about \$6M supports programs for other Federal and State Government Agencies and/or American industry on a cost reimbursable basis.

The Division serves as the Nation's reference laboratory for chemical measurements and standards to enhance U.S. industry's productivity and competitiveness, assure equity in trade, and provide quality assurance for chemical measurements used for assessing and improving public health, safety, and the environment. The Division maintains world-class metrologically based core competencies in:

- Analytical Mass Spectrometry
- Analytical Separation Science
- Atomic and Molecular Spectroscopy
- Chemical Sensing Technology
- Classical and Electroanalytical Methods
- Gas Metrology
- Nuclear Analytical Methods
- Microanalytical Technologies

These competencies provide the capability to carry out the Division's broad mission and the flexibility to respond to changing and evolving national priorities. These core competencies reside in five Groups: [1] Spectrochemical Methods, [2] Organic Analytical Methods, [3] Gas Metrology and Classical Methods, [4] Molecular Spectrometry and Microfluidic Methods and [5] Nuclear Analytical Methods. The skills and knowledge derived from laboratory-based research concerning the phenomena that underpin the measurement of chemical species in a broad spectrum of matrices are applied to the development and critical evaluation of measurement methods of known accuracy and uncertainty. These internationally-recognized reference methods support the Division's infrastructure for providing traceability of chemical measurements used in programs of National/International importance through:

- Standard Reference Materials
- NIST Traceable Reference Materials (NTRMs)

- Measurement Quality Assurance Programs in critical areas
- Comparisons of NIST chemical measurement capabilities and standards with other National Metrology Institutes.

## **II. Division Project Areas in Support of Chemical Science and Technology Laboratory Programs**

### **Reference Methods and Standards for Clinical Diagnostics**

The objective of this project is to develop and maintain the measurements and standards infrastructure to facilitate accurate decision-making regarding the diagnosis, treatment, and prevention of diseases. Measurements are responsible for 10% - 15% of the \$1.3 T annual costs of healthcare in the United States. A significant portion (25% - 30%) of health-related measurements is performed for non-diagnostic reasons (re-tests, error prevention and detection). Even modest improvements in measurement accuracy and quality assurance will result in multi-billion dollar savings in health care costs. Project drivers are therefore, measurement reliability as it impacts healthcare costs and medical decision-making, regulatory requirements, and international trade and competitiveness-related issues.

Project components include [1] Reference Methods for selected health status markers (electrolytes, small organic markers, toxic and/or speciated metals, protein-based markers) [2] Standard Reference Materials (pure primary chemical standards, optical filter standards for instrument calibration/assessment, body fluid-based materials), [3] interactive measurement quality assessment activities, [4] strategic international comparison exercises

NIST works with other government agencies (e.g., CDC, NCI, NIH), professional organizations (e.g., AACC, CAP, NCCLS), and the private sector (e.g., AdvaMED, Mayo Clinic and the international community through the recently formed Joint Committee on Traceability in Laboratory Medicine prioritize measurement and standards needs.

### **Measurement Methods and Standards for Forensics and Homeland Security**

Forensic chemical analyses have become important tools for solving crimes and assuring justice. Today, most forensic analysis techniques are qualitative and are used to identify or confirm the presence or absence of certain materials. However, in many cases applying quantitative analytical techniques can provide important additional information about material sources or the significance of material identifications.

Project activities address measurement method and standards issues related to alcohol and drugs of abuse testing, human identity testing (DNA and chemical profiles in hair), crime scene investigations (gunshot and explosive residues, teleforensics), and chemical and biological weapons detection.

Project priorities are driven by input from the U.S. Departments of Justice and Defense.

### **Measurement Methods and Standards for Nutrients, Contaminants, and Adulterants in Foods**

The integrity of the nation's food supply is important for public health and safety. The development of reference methods and standards for nutrients, contaminants, and adulterants in foods is essential in this regard. Project components include measurement and standards issues related to nutrients in food products, contaminants and adulterants in food products, chemical composition and contaminants in herbal supplements/nutraceuticals, and detection of genetic modifications in food products. Project priorities are determined in consultation with the AOAC, FDA, NIH, and the National Food Processors Association.

### **Environmental Measurements and Standards**

Responsible stewardship of the environment is facilitated when measurements of known accuracy are used in monitoring and decision-making. Activities in this project involves the development of reference methods, developing Standard Reference Materials and the provision of other measurement quality assurance services to address measurement problems associated with Drinking Water Quality, wastewater Chlorination/Dechlorination Mechanisms, Atmospheric Monitoring and Global Climate Change Assessment, Automotive Exhaust Emissions, Atmospheric Particulate Characterization, Contaminants in Sediments and Soils, Contaminants in Biological Fluids and Tissues, Specimen Banking Technology.

Activities of this project support directly and indirectly the federal agencies that have stewardship responsibility for the environment (EPA, NOAA, DOE, DOD), state laboratories, and the large, environmental testing service sector that consists of private laboratories, secondary standards producers and proficiency testing firms. Our environmental projects also support industries and their consortia (e.g., AIGER, EPRI, etc.) that require high quality and/or traceable measurements to respond in a cost-effective manner to changing regulatory mandates concerning emissions and waste disposal. Our activities also support basic studies and models that track the fate of pollutants over both time and space and their impact on quality of life.

### **Methods and Standards for Advanced Materials Characterization**

Industrial and academic materials scientists often require accurate information regarding the chemical properties of advanced materials. These properties include chemical purity, trace element content, and the distribution of elements within the material. Such information not only allows optimization and control of production processes, but also facilitates a theoretic understanding of both chemical processes and material properties. As the Nation's reference laboratory for chemical measurements, CSTL must not only provide the fundamental basis of the nation's chemical measurement system, but also extend these capabilities to the real world. The Analytical Chemistry Division does this by direct measurements for customers and collaborators, by providing standard reference materials for instrument

calibration and for measurement quality assurance, and by making the latest measurement techniques and data available to the advanced materials

The Division has a broad array of analytical measurement tools including unique capabilities like neutron activation analysis, prompt gamma activation analysis, neutron depth profiling to provide analytical data and reference measurements needed to provide answers to important measurement problems.

### **Methods and Standards for Commodities Characterization**

The provision of accurate, accepted chemical characterization determinations is critical for those materials whose commercial value, properties, or suitability for use depend on their chemical composition. These characterizations may require measurement of major component(s), identification and quantification of contaminants, and/or determination of the spatial distribution of components. This project requires ongoing identification of those commodity areas with critical needs that can be addressed by NIST and strategic selection of project tasks from among these.

Current project activities focus on sulfur in diesel fuels, composition of metals/metal alloys (e.g., low carbon silicon steel, phosphorized copper), a series of cement SRMs, and an electronic scrap artifact material – the first of a new class of SRMs addressing industrial “green product” goals in which key decisions, with significant economic and ecological consequences, depend on the quality of analytical characterizations of materials to be recycled.

### **Microanalytical Technologies – Lab on a Chip**

This project is focused on facilitating the design and development of commercial plastic microfluidic systems by developing techniques to achieve greater control of microflow and microchemistries performed in plastic microchannels. In both the analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in microfluidic systems must be well controlled to provide accurate and reliable results. In response to this need, the goal of our multi-year program is to develop novel methods to evaluate and control chemistries in microscale systems. First, we will develop methods to understand and manipulate microflow in a predictable manner since a lack of flow control can lead to misinterpretation of analytical results. We will also develop methods to accurately evaluate and control temperature profiles in microchannels since temperature is a critical parameter that has a profound effect on reaction kinetics. Finally, we will develop methods to fabricate and integrate components that allow for finer control of chemical reactions including passive micromixers and microarrays. Based on input from a young microfluidics industry, the fundamental control of chemical reactions performed in

microchannels is the underpinning of a program designed to promote the commercial realization of plastic microfluidic systems.

Further discussions regarding the Division's core competencies, focused project areas, and products/services delivered to customers are provided in the Groups Section of this Overview and the Selected Technical Activity Reports.

### **III. SRMs: Tools for Providing Chemical Measurement Traceability**

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. Standard Reference Materials (SRMs) are certified reference materials (CRMs) issued under the National Institute of Standards and Technology trademark that are well-characterized using state-of-the-art measurement methods and/or technologies for chemical composition and/or physical properties. 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 ~700 different types of materials that are certified for chemical composition.

During the past year, measurements were made on ~140 SRMs. Approximately 40 high-priority renewals were completed along with 30 completely new SRMs. Other measurements were made for stability assessment and/or to address customer questions. In order to address questions from the international community concerning the quality of data provided on SRM certificates, NIST 260-136 "Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements" was developed. Completed in January 2000. In addition, a Division Quality Manual that summarizes and formalizes the Division's policies and approaches to addressing quality-related issues concerning the services that we provide was also completed and placed on the Division's internal website.

Since it has the world's leading, most mature, and most comprehensive reference materials program, most of the world looks to NIST as the de facto source for high quality CRMs to support chemical measurements. NIST has met the reference materials needs of U.S. industry and commerce for nearly 100 years. While our reference materials program has focused primarily on U.S. requirements, it is clear that these materials address international measurement needs as well. As demonstration of quality and "traceability" for chemical measurements have become increasingly global issues, the need for internationally recognized and accepted CRMs has increased correspondingly. Their use is now often mandated in measurement/quality protocols for analytical testing laboratories. Coupled with the fast pace of technological change and greater measurement needs, the demand for additional quantities and additional specific varieties of reference materials has mushroomed. NIST, by itself, does not have the resources to provide SRMs (exact sample types, unique compound combinations, concentrations, etc.) to meet

all these needs. Without a significant shift in paradigm, we will not be able to address future needs for reference materials; neither nationally nor internationally.

#### **IV. NTRM Programs for Leveraging NIST Resources and Efforts**

The NIST Traceable Reference Materials (NTRM) program was created to partially address this 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 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%.

Operationally, in the Gas NTRM Program, candidate gas mixtures are prepared in batches of ten or more cylinders, analyzed, and data submitted to NIST. NIST selects 10% of the cylinders in the batch and analyzes them so that NIST can value assign the batch. The cylinders are returned to the SGC with Certification documentation for the batch. The cylinders are under control of the SGC and can be sold to end users to provide a NIST traceable gas or the NTRM mixtures can be used by the SGC to analyze other mixtures that are then sold to provide traceability. The qualifier on the part of NIST is that NIST must have the primary standards available to perform the NIST analyses. Thirty-six NTRMs were value-assigned for four Specialty gas Companies during FY01. According to Stephen Miller, Technical Director, Scott Specialty Gases, "the NTRM program has served as an excellent vehicle for production of the high quality standards - of known pedigree - required by both industry and the regulatory community in the implementation of Title IV [SO<sub>2</sub> emissions trading] of the 1990 Clean Air Act."

The NTRM concept has been expanded to our Optical Filter Standards Program. Currently, this program is just getting underway with three vendors. In the Optical Filters NTRM program, each vendor is certified by the National Voluntary Laboratory Accreditation Program (NVLAP) to produce visible filter standards. Traceability is provided by the use of NIST SRM optical filters to calibrate vendor spectrophotometers, and quality assurance is afforded by NIST review of all vendor calibration, certification, and recertification data, NIST measurements (one set from each vendor production batch), and blind spot checks. In addition to NTRMs, approximately 200 sets (for 133 different companies) of optical filter standards were re-value assigned for wavelength and/or absorbance in the UV and NIR spectral region during FY02.

We had previously planned to also develop NTRMs in the Elemental Standards area. Those plans are being re-evaluated. Our single element spectrometric solution standards program (SRM 3100 series) is now on its firmest footing since its inception. All elements are now in stock and the uncertainty model was updated to bring the certificates into full ISO Guide to Uncertainty of Measurements (GUM) compliance. This uncertainty analysis includes incorporation of the new “Type B on Bias” method developed by the NIST Statistical Engineering Division, as well as a different treatment for transpiration correction. Improvements in packaging instituted several years ago are now paying off with longer shelf lives and larger lot sizes. This has reduced the need for replacement of expired or sold out SRMs. Two years ago over 20 SRMs in the series needed to be produced and certified over the course of a year. This year only 3 SRMs needed to be replaced. Emphasis has shifted to stability testing of SRMs in stock, and working with commercial standards producers to develop strong and defensible claims of traceability between their CRM products and the SRM 3100 Series. Toward that end we have developed a method for the rigorous comparison of single element solutions standards to the SRM 3100 Series, which has been accepted, for publication in *Analytical Chemistry*. The paper includes as supplemental information a software tool to help laboratories outside NIST implement the method to establish their claim of traceability to the SRM. Plans for the coming year call for the initiation of a new class of elemental standards, NIST Primary Standards (NPS); these materials will be certified for amount of material, as opposed to concentration.

We recognize that the NTRM model is only applicable in selected areas. In the remaining areas, such as complex matrix standards, we have begun to leverage our resources through increased strategic collaborations with other National Metrology Institutes and selected U.S. laboratories. Additional details concerning our collaborative activities with both private sector U.S. laboratories and commercial reference material and proficiency testing service providers as well as other National Metrology Institutes worldwide are provided in Technical Activity Reports that follow.

## **V. International Standards Activities**

International agreements and decisions concerning trade and our social well-being are increasingly calling upon mutual recognition of measurements and tests between nations. The absence of such mutual recognition is considered to be a technical barrier to trade and environmental and health-related decision-making. In recent years, mutual recognition agreements have been established related to testing and calibration services and in respect of the bodies accrediting such activities. All of these rest upon the assumption of equivalence of national measurement standards and reliability of the link between national measurement standards and the relevant testing services in each country. In October 1999, the Directors of National Metrology Institutes for the thirty-eight member states of the Meter Convention signed the mutual recognition arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). This MRA provides an open, transparent, and comprehensive framework for obtaining reliable quantitative information on the comparability of metrological services provided by the signatory NMIs. It also

provides governments and other parties with a secure technical foundation for wider agreements related to international trade, commerce, and regulatory affairs. Signatories to this MRA have some very special responsibilities:

- declaring and documenting their calibration and measurement capabilities (CMCs in Appendix C of the MRA)
- participating in relevant international comparisons to benchmark the claims made in the CMCs (MRA Appendix B)
- documenting the existence of a system for assuring the quality of the measurement services provided.

NIST has taken a leadership role 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. Analytical Chemistry Division staff are leading various activities within five of the seven working groups and Chairing the Organic Analysis Working Group. During the past five years, 53 comparison studies have been conducted under the auspices of the CCQM. The Analytical Chemistry Division has participated in 46 of these, serving as Pilot Laboratory in 18. An additional 36 studies are planned to be conducted over the next three years and NIST has committed to pilot 9 of these.

Analytical Chemistry Division staff have 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.

Participation in CCQM Key Comparisons is available only to top-tier NMIs around the world. Within the Americas, only the U.S., Canada and Mexico have well-established programs in chemical metrology. In order to most effectively address the unique needs of all 32 countries within SIM, whose capabilities in chemical metrology span a very broad range, we have initially focused the SIM program on training and capability assessment rather than participation in MRA- driven Key and Supplemental Comparisons. During the past three years, ten intercomparison exercises were carried out to assess the proficiency of SIM NMI's and/or their designated laboratories. While SIM is focussing entirely on training and capability assessment, Regional Chemical Metrology Working Groups in Europe and the Asian Pacific are forging ahead and conducting MRA-driven Key Comparison Studies. We have established agreements with the Chemical Metrology Working Group Leaders of both regions to allow non-CCQM member countries within SIM to participate in such studies as soon as they feel competent to do so. Once self-

assessed capabilities of SIM member states are at an appropriate level, as determined based on performance the SIM capability assessment studies, they can also request permission to participate in CCQM Comparisons.

We are 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 the various countries and regions around the world.

In addition to these global and regional activities, 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. 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 recently signed a Cooperative Arrangement with NIMC (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 /Standards laboratories are being discussed.

## **VI. Collaborations with Other Government Agencies and Professional Organizations**

Providing chemical measurement quality assurance services in support of other Federal and State government agency programs (on a cost reimbursable basis) continues to be an important part of our measurement service delivery portfolio. During the past year, we were involved with 25 projects with eleven federal and state government agencies. We also had technical interactions that involve laboratory research and measurement activities with more than 20 professional organizations and societies including the American Industry/ Government Emissions Research consortium (AIGER), American Association for Clinical Chemistry (AACC), American Society for Testing and Materials (ASTM), Certified Reference materials Manufacturers (CRMMA), Association National Food Processors Association (NFPA), National Council on Clinical Chemistry (NCCLS), and the National Environmental Laboratory Accreditation Council (NELAC) Specific details concerning many of these interactions are provided in the Groups Section of this Overview.

## **VII. Future Directions**

We will continue our strategy of maintaining core competencies in chemical measurement science to address measurement problems in areas of national priority. All

Division research and service projects will continue to be reviewed on an annual basis for match to mission, progress, quality and customer need. Projects in several new areas are being initiated or expanded.

In the **Forensics Standards** area, we are working with CDC to provide quality assurance and measurement proficiency assessment for the “Chemical Counter-Terrorism Laboratory Network” which will initially be comprised of several state public health laboratories and the CDC. In the event of a chemical terrorism attack, samples (urine or blood) would be shipped to CDC for analysis to determine what agents were used, who was exposed, and how much exposure occurred. CDC would most likely not be able itself to handle so many samples in a short time that would need the help of the state labs in the analyses. NIST’s responsibility would be to provide Reference Materials and QA samples to assist CDC in assuring the quality of results from such tests. In addition, activities concerning detection of toxins in the water supply, the establishment of a mass spectral database to facilitate rapid and unambiguous identification/detection of pathogens have are being expanded to address Homeland Security issues.

The primary focus of the Division’s component of CSTL’s **Food Safety and Nutrition Program** is being shifted from populating the AOAC Food Triangle with food-matrix reference materials (to address nutritional labeling issues) to reference methods and standards to address consumer safety and truth-in-labeling issues associated with Nutraceuticals/Herbal 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. 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. Priorities for activities in this new project area are being set in consultation with the U.S. Food and Drug Administration, and the National Institutes of Health’s Office of Dietary Supplements.

Our research project on **Microfluidic Devices**, methods, and applications for chemical analysis including studies of materials and material properties affecting the flow of liquids in microchannels will begin to focus more on the use of microchannel and other electrophoretic methods for forensic and toxicological applications and standards. Research in our new Competence area, **Single Molecule Measurements and Manipulations** will be expanded. Additional details concerning the latter two research activities are provided in the Group Sections of this Overview that follow later.

**Healthcare** is a major focus area for NIST, the Chemical Science and Technology Laboratory and the Analytical Chemistry Division. In addition to the clinical measurement reliability and cost issues that have driven our measurement and standards for clinical diagnostic markers project over the past twenty years, a very significant commerce and competitiveness issue has recently emerged -- the European Directive

98/79/EC on *in vitro* diagnostic medical devices. By December 2003, manufacturers must declare that any new IVD product to be sold within the EU complies with all “essential requirements” of this Directive. One of these requirements is that IVD products be traceable to “standards of the highest order”, e.g., nationally and/or internationally recognized reference methods and/or certified reference materials. At present, IVD devices are used in clinical laboratories to measure more than 300 different chemical or biochemical species. Reference methods and/or materials exist for about 30. Approximately 60% of the IVD products currently on the European market are imported from the USA. Excluding home diagnostics, the overall world wide in-vitro diagnostic market ("IVD") is approximately \$20 billion.

In response to this need we have committed to develop new reference methods and blood/urine-based SRMs for several new health-status markers while maintaining previously developed reference systems for calcium, chloride, cholesterol, creatinine, glucose, lithium, magnesium, potassium, sodium, triglycerides, urea, uric acid, and vitamins A, C, E and beta carotene as well as several therapeutic and drugs of abuse. Over the next two years, reference methods and/or new reference materials will be developed for:

- **Cardiac Troponin I** (*heart attack occurrence and damage*)
- **Cadmium** (*toxicity*)
- **Cortisol** (*endocrine function*)
- **Folates** (*neural tube defects*)
- **Prostate Specific Antigen** (*prostate*)
- **Glycated Hemoglobin** (*diabetes status*)
- **Homocysteine** (*heart attack risk*)
- **Ionized Calcium** (*important marker for diagnosis of various disease states such as skeletal resorption and stone formation in the urinary tract*)
- **Mercury** (*toxicity*)
- **Speciated Iron** (*anemia and hemochromatosis*)
- **Triiodothyronine and Thyroxine** (*thyroid function*)
- **Total and Speciated Selenium** (*to support clinical monitoring in the relatively narrow range of beneficial effect*)

Over the next decade, driven by the availability of new sensor-based measurement technologies, more and more clinical testing will be done outside the traditional clinical laboratory. The annual U.S. market alone for this new form of clinical measurements, called point-of-care testing (POCT), is currently a billion dollars and is growing at an annual rate of 10%. POCT is expected to be used extensively in the home as part of a self-care trend, which is currently experiencing a 70% growth rate. Published studies have concluded that POCT provides at least the same level of diagnostic value as centralized testing, but at half the cost. The standards infrastructure that has supported clinical chemistry for the past two decades must adapt to support POCT. Collaborative efforts need to be established among National Standards Laboratories, in-vitro diagnostic device (IVD) manufacturers, and others in the medical professional community to develop appropriate technologies and non-biohazardous standards to facilitate the

provision of data used in medical decision-making that are accurate and traceable to national/international standards. NIST leadership in developing traceable POCT standards will help assure continued U.S. dominance of the worldwide IVD market and foster more affordable healthcare both at home and abroad.

The most widely used home testing devices are for glucose. Diabetes affects more than 10 million Americans and its prevalence rose from ~5% to 7% during the 1990's. Although home blood glucose monitoring has clearly revolutionized diabetic care and changed both the therapy and outcome for this disease, use of these testing systems remains problematic. In the short term, we support will CDC and FDA in their investigations of the performance of home glucose monitors. We have been asked to provide the reference method and whole blood reference materials to support these studies. In the longer term, we will be developing optical artifact standards for non-invasive measurement technologies.

While focusing increased attention on the health-care/clinical diagnostics area over the next 3-5 years, we will still provide renewals of existing SRMs that support measurements in areas critical to the global community and address critical measurements and standards needs in new high priority areas such as:

- **Biotech Foods** (*reference methods and materials for detecting genetic modification of whole grains using transgenic proteins as markers in collaboration CSTL Biotechnology Division—who will be developing reference methods and materials for direct detection of DNA modifications*)
- **Detection of Chemical Agents in Body Fluids** *to support CDC's Chemical Counter Terrorism Network*
- **Fluorescence Intensity Standards** *for calibration of instrumentation widely used by clinical and biotechnology communities in collaboration with CSTL Biotechnology Division*
- **Gunpowder Composition** *to support NIST Office of Law Enforcement Standards programs for validating forensic methods used to identify gunpowder residues*
- **Low Sulfur and Mercury Coal** *to support lower EPA emissions regulations for sulfur and mercury*
- **Near Infrared Transflectance/Reflectance** *for calibration of single- and double-pass transmission instruments finding increasing use in sensing applications for both wavenumber and wavelength*
- **Nutraceuticals** *in collaboration with FDA and NIH to address consumer safety and truth-in-labeling issues*
- **Phosphorus Implant in Silicon Depth Profile Standard** *to complete suite of three depth proofing standards requested in the Semiconductor Industry Road Map; boron and arsenic implant SRMs already completed*
- **Raman Intensity Correction SRMs** *in response to priorities established by ASTM E13.08 Raman Spectroscopy Subcommittee for a suite of standards for providing relative Raman intensity corrections for Raman spectrometers at the commercially important excitation wavelengths. SRM 2241 at 785 nm has been completed. SRMs for excitation at 532 nm, 488 nm/514 nm, and 1064 nm remain.*

- **TiAl Alloy for Microanalysis and XRF** (*with CSTL Surface and Microanalysis Science Division to provide benchmark for aerospace industry for Ti, Al, Nb, W in USAF-supplied alloy material*)

We expect that the NTRM approach will continue to expand as the basis for allowing the commercial sector to provide reference materials to end-users with a well-defined traceability linkage to NIST. High quality SRMs will continue to be developed to address both national and international measurement problems, but they will be much more expensive and targeted for purchase primarily by other national metrology institutes and commercial producers/distributors. Increasingly, end-user needs will be met via NIST-traceable commercially produced reference materials.

The complex nature of chemical measurements coupled with the increasingly global nature of trade, health, and environmental issues speaks to the need for having the world make measurements using a common “meterstick”. The comprehensive nature of the NIST program in chemical measurements puts us in position to make a very significant contribution to the international chemical measurements community in this regard. In order to do this in both a cost-effective and internationally congenial manner, we are investigating opportunities for partnering with other highly qualified NMIs to provide high quality Certified Reference Materials in specific standards areas. Potential advantages of these proposed partnerships include increased supply, breadth and quality of reference materials for the worldwide chemical measurements community as well as less duplication of effort for more efficient use of limited resources.

This overview plus the Group-specific sections and Selected Technical Activity Reports that follow provide additional details concerning our work and reflect the high quality of our staff. This information also shows how our efforts are impacting U.S. industry's productivity and competitiveness as well as providing the measurement and standards infrastructural support for environmental quality and human health assessments. They also illustrate the critical synergistic relationships that exist among our research programs in chemical measurement science and the standards and quality assurance services that we provide to customers.

## **VIII. Group Overviews**

### ***Spectrochemical Methods***

Research activities in spectrochemical methods are directed toward the development, critical evaluation, and application of techniques for the identification and measurement of inorganic species using x-ray, optical, and mass spectrometries. The focus of this research is measurement accuracy and precision, benchmarking industry's needs for traceability, advanced materials characterization, and commodity value assignment and specification.

One of the new strategic thrusts at NIST in FY 2002 was Homeland Security. The increased National emphasis on this topic accentuated ongoing efforts in the Group and

resulted in the consideration and establishment of new research efforts. For instance, during the past two years we have collaborated with the Surface and Microanalysis Science Division on a project with the U.S. Army to critically evaluate the performance of gas masks in protecting humans from both particles and particle borne toxicants. The Group has joined the Division's efforts to respond to the threat of chemical terrorism in the U.S. It is imperative that the chemical measurement capabilities developed to meet this threat provide accurate and incontrovertible results. Discussions were initiated with the Centers for Disease Control and Prevention (CDC) to collaborate on quality assurance oversight for CDC's "Counter-Terrorism Laboratory Network (CTLN)". This network now consists of CDC and laboratories in five states (NY, VA, MI, NM, and CA) with the plan to expand the number of states participating in the coming years. CDC has transferred to the states methods for the determination of organophosphorus nerve agents (5 analytes) and sulfur mustard gas metabolites in urine. Additional methods to be developed and transferred to the states include lewisite, cyanide, and inorganics such as arsenic, mercury, and plutonium. CDC advised NIST that the development of standards and the validation of methodology for cyanide were their top priority. Thus, we initiated a project for development of cyanide in whole blood standards and proficiency testing materials. We have replicated the CDC headspace GC method with nitrogen-phosphorus detection and looked at improvements that might be provided through use of isotopically labeled internal standards and mass spectrometric detection.

The poisoning of our food, water, or air could come from more readily available chemicals -- used less for mass destruction and more for mass terror. Thus, our ongoing efforts to benchmark trace element detection and the characterization of "natural" levels of elements in the environment, body fluids, and in our foods take on increased relevance. For instance, we have added new certified values for Cd and Hg in our blood SRM 966 at concentrations of a few tens of parts-per-trillion, and have completed analyses for a suite of toxic elements in the urine SRM 2670a at levels as low as 5 parts-per-trillion. In the coming year, we are scheduled to provide supporting toxic element data for the nutraceutical standards under development. We are exploring the need for new water-based standards—for instance, ultra-low detection of plutonium, and for non-natural (depleted) uranium.

The Group has a long history of providing research and standards to support environmental measurements regulated by the Clean Water Act, the Safe Drinking Water Act, and the Clean Air Act. One of the more popular SRMs is SRM 1643 Trace Elements in Water; it sells more than 1000 units a year. A new method for the production of SRM 1643e was developed and tested this year that relied on both measurement and the use of primary materials, gravimetrically added. The results were surprisingly consistent and will allow the certification of this material more efficiently and with higher precision and accuracy than has been achieved in the past. Stability does not appear to be an issue. A new ICP-MS method for high precision comparisons of multielement standards was designed and applied this year to support the quality assurance and proficiency testing programs conducted by ACD for the Department of Energy and EPA. This method is capable of yielding 0.2 % uncertainty for multielement solution standards and it was

applied in the determination of five elements in 13 different mixtures analyzed for the EPA-PT program.

The emission of mercury into the environment is one of the most important regulatory concerns and the Group made measurements and certified standards in a range of projects using the recently developed method based on CV-ID-ICP-MS. The sensitivity of this method was improved by refinements in sample introduction and in the reduction of blank. The Department of Energy sponsored survey analyses of oils in the U.S. Strategic Petroleum Reserve. Although coal burning is believed to be the major anthropogenic source of mercury emissions, the refinement and combustion of liquid fossil fuels is a potential source for which sparse data exist. The NIST measurements will be extremely useful in this source term evaluation and expands on data collected last year on SRM oils. Mercury was also determined in a wide variety of SRMs: Urine, Inorganic Sediment, Crude Oils, Pine Needles, Bovine Blood, Mussel Tissue, Fish Tissue, and Coal.

The Group completed a project at its Charleston, South Carolina laboratory on the determination of mercury in seabird eggs. The transport and fate of airborne mercury in marine and coastal environments is an active area of research. Numerous agencies and research organizations are involved in mercury measurement. The scientific integration of these measurements requires quality assurance in terms of accuracy and sampling protocols that ensure representativeness. The unique application of the CV-ID-ICP-MS method to the eggs of common murrelets from the Bering Sea and Gulf of Alaska highlighted the use of high-accuracy methods and their ability to establish natural variability (temporal and geographic) of contaminants. Method reproducibility of better than 1.5 % was demonstrated at the 10 ng/g (ppb) range for mercury.

In the area of air quality, the Group has been working with the National Institute of Occupational Safety and Health (NIOSH) to develop a new series of SRMs: Silica on Filters. Respirable crystalline silica is an occupational hazard whose presence in the workplace is strictly regulated by the Occupational Safety and Health Administration. It is poorly measured by standard industrial techniques (XRD, IR, UV/VIS). Thus, both the demonstration of a safe workplace and effective enforcement of regulations have been frustrated. The new SRMs have been prepared by depositing SRM 1878a, which is certified 100.00 % +/- 0.21 % crystalline alpha-quartz, on PVC filters. A new sample preparation method was developed for the quantitative digestion of silica. Both ICP-MS and ICP-OES methods for the measurement of silicon have been developed and shown to be appropriate and comparable. Certification will be based on the chemical measurements and the gravimetric amounts of silica delivered. Measurements have been completed on five of the eight levels (between 5 and 1000 microgram loadings) in the series. When this series is complete, NIOSH has proposed to support the production of a second series—cristobalite on filters. Our experience with the development of the first series should make this project easier.

Measurement of the release of  $^{129}\text{I}$  into the environment, either inadvertently or deliberately, is a scientific challenge. A series of  $^{129}\text{I}/^{127}\text{I}$  isotopic standards was developed this year to benchmark the detection of  $^{129}\text{I}$ , a long-lived fission product, by

mass spectrometry. This work was a follow-on to last year's isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) method development and the determination of iodine in urine. The isotopic series will support development and testing of new instrumentation, especially ICP-MS, as well as provide calibration standards for accelerator mass spectrometry (AMS). This project was done in collaboration with the Physics Laboratory's Ionizing Radiation Division, and the AMS laboratory at Purdue University.

Accelerator mass spectrometry measurements of the primary ammonium iodide material were made at Purdue University. The measured  $^{129}\text{I}/^{127}\text{I}$  ratio of approximately  $10^{-15}$  was the lowest ratio they had ever measured—below their own historic blank level. Four isotopic  $^{129}\text{I}/^{127}\text{I}$  mixtures ( $10^{-6}$ ,  $10^{-8}$ ,  $5 \times 10^{-10}$  and  $10^{-12}$ ) were prepared from this ammonium iodide and the calibrated  $^{129}\text{I}$  spike (SRM 4949C). The ammonium iodide was assayed using gravimetry in order to confirm the manufacturer's purity specification; the iodine was determined by precipitation as palladium(II) iodide,  $\text{PdI}_2$ . The isotopic composition and concentration of the spike were measured using ICP-MS. The isotopic mixtures were ampouled with the assistance of the Standard Reference Materials Program and each unit consists of 5 mL of standard solution sealed under argon in a borosilicate amber glass ampoule. One hundred ampoules of each of the four standards, as well as 100 ampoules of the natural iodide (blank), were prepared.

Interface characterization and the analysis of thin layers and coatings is an important analytical problem in a wide variety of modern technologies. The need to develop better, faster, and more economical characterization methods to determine elemental composition quantitatively as a function of depth, will surely grow. On a limited basis, we have continued to investigate thin layer characterization this year—using glow discharge optical emission and XRF spectrometries. The sensitivity of GD-OES for depth profiling of SRM 2137, which is a B-doped Si wafer, proved to be borderline at best. Nonetheless, a depth profile could be produced by co-adding several sputtering runs together and low-pass filtering the combined data. The depth axis was calibrated independently from the glow discharge data by profilometry. The shape of the resulting depth profile compared favorably with SIMS. Supported by a NATO grant, Dr. Marek Lankosz rejoined the Group as a Guest Researcher for a month this year. During his visit, the problem of determining of both composition and thickness when one element is present in two layers was investigated. Data from thin films of Cu and Ti were collected. Analysis and modeling of the results is in progress.

The metal and metal alloy industries are important sectors of our economy and the economic impact of chemical measurements within these industries is very large. The wide range of commercial metal and alloy materials has made identification of an appropriate CCQM measurement activity in this area a challenge. The first 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. This sample was analyzed using a recently developed matrix independent XRF borate fusion method. The X-Ray Fluorescence (XRF) team continued to refine the development of a matrix-independent method, which provides direct traceability to primary reference materials in combination with the speed and

repeatability of XRF and has been demonstrated to be applicable to the determination of important constituents in cements as well as metal alloys

The Group Benchmarked its measurement capabilities through participation in four CCQM comparison studies during the past year: CCQM-K24 Cadmium in Rice, CCQM-P29 Zinc in Rice, CCQM-P13 Synthetic Food Digest, and CCQM-P25 Minor Elements in Steel. NIST used combined results from ID-ICP-MS and INAA based methods for the Cd in Rice Key Comparison. Our result was in excellent agreement with the 10 other laboratories [NIST: (14.36 +/- 0.23) nmol/g; Key Comparison Reference Value (14.40 +/- 0.09) nmol/g]. 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)  $\mu$ mol/g; comparison reference value: (0.3527 +/- 0.0024)  $\mu$ mol/g]. The major source of uncertainty was in achieving a dry-mass basis weight.

In the Pilot Study (CCQM-P13) on Metals in Artificial Food Digest we used ICP-OES methodology with standards addition calibration – as we have been using in our recent certification measurements for metals in SRM 2387 Peanut Butter, SRM 2385 Spinach, and Milk Powder. The results for Ca and Cu were +1.3 % and –0.8 % relative to the reference values, respectively. The average deviation of all participants from the reference values were –0.5 % and +3.5 % respectively. 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.**

### ***Nuclear Analytical Methods***

Research activities in this group are focused on the science that supports the identification and quantitation of chemical species by nuclear analytical techniques. Current laboratory research activities involve the full suite of nuclear analytical techniques, including instrumental and radiochemical neutron activation analysis (INAA and RNAA), prompt gamma activation analysis (PGAA), and neutron depth profiling (NDP). In addition, we are developing analytical applications of neutron focusing technology. The measurement capabilities that reside within this group provide an excellent complement to those in the Spectrochemical Methods Group in that nuclear analytical methods depend upon characteristics of the nucleus of the element rather than the electron shells, and therefore are insensitive to the chemical state of the analyte. In addition, the nuclear methods are generally nondestructive and do not require sample dissolution, thus providing an independent assay. NDP and focused beam PGAA provide unique capabilities at NIST for location sensitive analysis and elemental mapping.

INAA and RNAA are powerful reference techniques that have been used at NIST for many years. New developments continue to provide improvements in detection sensitivities, elemental specificity, precision, and overall accuracy that allow nuclear methods to address new measurement needs. During the last several years, we have been attempting to demonstrate that instrumental neutron activation analysis meets the CCQM definition of a primary ratio method of measurement. Our first step was to characterize all sources of uncertainty for INAA measurements and develop the tools to establish a

complete uncertainty statement in terms of SI units. We next applied INAA as a primary method for certification of the arsenic content of SRM 2134, Arsenic Implant in Silicon. The INAA results used for this certification included the first complete, quantitative evaluation of all sources of uncertainty in an INAA measurement. The expanded relative uncertainty for the mean value of this SRM was 0.38 % and approximates the 95 % level of confidence. While involved with this project, we found that the most recently published and compiled half-life of  $^{76}\text{As}$  did not describe our data as well as the earlier accepted value. We have redetermined this parameter using four Ge detector systems, and fitted an exponential to the decay data by two different nonlinear least-squares methods. We measured  $t_{1/2} = 1.0938$  d with an expanded uncertainty  $U = 0.0009$  d. This result is 1.5% higher than the recent value, and in agreement with the older, less precise, consensus value. Methods developed in this work are being applied to the accurate redetermination of other crucial half-lives, beginning with  $^{24}\text{Na}$  and  $^{42}\text{K}$ .

This year, we have determined the concentration of Cr in a ferrous metal, SRM 1152a, and provided a complete uncertainty evaluation, as a test of our capabilities in a difficult matrix. Analysis of a metal challenges the INAA measurement process in a totally different manner than does determination of arsenic in silicon. The high density of the metal increases the magnitude of both neutron and gamma ray self-shielding. The metal matrix also produces an elevated level of baseline radiation. An additional reason we have chosen to determine Cr in SRM 1152a is the small uncertainty in its certified value, which provides a good test of our analytical values. Measurements were conducted using crystalline chromium metal as a comparator standard, and were validated with weighed aliquots of SRM 3112a solution dried on filter paper pellets. The INAA results agree with the certified value within the stated uncertainty of this SRM ( $\leq 0.2$  % relative), and the complete assessment of the uncertainty budget indicates a relative expanded uncertainty of 0.2 %. This work is now being expanded to a participation in the CCQM P-25 pilot study for the determination of Cr, Mn, Ni, and Mo in low-alloy steel.

We have recently developed new INAA procedures and applied them to homogeneity studies of SRMs at small sample sizes. Many analytical techniques used in industry and academia, rely on the analysis of very small samples (i.e., 1 mg), typically in the solid (undissolved) form. Unfortunately, most SRMs are certified with minimum sample sizes of 100-500 mg, and are therefore unsuitable for use as control materials for these techniques unless additional information is made available. Taking advantage of the sensitivity and nondestructive properties of INAA, the use of this technique for homogeneity studies of small samples has been evaluated and implemented for the determination of sampling characteristics for a number of environmental SRMs. The small analytical uncertainty associated with the INAA measurements allows extraction of the variability due to material inhomogeneity from the observed total variability within a given set of measurements. We have investigated three sediment materials that have shown the potential for a high degree of homogeneity at very small sample sizes for possible use as a future SRM aimed at solid-sampling analytical techniques. From the candidate materials a portion of sediment from the Baltimore Harbor, certified for "normal" sample sizes (SRM 2702) was further processed as an SRM for microanalytical techniques. The proposed SRM 2703 Sediment for Microanalytical Techniques is being

characterized by NIST and collaborating laboratories using small sample analysis techniques. Evaluation of the Ingamells model describing homogeneity has been initiated with the Statistical Engineering Division.

Radiochemical neutron activation analysis has proven to be a powerful tool for measuring trace phosphorus in a variety of materials. An RNAA procedure has been developed to determine and value assign phosphorus in various SRMs. Phosphorus is quantified by beta counting of  $^{32}\text{P}$  after radiochemical separation. Originally developed for the analysis of metals, the method was used to value assign phosphorus in SRMs 861 (Aircraft Superalloy) and 2175 (Refractory Alloy), both containing phosphorus at low mg/kg levels. Modifications to the procedure have allowed us to value assign phosphorus in two new SRMs: 1575a (Pine Needles) and 2702 (Inorganics in Marine Sediment). This year, the radiochemical method for phosphorus has been critically evaluated, and shown to have the necessary sensitivity, chemical specificity, matrix independence, and precision to certify phosphorus at ion implantation levels in silicon. As a result, RNAA has been used for the first time as a primary method to certify the ion implanted phosphorus dose in SRM 2133, Phosphorus Implant in Silicon. Such a material has been a continuing high-priority need of the semiconductor industry for many years. It has been produced and characterized in collaboration with the Surface and Microanalysis Science Division, and is intended for use as a calibrant for secondary ion mass spectrometry (SIMS). The RNAA measurements used for certification include the first complete, quantitative evaluation of all sources of uncertainty in an RNAA measurement, and the relative expanded uncertainty for this SRM is 1.27 %.

Nearly thirty years ago a simple and rapid radiochemical procedure was developed at NIST, using oxygen combustion to separate mercury quantitatively from interfering radioactivities. Mercury was subsequently determined by counting the 64-hour  $^{197}\text{Hg}$  with a low-energy Ge gamma detector. This method was used extensively for the certification of mercury in biological materials and other reference materials. However, the published combustion method suffers from problems due to high levels of activity from  $^{82}\text{Br}$ , which elevates the baseline level under the peaks of interest. This radiochemical procedure is not optimum for determination of mercury via 47-day  $^{203}\text{Hg}$ , because selenium follows mercury efficiently in the combustion (indeed, an almost identical procedure was developed for selenium determination). The sole  $^{203}\text{Hg}$  gamma is interfered with by 120-day  $^{75}\text{Se}$ , so correction for this interference is imprecise in high-selenium matrices. In recent NIST work selenium is removed by precipitating mercury as periodate. The chemical yield of each sample is measured gravimetrically. The procedure has been applied to the certification of mercury in SRMs 1632c Coal, 2702 Sediment, and 1575a Pine Needles. Expanded uncertainties of 2 to 3 % have been achieved via this method.

Pioneering research is conducted by the Nuclear Methods Group on the use of cold neutron beams as analytical probes for both PGAA and NDP. PGAA measures the total amount of an analyte present throughout a sample by the analysis of the prompt gamma rays emitted immediately following neutron capture. NDP, on the other hand, determines the concentrations of several important elements (isotopes) as a function of depth within

the first few micrometers of the surface by energy analysis of the prompt charged particles emitted during neutron bombardment. Both of these techniques continue to make important contributions in the characterization of advanced materials, particularly with the enhanced sensitivities now available using cold neutrons from the NIST Reactor.

A method has been developed and apparatus built to produce titanium (and other metal) SRMs of known hydrogen concentration on the few-kilogram scale. The method is based on the controlled reaction of hydrogen with titanium in a closed system. After preparation, the hydrogen concentration is verified by cold-neutron prompt-gamma activation analysis and gravimetry. The first SRM, 2453 (Hydrogen in Titanium Alloy) has been prepared near the critical level of approximately 100 mg/kg. Cold neutron PGAA, neutron incoherent scattering, volumetry and gravimetry have been used together to certify a hydrogen mass fraction of  $114 \pm 5$  mg/kg. Two additional hydrogen in titanium SRMs are in preparation at bracketing levels. This apparatus has also been used to prepare standards for neutron-tomographic nondestructive analysis of turbine blades at McClellan Air Force Base.

The neutron depth profiling facility continues to provide unique measurement capabilities directly to US industries. Current experiments of interest at the NDP instrument include the measurement of lithium concentration and distribution in thin films being studied for battery applications, studies of boron mobility in tungsten with the Army Research Laboratory, shallow-doped boron content in silicon in conjunction with Advanced Micro Devices, the study of lithium distribution in lithium niobate, and the measurement of nitrogen in layers such as TiN and GaN. As a recent example, we have used NDP to measure nitrogen distributions in GaN/GaAs bilayers with Corning. This material is a base material for construction of devices such as blue light emitting lasers. The N concentration, which we can determine quantitatively, is a crucial parameter for establishing the device characteristics.

We have also measured the nitrogen concentration of MnN/ScN in conjunction with scientists from the NCNR and Ohio University. MnN is a metallic anti-ferromagnetic material that can be used with ferromagnetic semiconductors to make spintronic devices for data storage systems. The magnetic transition point of thin films made with this material is different from that of the bulk material. To measure this transition, which occurs at an elevated temperature, annealing is required. However, the annealing process is suspected of causing the nitrogen to escape, which in turn changes the transition temperature. Using NDP, we have determined that the original films were indeed nitrogen-deficient. Subsequent films were then made with a ScN layer and were again measured with NDP. These measurements show that this problem has been corrected. A joint publication on this work is in preparation. The total film thickness was determined (330 nm) and the total nitrogen concentration to a precision of better than 1% (1 $\sigma$ ).

As part of a continuing collaboration between NIST and Tufts University, we have employed NDP to measure elemental concentrations versus depth for different lithium ion battery materials. For example, NDP was used to determine the distribution of lithium

and nitrogen simultaneously in LiPON. This study has shown that the ion beam assisted deposition technique used for the manufacture of LiPON films allows the incorporation of nitrogen in concentrations that can exceed those reported for sputtered films. We are also studying LiCoO<sub>2</sub> films (a material that can be used as an electrode in batteries) and determined that if the ion beam assisted deposition process being used has the capability of controlling not only the degree of crystallinity and orientation of crystallites, but also the Li/Co ratio.

We have just concluded an assessment of long-term stability of primary boron and lithium thin film standards for NDP. Results show that the lithium standards have not changed over a ten year period, while the boron standards have lost from 0.1 to 0.5% of their original value. These results had an important impact on a recent NIST measurement of the neutron lifetime.

In collaborations with the Statistical Engineering Division and the Center for Neutron Research, we have combined NDP with neutron reflectometry to obtain a more complete picture of a BPSG thin film. The BPSG density profile obtained from neutron reflectometry is used as a “true” boron distribution in an NDP simulation to predict the convoluted spectrum to compare with the experimental NDP energy spectrum. Excellent agreement was obtained with complementary information on the films from each technique, verifying that there is little diffusion of boron into the adjacent layers. This study provides a basis for future NDP spectrum deconvolution for systems with a near-step function distribution.

Staff in the Nuclear Methods Group have also worked on a number of high priority projects with more than 20 “outside clients” as part of our responsibility for supporting the NIST Center for Neutron Research “National Users’ Facility. Many of our current PGAA collaborations involve determining hydrogen in a wide variety of materials for different applications. We are currently collaborating with Jefferson Laboratory to monitor the hydrogen content of niobium that was used in the construction of the accelerator for the Spallation Neutron Source at Oak Ridge National Laboratory. This work is of extreme importance because of the detrimental effects introduced by hydrogen in the metal. PGAA has also been used to determine the hydrogen content of carbon nanotubes (a potential hydrogen storage material) and to study hydrogen uptake by solid proton conductors of formula BaPr<sub>1-x</sub>Y<sub>x</sub>O<sub>3</sub> for fuel cell applications. Other measurements made at the PGAA facility this year include H, S, Ca, and K in Nafions, which have potential use as membranes in electrochemical separations and in fuel cells, H, C, N, and Cl in cattails for the purpose of monitoring environmental conditions, and Na, Si, and H in a sodium disilicate used in the manufacture of concrete structures.

PGAA with a focused beam has been used to determine the spatial distribution of chlorine in high-purity quartz for an industrial customer. Chlorine is a key element in the removal of OH whose presence is detrimental to optical applications that transmit light in the 1.3 to 1.5 μm wavelength range. Focused beam PGAA has the advantage over bulk INAA and PGAA measurements in that it provides spatial information, and has been employed as a calibration technique for micro-XRF measurements of test samples.

### ***Organic Analytical Methods***

Activities in organic analytical methods are directed toward the development, critical evaluation, and application of methods for the identification and measurement of organic and organometal species using mass spectrometry and analytical separation science-based methods. These methods are applied to the development and/or provision of reference methods, SRMs, and other quality assurance program in the clinical, food and nutritional, environmental, forensic, and homeland security areas.

Research activities in separation science continue to focus on investigations of the physical and chemical processes that influence retention in LC, GC, SFC, CE, and CEC. Results from these fundamental studies are used to design stationary phases tailored to solve specific separation and analysis problems, and to assist in method development and optimization. Recently we have explored a novel approach to the synthesis of LC stationary phases based on polymer immobilization. Polyethylene acrylic acid copolymers were immobilized on silica as an alternative to conventional silane surface modification chemistry. The resulting columns were evaluated for the LC separation of carotenoid isomers, and preliminary results indicate exceptional selectivity for this class of compounds. Further optimization of this promising stationary phase should result in improved measurement capability for carotenoids and related nutrients in food and tissue samples.

Recent investigations of alkyl bonded phases for LC involved the synthesis of a series of consecutive length alkyl phases ranging from C<sub>13</sub> through C<sub>18</sub>, with three different bonding chemistries (monomeric, polymeric, and self-assembled monolayer) at each phase length. The phases were characterized in terms of methylene selectivity, shape selectivity, and band broadening. It has been concluded that the performance of stationary phases in LC is influenced in different ways by the bonding chemistry, surface coverage, and alkyl chain length of the sorbent. 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 mmol/m<sup>2</sup> to 8 mmol/m<sup>2</sup>, a linear increase in methylene selectivity was observed with increasing bonding density. In general, retention behavior towards nonpolar solutes was most strongly influenced by the density of the alkyl chains at the silica surface. Dramatic changes in shape selectivity were observed, however, as a function of temperature for alkyl phases that differ by only one carbon in length.

Research in chiral separations is continuing in several areas using LC, CE, and GC. The determination of chiral drug species in hair samples using LC may permit environmental exposure to be distinguished from illicit use. A liquid chromatographic method has been developed for the determination of amphetamine and methamphetamine enantiomers in hair extracts. This method was applied to the analysis of an SRM that contains several drugs of abuse. In other studies, modified cyclodextrins have been evaluated as chiral selectors in CE. Three commercially available sulfated cyclodextrins with similar degrees of sulfation were chosen for study. A capillary electrophoresis method with

indirect detection was used to characterize the patterns of sulfate substitution of these materials. The selectors were then investigated as chiral additives in capillary electrophoresis. The electrophoretic results highlighted the importance of both the degree of substitution and the location of sulfate substituents on the cyclodextrin. Although the randomly substituted material generally yielded the best enantioresolution, the heterogeneity of this additive could pose problems for development of reproducible methods. These studies emphasized the importance of the use of well-characterized selectors for reproducible results in chiral CE. A gas chromatographic method incorporating a chiral stationary phase method was developed for the determination of chiral ephedra alkaloids as part of our development of a suite of ephedra-related SRMs. The alkaloids of interest consist of pairs of diastereomers that can be difficult to separate on nonchiral stationary phases.

Results of our research efforts in separation science have been applied to development of improved methodology for the determination of nitro-substituted polycyclic aromatic hydrocarbons (nitro-PAHs) and high molecular weight PAHs in environmental matrices. For the nitro-PAHs a normal-phase LC isolation of the nitro-PAHs was implemented prior to measurement by GC/MS with negative ion chemical ionization detection. An improved separation of the isomeric nitro-PAHs, particularly the nitropyrene and nitrofluoranthene isomers, was achieved using a 50% phenyl-substituted methylpolysiloxane. This improved methodology was used to assign values for concentrations of 25 mononitro-PAHs and 3 dinitro-PAH isomers in three diesel particulate-related SRMs (SRM 1650a, SRM 1975, and SRM 2975) and two air particulate SRMs (SRM 1649a and SRM 1648). Improved separation of PAHs isomers of MW 302 has resulted from the use of a 50% phenyl methylpolysiloxane phase instead of the common 5% phenyl phase. GC/MS analysis of several environmental matrix SRMs (air and diesel particulate matter, two sediments, and coal tar) provided identification and reference values for 23 MW 302 isomers. Both the nitro-PAH and the MW 302 PAH studies resulted in the most extensive characterization of these groups of compounds in any environmental matrix and have provided reference values for these SRMs.

Recent research activities in organic mass spectrometry have focused on the development and critical evaluation of new approaches to the quantitative determination of biomolecules (e.g. proteins) in biological matrices. The recent acquisition of a LC with tandem mass spectrometry (LC/MS/MS) system and a matrix assisted laser desorption time-of-flight mass spectrometer (MALDI-TOF), has significantly increased our capabilities for the determination of trace-level analytes of health, nutritional, forensic, and environmental importance, as well as for structural studies of natural products. The MALDI-TOF has greatly enhanced our capabilities for structural characterization of biomolecules.

A new generation of health status markers shows great promise from the clinical diagnostic perspective, but offers new and more difficult challenges for standardization. Many of the new markers are proteins, peptides, or other large biomolecules and are usually present at very low concentrations. Most are thermally labile, very polar, and

heterogeneous, both in conformation and in other molecules attached or otherwise associated with them. With a vast potential market for tests for these new markers, many different approaches are being developed in a short time frame and these approaches often provide different “answers”. These differences can lead to erroneous diagnoses and/or the need for retesting—both very costly. Recent efforts have been directed toward the development and critical evaluation of reference methods for troponin I (a new marker of myocardial infarction), thyroxine and triiodothyronine (thyroid function), cortisol (a marker for endocrine function), speciated iron (Anemia and Hemochromatosis), homocysteine (a risk factor for myocardial infarction), folic acid (an essential nutrient that reduces the risks of heart disease and neural tube defects), and prostate specific antigen (PSA) for prostate cancer.

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 they were 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. Because of the extensive use of the LC/MS/MS in the development of these methods, we plan to purchase a second instrument in the coming year. Development will begin in FY 03 for two human serum-based SRMs for these markers, one for homocysteine and folates, and the other for T3, cortisol, and several other important hormones.

A proteomics approach using LC and MS was investigated for the detection of transgenic proteins in whole grains. The immunoassays and lateral flow test strips that are used in 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. Measurement procedures that do not rely upon antibody selectivity need to 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. 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. These results demonstrate that transgenic proteins can be detected without the use of antibodies and immunoassays and we will continue to investigate this approach in the coming year.

Our research in organometal speciation has continued with improvements in the GC-atomic emission (AED) method for methylmercury and alkyltin species. The new approach involves derivatization and a solid phase microextraction (SPME) step to concentrate the analytes prior to GC-AED analysis. This approach has been used to

provide data for certification of methylmercury in SRM 2977 Mussel Tissue, SRM 1566b Oyster Tissue, and SRM 1946 Lake Superior Fish Tissue. This same general approach has also been used to measure butyltin species in several of the sediment-matrix SRMs (i.e., SRM 1941b Organics in Marine Sediment, SRM 1944 New York/New Jersey Waterway Sediment, and SRM 1646a Estuarine Sediment). The butyltin results are provided as reference values for these SRM. This methodology was recently published in *Analytical Chemistry* [74:4694-4701 (2002)].

Our Division has been involved in environmental specimen banking and marine analytical quality assurance programs for over two decades; these activities are collaborative efforts supported by both NIST resources and by funding for the other federal agency partners (see below for Other Agency Activities). We currently maintain the National Biomonitoring Specimen Bank (NBSB) at two locations, the NIST Gaithersburg campus and the Hollings Marine Laboratory (HML) in Charleston, SC. The NBSB is a cryogenic environmental specimen bank that resulted from the pilot Environmental Specimen Bank Program, which was established in 1979 with support from EPA, to develop collection and banking protocols for human liver specimens and to cryogenically store these specimens for retrospective contaminant analysis. Since 1982, the specimen bank has grown in number and types of specimens (e.g., sediments, fish tissues, mussels, oysters, human diet samples, marine mammal tissues, and seabird eggs) collected as part of several monitoring and research programs. The National Oceanic and Atmospheric Administration (NOAA), Minerals Management Service (MMS), and the U.S. Geological Survey (USGS) have been major consistent supporters since the mid-1980's. A major expansion in environmental specimen banking occurred in 1995 with an agreement between NOAA and NIST to establish an environmental specimen bank in Charleston, SC, specifically designed for marine specimens. ACD currently has four permanent staff members as well as several students working in the HML on specimen banking and marine quality assurance activities. Presently the primary specimen banking activities involve tissues collected from marine mammals throughout the U.S., including Alaska, and seabird eggs collected from seabird colonies in Alaska. There are presently 2,087 marine mammal tissue specimens banked in the NBSB, representing 737 individual animals and 34 species, and 188 seabird eggs from 3 species. These banked specimens represent a resource that has the potential for addressing future issues of marine environmental quality and ecosystem changes through retrospective analyses. As part of the specimen banking and marine QA activities, analytical methods have been developed and implemented for the measurement of organic contaminants in marine mammal tissues and seabird eggs. Specimen bank samples are regularly analyzed for both organic (PCBs and pesticides) and inorganic contaminants. Recent activities include the development and application of analytical methods for "new" organic contaminants such as toxaphene, polybrominated diphenyl ethers (PBDE), and polychlorinated naphthalenes (PCNs).

During the past year, significant efforts were expended toward the development of SRMs in the following areas:

- **Clinical Diagnostics**

Troponin SRM. 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 US, 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. This material will be prepared and characterized for distribution as the SRM in early 2003.

- **Environmental Monitoring**

SRM 1941b Organics in Marine Sediment. SRM 1941b is the third issue since 1989 of the popular marine sediment SRM for organic contaminants. Results from multiple analytical methods were combined to provide certified values for 24 PAHs, 29 PCB congeners, and 7 chlorinated pesticides. Reference values are also provided for 60 additional PAHs, PCBs, pesticides, and butyltins. The PAH, PCB, and pesticide concentrations are approximately 2 to 20 times lower than those in SRM 1944 New York/New Jersey Waterway Sediment, thus these two sediment materials will serve the needs of the marine environmental measurement community for both high and moderate levels of organic contaminants.

SRM 1946, Lake Superior Fish Tissue. SRM 1946 is a cryogenically homogenized fish fillet prepared from adult lake trout (*Salvelinus namaycush*). The fish tissue homogenate is indicative of urban contamination levels and will be beneficial to both the environmental and food/nutrition measurement communities. Certified values have been determined for 30 PCB congeners (including 3 non-ortho congeners), 15 chlorinated pesticides, methylmercury, and total mercury. This is the first SRM available with certified concentrations for the non-ortho PCB congeners, which are considered the most toxic, similar in structure to polychlorinated dibenzo-p-dioxins.

SRM 1974b, Organics in Mussel Tissue (*Mytilus edulis*). SRM 1974b is the third issue since 1990 of the mussel tissue SRM for organic contaminants. Results from multiple analytical methods were combined to provide certified values for 22 PAHs, 31 PCB congeners, and 7 chlorinated pesticides. Reference values are also provided for 30 additional PAHs, PCBs, and chlorinated pesticides. Values will also be assigned for methylmercury, total mercury, and additional trace elements.

- Instrument Calibration Standards (SRMs 3061-3086). A suite of 26 organic calibration solution SRMs has been developed to support the recently externalized Water Performance Evaluation (PE) Studies Program orchestrated by the US Environmental Protection Agency (USEPA). These SRMs are intended to support the PE program; however, it is anticipated that widespread usage will result after issuance. These SRMs include: six Aroclor mixtures in methanol and in oil; chloral hydrate, haloacetic acids, dioxin, endoHall, chlorinated herbicides, toxaphene, chlordane, and adipate/phthalates in methanol; organochlorine pesticides in acetone; glyphosate and diquat dibromide in water; and vydate and carbamates in acetonitrile.

- **Food Safety and Nutrition**

SRM 2384 Baking Chocolate. SRM 2384 has values assigned for proximates, 15 fatty acids, total dietary fiber, 5 vitamins, 3 catechins, theobromine, caffeine, and 9 elements of nutritional interest.

SRM 1946 Lake Superior Fish Tissue. SRM 1946 is described above as an environmental matrix SRM, but is also useful as a food-matrix. Therefore SRM 1946 also has values assigned for nutrients including proximates (fat, protein, etc.) and 25 fatty acids, including several omega-3 fatty acids.

SRM 2385 Spinach and 2387 Peanut Butter. Measurements have been completed for nutrients, carotenoids, and pesticides in the spinach and for nutrients, allergens, aflatoxins, and amino acids in the peanut butter.

- **Forensic**

SRM 2379 Cocaine, Benzoylcegonine, Cocaethylene, Amphetamine, Methamphetamine, and PCP in Hair

SRM 2380 Codeine, Morphine, Monoacetylmorphine, and THC in Hair of abuse in hair.

Three new SRMs are currently under development with support from the NIST Office of Law Enforcement Standards (OLES)

- an updated and redesigned SRM 1828b Ethanol in Water 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 the blood and breath alcohol testing)
- an SRM designed for qualitative arson analysis
- an SRM for drugs of abuse in human serum/blood to complement the current SRMs for drugs of abuse in urine because forensic laboratories often receive blood instead of urine for analysis.

The Organic Analytical Methods Group has led many of the organic analysis international comparison studies over the past two years:

- P20 Investigation of Purity Assessment Methods (leader)
- P27 LSD in Urine
- K6 Cholesterol in Human Serum (leader)
- K11 Glucose in Human Serum (leader)
- K12 Creatinine in Human Serum (leader)
- K21 p,p'-DDT in Fish Oil
- P35 and K27 Ethanol in Aqueous Matrix (at forensic and commodity levels)
- K25 PCBs in Sediment (leader)

We are committed to lead pilot studies and the Key Comparisons that will follow for Organic Contaminants in Tissue (P40) and Organic Calibration Solutions (P31).

The Group has also organized and led proficiency assessment studies for pesticides in solution, pesticides in spinach, and vitamins in milk for the 32 member-states within the Interamerican System for Metrology (SIM).

### ***Gas Metrology and Classical Methods***

Research and measurement service activities within the Gas Metrology and Classical Methods Group are focused on gas metrology, wet chemical methods (gravimetry, titrimetry), coulometry, and maintenance of the theoretical infrastructure for pH and conductivity measurements.

During the past year, 12 gas mixture standards, 7 conductivity solution standards, 1 anion solution standard, 3 pH materials, and a Sodium Oxalate Reductometric standard were completed. In addition, we recertified 42 gas mixture standards for various clients. We also worked with five Specialty gas Companies in development of 35 batches of NTRMs. The over 1000 individual gas cylinders that comprised these 35 NTRM batches will be used to produce approximately 100,000 NIST-traceable gas standards for end-users.

In the area of electrolytic conductivity, we continued our investigations of new standards packaging technology. The packaging used in the past was not suitable for storage of conductivity solutions for more than one year. Each conductivity solution standard had to be remade every year and uncertainties could not be lowered below 1%. During the past year we decided to package all conductivity solutions greater than 500  $\mu\text{S}/\text{cm}$  in 50 ml glass ampoules. We are still researching the problem for lower conductivity solutions. In sealed ampoules the transpiration problem, which has plagued us in the past, is solved and the solutions have multiyear self-lives. This should reduce the reissue rate and allow us to devote more time to the development of low conductivity standards being requested by the pharmaceutical industry to address new water purity specifications. We benchmarked our capabilities for the preparation and delivery of electrolytic conductivity standards with that of other National Metrology Institutes through participation in an international comparison carried out under the auspices of the CCQM Electrochemistry Working Group (CCQM-P22). The results, to be published in early 2003, show very good comparability between the major NMIs.

In the area of pH we continue to be very active in the international arena. During the past year, we participated in four CCQM comparison activities:

- CCQM-K17 (phthalate buffer at nominal pH of 4.0)
- CCQM-P37 to elucidate systematic effects that contributed to unexpectedly large deviations of certain NMIs in CCQM-K17
- CCQM-P19.1 (purity assessment of 0.01 mol/kg hydrochloric acid)
- CCQM-P36 (purity assay of potassium acid phthalate)

In CCQM-K17, NIST's pH values showed a very small bias from the Key Comparison Reference value with a greater overall uncertainty than other major pH programs around the world. This might be a result of the design and/or age of the pH cells used at NIST.

Over the next two years, we will be upgrading and redesigning our apparatus for the primary realization of pH. The new system will provide better control of all critical parameters and be automated to allow overnight and unattended measurements.

During the past year, we made the difficult decision to shut down our future production of anion solution standards. This decision was not easily nor hastily made, and we realize that there are many clients that have come to rely on these SRMs. However, we are not abandoning our responsibility or our customers in this area. We will maintain the capability of making world-class measurements in this area and there is currently a 42+ years supply for all on the ---6- individual SRMs in this series. Many of the anion SRMs could be easily prepared directly by customers using available pure chemical SRMs. We will be evaluating whether a NIST published procedure will assist customers in preparing these solutions. In addition, in a recent CCQM study several NMIs demonstrated capabilities for making and value-assigning anion solution standards that are comparable to NIST. We plan to establish strategic partnerships with one or two of them whereby they would provide anion solution SRMs to U.S. customers and we would make any measurements required to assure U.S. quality and traceability requirements.

We have continued our collaboration with the U.S. Environmental Protection Agency and the remote sensing community in the development of a quantitative database of infrared spectra for calibration of IR-based technology used for real-time monitoring for airborne chemical contaminants along plant boundaries and within plant facilities. The spectra are being prepared using NIST primary gas standards. These standards have been critically evaluated at NIST and intercompared internationally. The use of SRD-79 to establish traceability of open-path IR measurements will be required in the update of EPA method TO-16.

SRD-79 currently has data for 40 compounds. During the past year, we have worked to data for dichloromethane, carbon monoxide, ethanol, benzene, ethylene, and hydrogen chloride at ambient and elevated temperatures. This database is currently available only in CDRom format. In the near future we hope provide SRD-79 on the web.

Improved analytical tools for real-time measurement of trace-level vapors in the atmosphere are critical for evaluation of new technologies for reduction of hazardous industrial emissions. Towards this end, we are critically evaluating the capabilities of Fourier-Transform microwave (FTMW) spectroscopy for real-time sensing applications. The high spectral resolution and high sensitivity of FTMW spectroscopy suggest that the technique can provide unambiguous identification of vapor phase analytes. Current work has focused on improving the signal intensity and optimizing the instrument for gas samples with nitrogen as the primary balance gas. The original goal of this project was to address the needs of the automobile industry to identify and quantify trace levels of oxygenates in exhaust emissions. Success of this work will also impact many other critical applications including the detection of chemical warfare agents.

Our Ozone Standards Program continues to expand. Many more countries have requested NIST Standard Reference Photometers (SRPs) for establishing traceability for their

atmospheric ozone measurements. New SRPs have been constructed and delivered to the BIPM in France, UBA in Germany, METAS in Switzerland, and Taiwan Environmental Protection Agency in Taiwan. Italy, Hong Kong, and Brazil are in line to receive new SRPs this next year.

During the past year, we introduced an upgrade to the SRP control program and data acquisition computer boards. With the advent of the new Windows operating systems based on Window NT, and the disappearance of the ISA backplane on newer computers, the MS DOS based control program became obsolete. A new control program was created that will operate in the modern operating systems and on currently available computers. This control program was designed to automate much of the data acquisition and reporting for calibrations done using the Standard Reference Photometers (SRP). The program was also designed to allow the comparison of up to 4 SRPs concurrently. This enabled the comparison of the two NIST SRPs with the two new BIPM SRPs. It also automates the calibration of ozone monitors against the primary SRP. We also began the transfer of some of our responsibility for providing traceability for atmospheric ozone measurements to the BIPM in Paris, France. We constructed and installed two new NIST SRPs at BIPM and they will soon begin to provide ozone photometry measurements for many of the smaller Meter Convention Member economies and SRP recalibration services for many non-US customers. NIST will continue to build and service SRPs for the international community but we will no longer provide the SRP recalibration service outside the Americas. In addition to establishing an agreement concerning SRP recalibration responsibilities, NIST and the BIPM are Co-Piloting a CCQM comparison on Ozone Photometry and collaborating on the development of a next generation Ozone Photometer.

We also continue to support U.S. industry through development and dissemination of high priority reference materials based on input from organizations such as the American Industry Government Emissions Research (AIGER) consortium and ASTM. Over the past two years we have developed two new low concentration nitric oxide gas SRMs. These SRMs are needed by the automotive industry in new car development and to meet new regulations in California. These standards also are required by Industry to meet new regulations covering stack gas emissions. These gas SRMs, one at 0.5 ppm (SRM 2737) and one at 1.0 ppm (SRM 2738), will be available for sale late in FY03. These SRMs are the result of an active collaboration between NIST and the AIGER consortium, which donated the candidate gas mixtures for the SRM. It also involved collaboration with Scott Specialty Gases to develop the technology used in passivating the cylinders.

One way to meet the increasing needs of industry for standards at much lower concentrations is through dynamic dilution technology. Over the past two years we have collaborated with the United Kingdom's National Physical Laboratory (NPL) on the critical evaluation of a binary network dilution device that they patented in 2001. This dilutor consists of 6 mass flow controllers (MFC) arranged in series. Each MFC, or bit, is adjusted to deliver precisely twice the flow of the preceding MFC. Thus calibration consists of matching flows precisely, and does not rely on absolute flow calibration. The binary network behaves like a binary number, and dilution is easily adjusted through

switching the MFCs between the dilution gas and the standard gas. . We have been working to further develop this concept into a “primary” dilutor. Work using Mol Bloc flow measurement technology and new state of the art mass flow controllers, shows great promise for reducing the uncertainty in the composition of gas mixtures delivered

We participated in three gas mixture comparison studies during FY02. We were a participant in a CCQM key comparison on Natural Gas composition. Our results were in good agreement with those from other NMIs considerable experience in gas metrology. We piloted a pilot study on natural gas composition measurements within SIM. The results indicated that additional work and training would be needed to for any of the participants to reach the capability level demonstrated by the participants in the CCQM study.

The third study was a CCQM pilot comparison on the gravimetric preparation of carbon monoxide primary gas standards. This study was intended to identify sources of uncertainty in the production of primary standards. Carbon monoxide (CO) was chosen since it was thought to be the simplest molecule, and the gas standards were relatively easy to prepare and analyze. The study turned out to be the most useful comparison done to date by the gas working group. It was determined that two NMIs, NIST and KRIS (Korea), were significantly biased, up to 1 %, from the other participants. Through additional work done by the Pilot Laboratory, NMI (the Netherlands) NIST, and KRIS, the cause of this bias was eventually determined to be the isotopic abundance of C13 CO. In the US and Korea, suppliers of pure CO had stripped the gas of the C13 CO to sell to suppliers of C13 labeled organic compounds. The depleted CO primary standards made from this depleted CO produced a biased signal in the non-dispersive infrared (NDIR) monitors used to analyze the primary standards in the comparison. This has huge implications to industry and to the regulatory agencies. Industry, especially the automotive industry, relies on NDIR monitors for compliance to EPA regulations. A 1 % bias is a large concern to this industry, and the C13 CO problem discovered through the CCQM study is very prevalent in the CO gas standards supplied by specialty gas companies. We immediately informed the AIGER consortium, EPA, and the specialty gas companies of the problem identified in this study and rejected four SRM lots that contained depleted C13 CO that were in progress, and ordered and certified new lots. We will continue to work with all concerned parties to solve this problem and will insist that the Specialty Gas Companies test all pure CO they use in gas standards lots that they prepare for NIST.

### ***Molecular Spectrometry and Microfluidic Methods***

The Molecular Spectrometry and Microfluidic Methods Group conducts research on the metrology of molecular spectrometry and develops standards for calibration, validation, and performance of instruments for measuring molecular spectra; conducts research on microfluidic devices, methods, and applications for chemical analysis including studies of materials and material properties affecting the flow of liquids in microchannels and the use of microchannel and other electrophoretic methods for forensic and toxicological

applications and standards; has responsibility for the development and certification of optical transmittance and wavelength standards in the ultraviolet, visible, and near-infrared spectral regions, of Raman intensity correction standards, and of fluorescence wavelength and intensity standards; and works with users and manufacturers of analytical instruments to assess and measure the performance of analytical methods and to determine and address existing and future needs for analytical instrument standards ranging from device calibration and instrument performance through specifications for remote device control and data interchange.

In FY02, 68 solid absorbance filter SRMs were certified, and 202 optical filter sets were recertified. Continuing measurements were made on a number of other filter sets. Additionally, 246 units of SRM 2034 (holmium oxide UV-Vis wavelength standard) were certified and delivered to SRMP. Certification measurements were completed on 100 units of SRM 2036 (UV-Vis-NIR transreflection wavelength standard), and 200 units of SRM 2242 (Relative Intensity Standard for Raman Spectroscopy with 532 nm Excitation).

Renovations continued on the original high accuracy reference spectrometer (HAS-I) with the reconstruction and modification of the original enclosure to accommodate the new transport mechanism and interlock system, replacement of the deuterium lamp source, and installation of the data acquisition system. The goal for HAS-I renovation is to reach the level of automation and control currently available for HAS-II so that HAS-I can be used for research on spectrometric measurements. Since HAS-II is used for production (certification and recertification) activities, it cannot be (and should not be) used for research.

Research continued to develop an extended set of visible filters (0.1%, 0.3%, and 70% transmittance) to enlarge the range of transmittances covered and fill in a gap in our coverage. This is being done to make the range of our visible filter offerings more compatible with those of other NMIs (e.g., NPL) and to meet partially requests by CORM, our NTRM vendors, and others for broadening the range of our standards. Modifications to the HAS-II software were completed enabling certification of these darker filters. We are now sufficiently confident about the filter glasses and the measurement technology that we proposed certifying these filters as SRM 2930 in FY03.

Two of the initial vendors dropped out of the Optical filters NTRM program citing costs of participation and lack of NTRM filter sales. PerkinElmer joined the program and over the course of the year, we completed their NVLAP assessments and accreditation. Both of the current NTRM vendors are instrument companies that have motives other than profits from direct sales and recertifications of NTRM filters sets for being in the program. Nonetheless, we must expand the offerings of the NTRM program to other materials so that the costs of operating the program can be amortized over more than just glass absorbance standards. Discussions with the vendors suggest that holmium oxide solution wavelength standard and the darker filters mentioned above are the highest priority for future offerings.

Measurements for the SIM intercomparison of holmium oxide solutions for UV/Visible wavelength standards were completed. Report preparation is underway. The report of the similar NORAMET study was published in Analytical Chemistry, and the results were used to establish new uncertainty values for the wavelength assignments of SRM 2034, Holmium Oxide Solution. To this end, we have proposed that the spectrum of holmium oxide may be useful as an “intrinsic” standard—a standard whose purity can be assessed inherently and whose wavelength “peak” values at given spectral slit widths can be certified independently and published as standard reference data. Therefore, a given artifact, independent of source, can be accurately assessed and, if found suitable, can be utilized as a standard. To substantiate this concept, we need to assess the extent of the international agreement on the wavelength assignments for holmium oxide solutions. Accordingly, we have begun a holmium oxide wavelength intercomparison with several NMIs around the world. If this concept proves correct, we will publish the wavelength values as a function of spectral slit width as a standard reference dataset. NIST will then cease to certify SRM 2034.

This year we set out to certify the second Raman Intensity Correction material, a uranium glass for excitation at 488 nm to 532 nm, SRM 2242. Unfortunately, the fluorescence fit for the Raman shift ranges spanning this 44 nm excitation range was too wide for this uranium glass. Accordingly, we developed a manganese glass with a redder fluorescence specifically to handle Raman intensity correction with 532 nm excitation. In FY03, we plan to certify the original uranium bearing glass as SRM 2243 for Raman intensity corrections with 488 to 514 nm. We continue to study materials, including europium-based glasses, for similar use at 1064 nm excitation.

We continued our search for candidate materials for luminescence standards and examined several transition-metal-, rare-earth-, and actinide-doped glasses. We have found a uranium-based glass and a manganese-bearing glass that appear useable for fluorescence spectral emissivity standards in the green and yellow spectral regions respectively. We have proposed certifying the manganese glass in FY03 and the uranium glass in FY04. Potential candidate glasses for fluorescence spectral emissivity standards in the red and blue spectral regions have been identified for further study in FY03.

We continued to qualify fully the SPEX-JY fluorometer. Last year we had a small integrating sphere/photodiode assembly calibrated in PL and have used it to quantify the light from the excitation monochromator and to calibrate the internal photodiode/beamsplitter assembly that relates the fluorescence signal to the excitation output. By using a highly diffuse reflective material (Spectralon) of known reflectance in the sample position to reflect light from the excitation monochromator to the emission monochromator/detector, we can calibrate the whole system with traceability to the SI. As a second, more traditional means, of calibrating the emission monochromator/detector assembly, we constructed a dual-integrating-sphere light source and had it calibrated in PL for both radiance and irradiance from 300 nm to 800 nm. We can utilize either the sphere source's radiance calibration and a mirror in the sample position or its irradiance calibration and Spectralon in the sample position to calibrate the emission

monochromator/detector assembly. This will provide an independent, SI-traceable calibration check.

The certification measurements for SRM 1932 Fluorescein Solution were completed, and around 300 units were put on the shelf for sale. Purity determinations were done using <sup>1</sup>H NMR, MS, LC, LC-MS, flame atomic emission spectrometry, ICP-MS, X-ray fluorescence, Karl Fisher titration, argentimetric titration, UV/visible spectroscopy, fluorescence spectroscopy, and elemental analysis. Several ACD and Biotech staffers collaborated on the certification measurements.

We continued to provide statistics and data representation studies for FBI, NIST Office of Law Enforcement Standards, and other agencies investigating the use of DNA methods of forensic analysis. In close collaboration with the NIST Biotechnology Division, significant progress has been made on three projects:

- (1) The AFDIL-sponsored study of DNA extractability from archival media was completed and the final report published.
- (2) Data entry and validation for the OLES-sponsored Mixed Stain Study #3 interlaboratory challenge exercise has been completed.
- (3) Database and quality assurance procedures have been (and are being) developed for the Biotechnology Division's NIJ-sponsored Y-STR data bank.

This year was another banner year for our microfluidics projects resulting in eleven publications, four patent applications, and ten talks and posters. Funding for this area has come from the Microscale Analytical Laboratory Competence, an ATP intramural grant, the Single Molecule Manipulation and Measurement Competence, and STRS. This program area with collaborations in the Process Measurements Division, the Biotechnology Division, the Optical Technology Division, and the Semiconductor Electronics Division continues to attract NRC postdocs, adding one more this year to bring the MS&MM total to four.

We began the Single Molecule Manipulation and Measurement Competence this year with several solid accomplishments: We developed chips for the handling of water in fluorocarbon emulsions using optical tweezers; developed protocols for wet chemical bonding of PDMS to PDMS and PDMS to glass; developed protocol for the production of ultrathin, laminated chips with integral capillary ports for high pressure, low dead volume applications; developed model for a filled loop injector for two phase fluid systems; and developed a protocol for the bonding of biaxially oriented poly(ethylene terephthalate). In the area of self-assembled polymeric nanofluidics, we developed a one-pot, living polymerization of poly(butadiene-b-ethylene oxide) and established a protocol for the production of large vesicles from the poly(butadiene-b-ethylene oxide). We extended our previous studies on polyelectrolyte multilayers (PEMs) by creating a robotic system for the automated production of both simple and complex PEMs; by synthesizing fluorescently labeled poly (allylamine) for visualization of PEMs; and by synthesizing a diazo-resin for the production of photocrosslinkable PEMs

We continue to study the parameters that affect flow in plastic microchannel devices. This year major progress was made in applying of UV-laser ablation for fabricating microdevices, for studying parameters important to the post-machining properties of microdevices, and for carrying out both physical and chemical modifications to the surfaces of microdevices. The laser micromachining system was upgraded this year with a new fully automated laser gas handling system. Furthermore, the 248 nm krypton fluoride laser was replaced with a 193 nm argon fluoride laser, which primarily improves the precision and smoothness of the micromachined surfaces.

A formal collaboration with the Division of Bioengineering and Physical Science at the National Institutes of Health was established to address the need for microfluidic devices capable of analyzing 25-50 microliter cervical secretions to study the epidemiology of the immune response to Herpes Papilloma Virus (HPV) infection and the relationship between this response and the development of cervical cancer. Studies of lymphocytes taken from peripheral blood samples have suggested a difference between patients whose cells respond to HPV peptides with a T lymphocyte helper cell type 1 compared to those with a Th-2 type response. The Semiconductor Electronics Division of EEEL is also participating in this collaboration.

## Measurement Quality Assurance Programs for Contaminants in Environmental Samples

**CSTL Program:** Environmental Technologies and Systems

**Authors:** *M. Schantz, J. Kucklick, D. Poster, S. Christopher, and S. Wise*

**Abstract:** The Analytical Chemistry Division currently coordinates three quality assurance programs for contaminants in environmental samples: (1) NIST Intercomparison Program for Organic Contaminants in the Marine Environment; (2) NIST/NOAA Intercomparison Exercise for Organochlorines and Trace Elements in Marine Mammal Tissues; and (3) NIST/EPA Intercomparison Program for Organic Contaminants in PM<sub>2.5</sub> Air Particulate Matter. The first of these three programs started in 1987 with partial support until 1999 from the National Oceanic and Atmospheric Administration (NOAA) and the Environmental Protection Agency (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 second program was established with NOAA 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 third program 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.

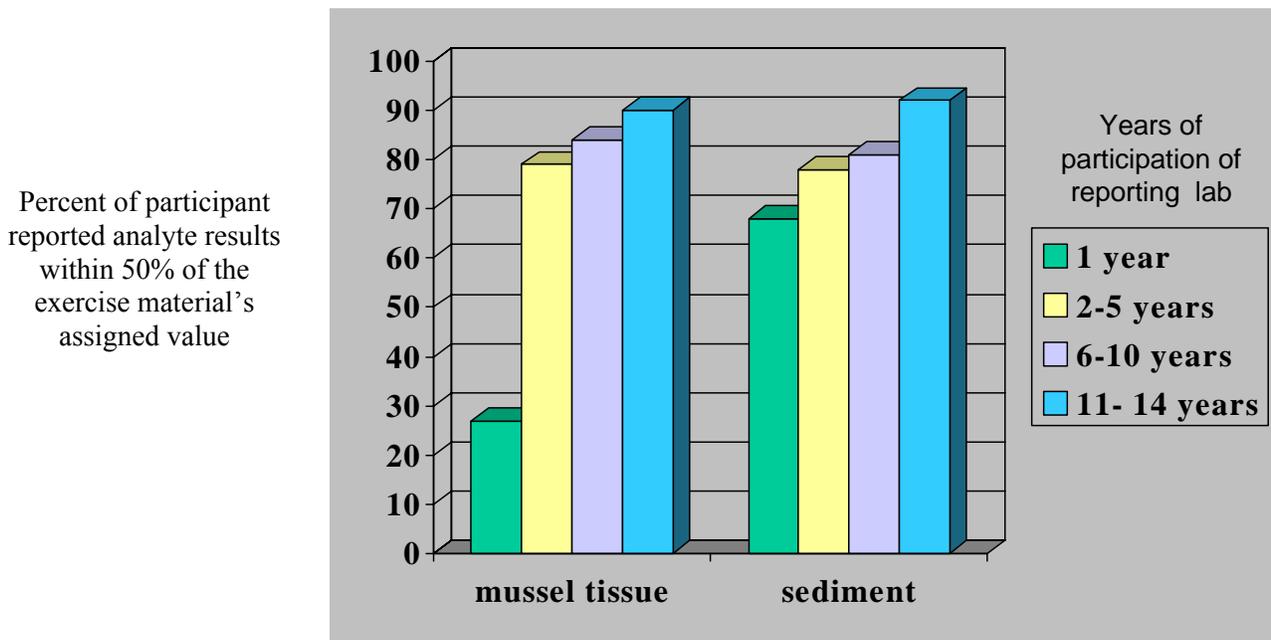
**Purpose:** Environmental monitoring programs sponsored by the National Oceanic and Atmospheric Administration (NOAA) and the Environmental Protection Agency (EPA) rely on contaminant measurements performed by numerous laboratories and therefore need to assess the quality and comparability of the analytical measurements from the various laboratories. NIST currently coordinates three quality assurance (QA) programs in support of NOAA and EPA monitoring programs for contaminants in the marine environment and in atmospheric particulate matter. These QA programs consist of annual interlaboratory comparison exercises to assess the comparability and improve the accuracy of contaminant measurements. These intercomparison exercises are performance-based so laboratories use the analytical methods that they would normally use for the analysis of similar matrices. Annual workshops are conducted for the participants to discuss results and to provide a forum for cooperative problem solving. By participating in these QA programs, laboratories demonstrate and document their measurement capabilities for specific ongoing monitoring programs, as well as for competing for grants and contracts.

**Major Accomplishments:** Currently over 75 laboratories participate in these three QA programs for environmental contaminant measurements. In all of these intercomparison

exercises, participants are required to analyze a similar matrix SRM in conjunction with the unknown sample to validate the measurement process. 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. For example, SRM 1941b Organics in Marine Sediment, SRM 1946 Lake Superior Fish Tissue, and SRM 1974b Organics in Mussel Tissue were all used in these exercises in recent years and the consensus results from the study were used in assigning the certified concentrations for various contaminants. Improvement in the accuracy of a laboratory's measurement capabilities has been shown to correlate with the number of years of participation in these exercises as shown in Figure 1 for the NIST Intercomparison Program for Organic Contaminants in the Marine Environment.

**Impact:** Laboratory measurement accuracy has improved by participation in these 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.

**Future plans:** The programs will continue on an annual basis with interlaboratory exercises and workshops. The lists of analytes of interest will continue to expand, and the need for additional reference and control materials will continue to be addressed.



## **Environmental Specimen Banking**

**CSTL Program:** Environmental Technologies and Systems

**Authors:** *P. Becker, R. Pugh, B. Porter, J. Kucklick, S. Christopher, S. Vander Pol, K.J. Tuerk, E. Mackey, and S. Wise*

**Abstract:** The Analytical Chemistry Division currently maintains the National Biomonitoring Specimen Bank (NBSB) at two locations, the NIST campus in Gaithersburg Maryland, and the Hollings Marine Laboratory in Charleston, South Carolina. The NBSB is a cryogenic environmental specimen bank that resulted from the pilot Environmental Specimen Bank Program, which was established in 1979 sponsored by the Environmental Protection Agency, to develop collection and banking protocols for human liver specimens and to cryogenically store these specimens for retrospective contaminant analysis. Since 1982, the specimen bank has grown in number and types of specimens (e.g., sediments, fish tissues, mussels, oysters, human diet samples, marine mammal tissues, and seabird eggs) collected as part of several monitoring and research programs. The National Oceanic and Atmospheric Administration (NOAA), Minerals Management Service (MMS), and the U.S. Geological Survey (USGS) have been major consistent supporters since the mid-1980's. A major expansion in environmental specimen banking occurred in 1995 with an agreement between NOAA and NIST to establish an environmental specimen bank in Charleston, South Carolina, specifically designed for marine specimens. Presently the primary specimen banking activities involve tissues collected from marine mammals throughout the U.S., including Alaska, and seabird eggs collected from seabird colonies in Alaska. There are presently 2,087 marine mammal tissue specimens banked in the NBSB, representing 737 individual animals and 34 species, and 188 seabird eggs from 3 species. These banked specimens represent a resource that has the potential for addressing future issues of marine environmental quality and ecosystem changes through retrospective analyses.

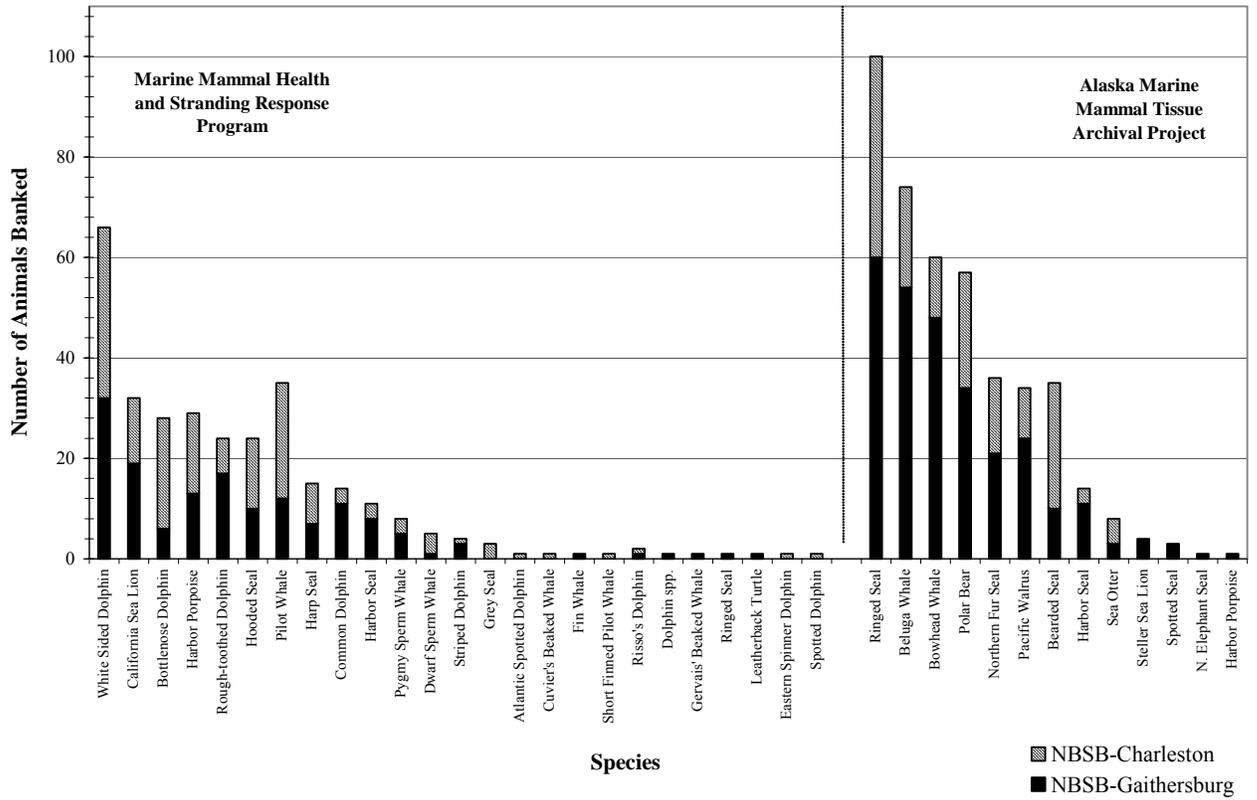
**Purpose:** The banking of a subset of specimens collected as part of an ongoing environmental monitoring or research program provides an important quality assurance function in allowing for retrospective analysis to verify results. In addition, the banked specimens provide samples for future retrospective analyses for new analytes of interest, provide samples for future analyses using improved analytical techniques, and provide a resource of samples that have been collected and stored in a systematic and well-documented manner for comparing results over time to identify whether environmental trends exist.

**Major Accomplishments:** Additional marine mammal tissue specimens and seabird eggs were added to the NBSB in FY2002. These materials were collected and provided to the bank by scientists and technicians from collaborating institutions trained by NIST in proper field collection protocols. These collaborating institutions include various federal, State, and local agencies, and private organizations located on both the west and east coasts of the U.S., including Alaska. The major programs providing specimens to the bank in FY 2002 were: the Marine Mammal Health and Stranding Response Program (sponsored by NMFS), the Alaska Marine Mammal Tissue Archival Project (sponsored

by USGS), and the Seabird Tissue Archival and Monitoring Project (sponsored by USGS and USFWS). There are presently 2,087 marine mammal tissue specimens banked in the NBSB, representing 737 individual animals and 34 species. The distribution of these specimens is illustrated in figure below. The Seabird Tissue Archival and Monitoring Project began in 1999. Eggs are collected every year from target seabird colony locations in Alaska. Egg contents are banked and aliquots are routinely analyzed by NIST for organic contaminants and mercury. Presently there are 188 eggs banked in the NBSB, representing three seabird species.

**Impact:** The specimens in the NBSB are recognized as the highest quality samples for contaminants analysis. Aliquots of these marine mammal specimens have routinely been requested for analysis by NMFS, USGS, USFWS, Department of Fisheries and Oceans, Environment Canada, and others. The seabird eggs and the analytical data resulting from these specimens represent the only resource of this type for Alaska (95% of seabirds breeding the continental U.S. nest at colonies in Alaska). Contaminant data generated by NIST from aliquots of these banked eggs were a recent contribution to the international Arctic Monitoring and Assessment Programme.

**Future plans:** Environmental specimen banking for marine environmental monitoring and research will continue. Support by the Marine Mammal Health and Stranding Response Program, Alaska Marine Mammal Tissue Archival Project, and the Seabird Tissue Archival and Monitoring Project will continue. In FY2003, protocols will be developed to incorporate marine animal blood for organic contaminant analysis, trace element analysis and immunological studies into the specimen bank. Routine banking of blood under sponsorship of NMFS will begin in FY2004.



**Figure 1.** Distribution of Banked Marine Mammal Tissue Specimens collected by the Marine Mammal Health and Stranding Response Program and the Alaska Marine Mammal Tissue Archival Project.

## SRMs for Contaminants on Air Particulate Matter

**CSTL Program:** Environmental Technologies and Systems

**Authors:** *S. Wise, R.D. Oflaz, J. Kucklick, B. Porter, D. Poster, M. Schantz, and R. Zeisler*

**Abstract:** 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  $\mu\text{m}$  ( $\text{PM}_{2.5}$ ), which is the respirable PM fraction, in addition to the existing  $\text{PM}_{10}$  standards. Research recommendations have already been made by the National Research Council at 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. The Analytical Chemistry Division is collaborating with EPA to provide SRMs and quality assurance tools for measurements of inorganic and organic species in  $\text{PM}_{2.5}$ . SRM 2783 Air Particulate on Filter Media has been issued with certified concentrations of 20 toxic and environmentally important elements. Development of a bulk  $\text{PM}_{2.5}$  SRM for organic contaminants is underway. An interim reference material for bulk  $\text{PM}_{2.5}$  has been prepared and used as part of a NIST/EPA interlaboratory comparison exercise to assess the comparability of measurements of organic constituents in PM.

**Purpose:** 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.

**Major Accomplishments:** ACD has completed SRM 2783, Air Particulate on Filter Media, which 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. 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 2-3 years, an interim reference material was prepared from a 20-g batch of  $\text{PM}_{2.5}$  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. 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. This interim reference material will be available to laboratories involved in EPA PM<sub>2.5</sub> research programs for use as a control/reference material.

**Impact:** The availability of SRMs for contemporary PM<sub>2.5</sub> will improve the accuracy and comparability of inorganic and organic measurements associated with PM research and monitoring.

**Future Plans:** The collection of the bulk SRM will be completed in fall 2003 and the certification measurements planned for 2004. Initial characterization of the interim material for toxic metals may be extended to the bulk materials leading to the development of new SRMs that can be used for the determination of inorganic species. The NIST/EPA interlaboratory comparison exercises for organic constituents in PM will continue for the next two years with annual exercises planned.

## SRMs for Contaminants in Soils, Sediments and Tissues

**CSTL Program:** Environmental Technologies and Systems

**Authors:** C.Beck, II, T. Butler, R. Oflaz, R.Greenberg, M. Howard, W.R. Kelly, J. Kucklick, R. Lindstrom, S. Long, E. Mackey, J. Mann, A. Marlow, K. Murphy, R. Paul, B. Porter, D. Poster, M. Schantz, J. Sieber, G. Turk, S. Tutschku, R. Vocke, S. Wise, L. Wood, L. Yu, and R. Zeisler

**Abstract:** Recent soil, sediment, botanical, and tissue matrix SRMs have been developed to address the need for natural matrix SRMs for quality assurance in environmental measurements including 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.

**Purpose:** Validation of the complete analytical procedure 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. 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. Expectations are that species-based regulation is imminent despite the uncertainties in the underpinning measurement science. SRMs with values assigned for speciated metals are required to address these measurements needs.

**Major Accomplishments:** The certification of several tissue, botanical, soil, and sediment matrix SRMs has been completed in the past year including three renewals of previous SRMs and three new materials. SRM 1941b Organics in Marine Sediment and SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*) are the third issue of these popular marine matrix SRMs for organic contaminants, which have found widespread use in a number of marine monitoring programs supported by the Environmental Protection Agency (EPA) and the National Oceanic and Atmospheric Administration (NOAA). Both SRM 1941b and SRM 1974b have certified concentrations for 60 polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides compared to only 11 and 9 certified constituents in the original sediment and mussel tissue SRMs issued in 1989 and 1990, respectively. Trace Elements in Pine

Needles, SRM 1575a, was renewed through a collaboration with US Geological Survey (USGS) and is intended for use in the evaluation of techniques employed in the analysis of pine needles and similar matrices; this botanical matrix challenges analytical methods in a very different manner than do the other leaf-based botanical SRMs. Certified concentrations are provided for 12 elements, reference values for 11 elements, and information values for 2 elements. Three new SRMs were issued in the past year including SRM 1946 Lake Superior Fish Tissue, SRM 2702 Inorganics in Marine Sediment, and SRM 2780 Hard Rock Mine Waste. SRM 1946 is the first NIST fish tissue SRM and is intended for both the environmental measurement community with certified values assigned for 30 PCBs, 15 chlorinated pesticides, total mercury, and methylmercury, and the food and nutrition measurement community with certified values assigned for proximates and 13 fatty acids. Both SRM 1946 and SRM 1974b are issued as frozen tissue homogenates rather than freeze-dried tissues to provide a matrix similar to that typically analyzed in analytical laboratories. SRM 2702, which replaces the popular SRM 2704 Buffalo River Sediment, has certified values for 25 elements, reference values for 8 elements, and information value for 11 elements covering all of the priority pollutant elements. SRM 2780 Hard Rock Mine Waste was developed, in conjunction with EPA and the U.S. Geological Survey (USGS), to improve the quality of the heavy metal measurements associated with evaluating the environmental impact of mine waste drainage, and will be issued in late 2002 with certified, reference, and information concentrations for 47 elements.

To address the need for reference materials for speciated metals, we have 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.

**Impact:** There are an estimated 500,000 abandoned mines in the western U.S., and these sites commonly contain significant quantities of discarded mine waster, 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. SRM 2780 was developed to support these measurements. SRMs 1946, 1941b, 1974b, and 2702 were developed to support the numerous laboratories involved in marine environmental monitoring.

**Future Plans:** 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.

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

**CSTL Program:** Environmental Technologies and Systems

**Authors:** *S. Long, and W. R. Kelly*

**Abstract:** 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 new 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.

**Purpose:** The development and implementation of a new method combining total oxidation of the sample matrix with the accuracy of isotope dilution analysis and the sensitivity of inductively coupled plasma – mass spectrometry (ICP-MS) fulfills an urgent need for more accurate measurements of mercury in environmental materials. Data provided by the method are providing a much improved traceability link for industrial EPA compliance monitoring and for calculating anthropogenic mercury releases for risk assessment studies.

### **Major Accomplishments:**

- 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

**Impact:** Sales of NIST fossil fuel SRMs have dramatically increased following the addition of certified mercury data using this method. These are being used by external customers in response to new EPA initiatives to control mercury emissions from coal-fired power stations.

**Future Plans:** We 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.

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

**CSTL Program:** Environmental Technologies and Systems

**Authors:** *S. Tutschku, M. Schantz, and S. Wise*

**Abstract:** 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). 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  $13.0 \pm 1.0$  :g/kg to fish tissue at  $397 \pm 13$  :g/kg (as Hg dry mass). Results from the SPME-GC-MIP-AES method were used in conjunction with results from other 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  $0.08 \pm 0.03$  mg/kg to  $0.35 \pm 0.05$  mg/kg (as Sn dry mass).

**Purpose:** 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 organometals are required to address these measurements needs.

**Major Accomplishments:** To address the need for reference materials for speciated metals, NIST issued the first SRMs with certified concentrations for methylmercury in 1997, SRM 1974a Organics in Mussel Tissue and SRM 2976 Mussel Tissue (Trace Elements and Methylmercury). Methylmercury was certified in these SRMs using a combination of results from NIST using gas chromatography with microwave induced plasma atomic emission spectrometric detection (GC-MIP-AES) and instrumental neutron activation analysis (INAA) in combination with results from two other laboratories using different analytical techniques (*Fresenius J. Anal. Chem.* 358:424-430, **1997**). Recently we developed an improved GC-MIP-AES method for the determination of methylmercury and used this method for certification measurements in three new marine tissue SRMs. The improved GC-MIP-AES method includes microwave-assisted acid extraction or digestion and solid phase microextraction (SPME) followed by analysis using GC-MIP-AES (*Anal. Chem.* 74:4694-4701, **2002**). 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 to 2 :g/kg (methylmercury) and 10 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 three new marine tissue SRMs, and the results were used in conjunction with results from other techniques to assign certified values for methylmercury as follows: SRM 1566b Oyster Tissue (13.2  $\nabla$  0.7 :g/kg dry mass), SRM 2977 Mussel Tissue (Organic Contaminants and Trace Elements) (36.2  $\nabla$  1.7 :g/kg dry mass), and SRM 1946 Lake Superior Fish Tissue (393  $\nabla$  15 :g/kg wet mass) (*Fresenius J. Anal. Chem.* 369:364-369, **2001**). Mono-, di-, and tributyltin were measured in three sediment SRMs at concentration levels of 0.08  $\nabla$  0.03 mg/kg to 0.35  $\nabla$  0.05 mg/kg (as Sn dry mass).

**Impact:** A suite of five marine tissue SRMs is now available with certified methylmercury concentrations ranging from 13.2  $\nabla$  0.7 :g/kg dry mass to 1380  $\nabla$  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.

**Future Plans:** 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.

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

**CSTL Program:** Environmental Technologies and Systems

**Authors:** *S. Christopher, P. Becker, S. Vander Pol, R. Pugh, and R.D. Day*

**Abstract:** 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 for this expanding 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.

**Purpose:** 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.

**Major Accomplishments:** 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 1), 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.

**Impact:** 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 was 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.

**Future Plans:** Specimen archival and analysis at NIST will expand. The collaborating partners plan to increase the number of seabird species and colony locations to be monitored throughout AMNWR (see figure 2). In FY2003 methylmercury will be determined for the eggs analyzed for total Hg in FY2002.

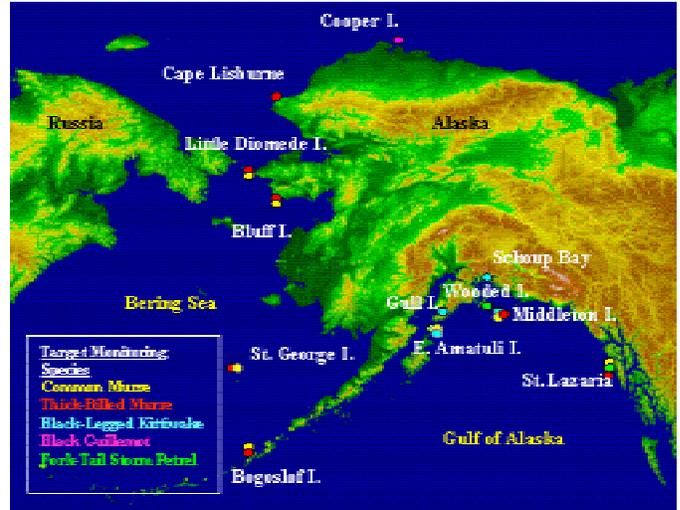
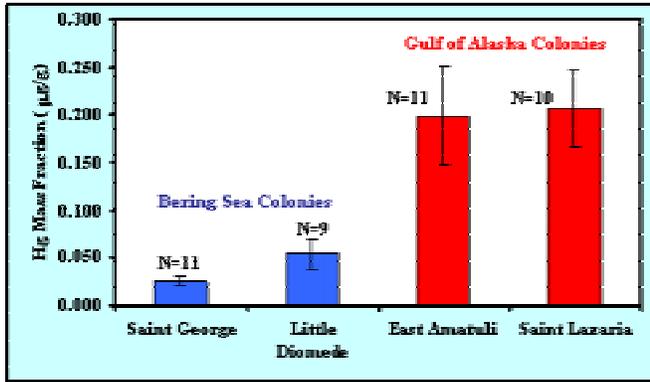


Figure 2. Projected sampling sites and species for STAMP.

## Standards to Support Stack Emissions Testing

**CSTL Program:** Environmental Technologies and Systems

**Author:** *W. Dorko*

**Abstract:** Calibration of instrumentation used for the monitoring stack gas emissions has to be traceable to NIST. One or more standards programs had to be developed in order to provide the mandated NIST traceability.

**Purpose:** Stack gas emissions are regulated by the US 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. 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 included arrangements for emissions trading whereby a power plant could sell permits to emit specific amounts of SO<sub>2</sub> if it did not emit as much as it was allowed too. The foundation for these emissions trading is the measurements made against NIST traceable calibration gases.

**Major Accomplishments:** When the original Clean Air Act was passed the traceability requirements were met by NIST developing Standard Reference Materials. NIST developed technical specifications for the Specialty Gas Industry (SGI) to produce required gas mixtures. NIST developed primary standards 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. The amendments to the Clean Air Act required increased usage of traceable gas standards and the SRM program could not meet this increase. NIST did not have sufficient resources. NIST worked with the SGI and EPA to develop a new program whereby the SGI took on more responsibility. Whereas with the SRM program NIST invested all of the resources and NIST performed 100% of the certification analyses. In the new program, the NIST Traceable Reference Material (NTRM) program, the SGI invests most of the resources and NIST does only 10% of the analyses for certification. The NIST resources are leveraged with the new program but the SRM program was also maintained. The demand for calibration gases

**Results and Future Plans:** Since the inception of the NTRM program 12 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 a SRM or a 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. The effect has been to reach the ambient air quality that is desired. It has also resulted in a smooth functioning emissions trading program

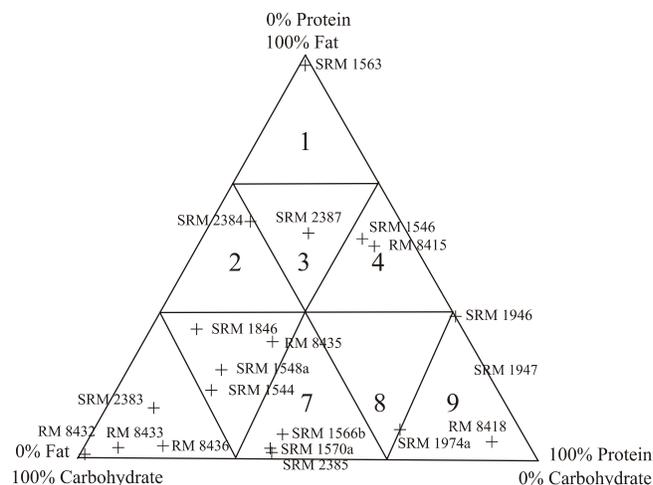
whereby companies that introduce new technology are rewarded and can sell the excess permits to emit to companies that have not yet capitalized for plant improvement. NIST is currently working to develop calibration gas mixture standards for chlorine, hydrogen chloride and ammonia. The magnitude of the demand for these will not be as large as for the other gases so we may have to find a way to provide traceability outside of the existing NTRM and SRM programs. One other approach that is being pursued is quantifying remote sensing. NIST is 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.

## Completion of Suite of SRMs to Support Nutritional Labeling Issues

**CSTL Program:** Food Safety and Nutrition

**Authors:** K. Sharpless, J. Brown Thomas, S. Margolis, B. Nelson, C. Phinney, J. Sieber, and L. Wood

**Abstract:** The Analytical Chemistry Division (ACD) 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 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. Value-assignment of two

food-matrix SRMs was completed this year: SRMs 1946 Lake Superior Fish Tissue and 2384 Baking Chocolate. Their positions in the AOAC triangle, along with those of the other food-matrix reference materials available from NIST, are shown.

**Purpose:** To develop 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, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices

**Major Accomplishments:** Value-assignment of two food-matrix SRMs was completed this year: SRMs 1946 Lake Superior Fish Tissue and 2384 Baking Chocolate.

**Impact:** A number of food-matrix reference materials with values assigned for nutrient concentrations are now available. These materials will ideally improve the accuracy of nutrition information provided on product labels.

**Future Plans:** Two food-matrix SRMs are currently nearing completion: SRMs 2385 Spinach and 2387 Peanut Butter. Following the release of these two materials in early FY03, nutrition information will be available for at least one reference material in each sector of the AOAC triangle. 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 Standards for Biotech Foods

**CSTL Program:** Food Safety and Nutrition

**Author:** *D. Bunk*

**Abstract:** A proteomics approach was investigated for the detection of transgenic proteins in whole grains. Without the use of antibodies, transgenic proteins were detected in glyphosate-tolerant soybeans and corn through a combination of multi-dimensional chromatography and mass spectrometry (MS). These results provide the groundwork for the development of quantitative reference methods based on chromatography and MS for transgenic proteins in biotech foods.

**Purpose:** To develop analytical methods based on chromatography and mass spectrometry to detect transgenic proteins without the use of antibodies and immunoassays.

**Major Accomplishments:** The immunoassays and lateral flow test strips that are used in 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, need to 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. 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. These results demonstrate that transgenic proteins could be detected without the use of antibodies and immunoassays.

**Impact:** 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. 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).

**Future Plans:** The next step in the development of a reference method for the transgenic proteins in biotech foods is to expand the qualitative proteomics methodology to a quantitative procedure.

## Reference Methods and Materials for the Chemical Characterization of Dietary Supplements

**CSTL Program:** Food Safety and Nutrition

**Authors:** *S. Wise, K. Phinney, L. Sander, and K. Sharpless*

**Abstract:** 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. 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 Standard Reference Materials (SRMs) and analytical methods for a number of botanical and botanical-containing dietary supplements. 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.

**Purpose:** 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.

**Major Accomplishments:** 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).

**Impact:** The availability of SRMs with certified concentrations of active/marker constituents and contaminants will provide the measurement tools necessary to assess the quality of dietary supplements.

**Future Plans:** This program is a six-year effort to develop SRMs for eight to ten different botanical dietary supplements. The next SRM suite will be identified in late 2002 by NIH-ODS and FDA. We anticipate that the ephedra SRM suite will be completed by September 2003.

## Reference Methods and Standards for Alcohol and Drugs of Abuse Testing

**CSTL Program:** Forensics and Homeland Security

**Authors:** *M. Schantz, J. Goodpaster, L. Sniegowski, S. Tai, and M. Welch*

**Abstract:** A number of the Standard Reference Materials (SRMs) have been developed within the Analytical Chemistry Division (ACD) for use in the forensic sciences. Examples 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. 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. In addition, three new SRMs are currently in progress that will further benefit the forensic science community: (1) an updated and redesigned SRM 1828b for blood- and breath-alcohol testing, (2) an SRM designed for arson analysis, and (3) an SRM for drugs of abuse in human serum to complement the current SRMs for drugs of abuse in urine.

**Purpose:** Blood and breath alcohol testing can be imposed on individuals operating private vehicles or operators of commercial vehicles. The various levels of blood-alcohol that determine whether these individuals are legally impaired vary depending on the circumstances, state, and sometimes month in which testing is occurring. Illegal use of controlled substances remains a serious problem in the US. Millions of tests are run annually to determine whether a person has ingested or has in their possession 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.

**Major Accomplishments:** During the past year measurements were completed for all six target compounds (cocaine, benzoyllecgonine, cocaethylene, amphetamine, methamphetamine, and phencyclidine) in SRM 2379 and for all four target compounds (morphine, codeine, monoacetylmorphine, and tetrahydrocannabinol) in SRM 2380. Two methods were used for each analyte, one based upon gas chromatography/mass spectrometry and one based on liquid chromatography/mass spectrometry which allowed the assignment of certified values. These two SRMs replace RM 8448 and RM 8449 Drugs of Abuse in Hair (segments and powder, respectively), which were the first drugs of abuse in hair reference materials and had values assigned for only four drugs. At the request of OLES, ACD evaluated the specificity and sensitivity of four commercial spray reagents test kits 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 claims.

Three new SRMs are currently 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 the blood and breath alcohol testing), (2) an SRM designed for qualitative arson analysis, 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

**Impact:** The law enforcement community will have several new tools to assure the accuracy of the methods used to detect drugs and substance abuse.

**Future Plans:** The three new forensic SRMs described above will be completed in FY03.

## New Measurement Technologies and Standards to Aid in Crime Scene Investigations

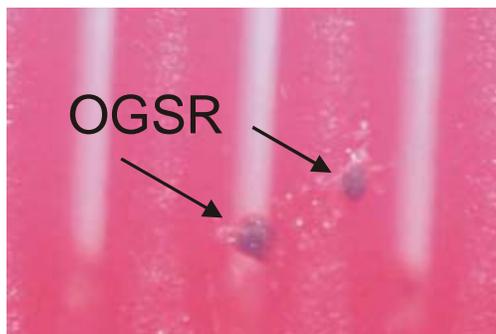
**CSTL Program:** Forensics and Homeland Security

**Authors:** *W. MacCrehan, M. Layman, J. Secl, and A. Dorsey*

**Abstract:** 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. In addition, CSTL is also developing a reference material, RM 8107 Additives in Smokeless Powder, to support accurate measurements in explosives investigations. Currently, there are no quality assurance materials for the validation of analytical procedures for explosives determination or for the testing of laboratory proficiency. In this year, the first set of compositional measurements of the organic additive content was made on the candidate smokeless powder for the RM.

**Purpose:** To help forensic investigators achieve better information from the collection of gunpowder residues at shooting and explosives crime scenes.

**Major Accomplishments:** 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. Figure 1 shows OGSR particles collected from combing a revolver shooter's hair. 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 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). 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. In the past year, the initial measurement of the composition of a candidate material for RM 8107, a smokeless rifle powder, was completed.

**Impact:** The CSTL GSR research will provide forensic scientists with a new tool to identify the perpetrators of gun crime. The reference material will help assure the best laboratory measurements to support the investigation of explosives incidents.

**Future Plans:** Research on the collection of OGSR from hair is being completed with publication of the study results. Certification measurements on RM 8107, including stability and homogeneity will be made in the coming year. 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.

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

**CSTL Program:** Forensics and Homeland Security

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

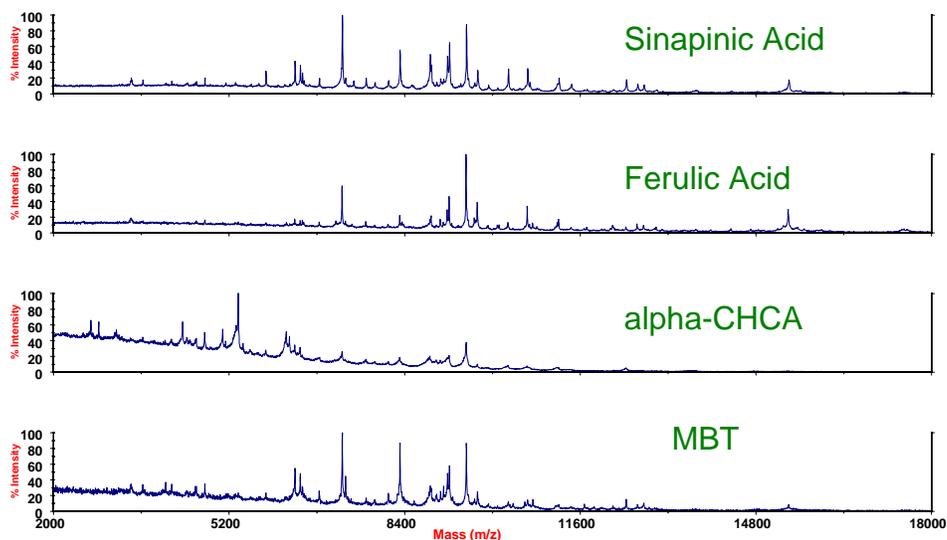
**Abstract:** The rapid and reliable detection and identification of microorganisms has become a crucial issue for homeland security. Current ACD/CSTL efforts, supported by funding from the Defense Threat Reduction Agency (DTRA), are focused on standardization of methods for identification of microorganisms 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 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 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).

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

**Major Accomplishments:** 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.

**Impact:** Standardization of bacterial sample preparation and analysis by MALDI-TOF MS, along with algorithmic development for library searches, could aid in the identification of microorganisms used as bioterrorism agents and may also have wider implications in other areas (e.g. biological weapons treaty violations, environmental, biomedical research, clinical, food industry).

**Future Plans:** With the support of the DTRA Chemical/Biological Arms Control Program (OST), this project is expected to continue for the next 2-3 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 eventually create a library of MALDI-TOF MS for a wide variety of microorganisms.



**Figure 1.** 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).

## **Development of Graphical Tools for the Analysis of Data from Interlaboratory Comparisons**

**CSTL Program:** Forensics and Homeland Security

**Authors:** *D. Duewer, and M. Kline*

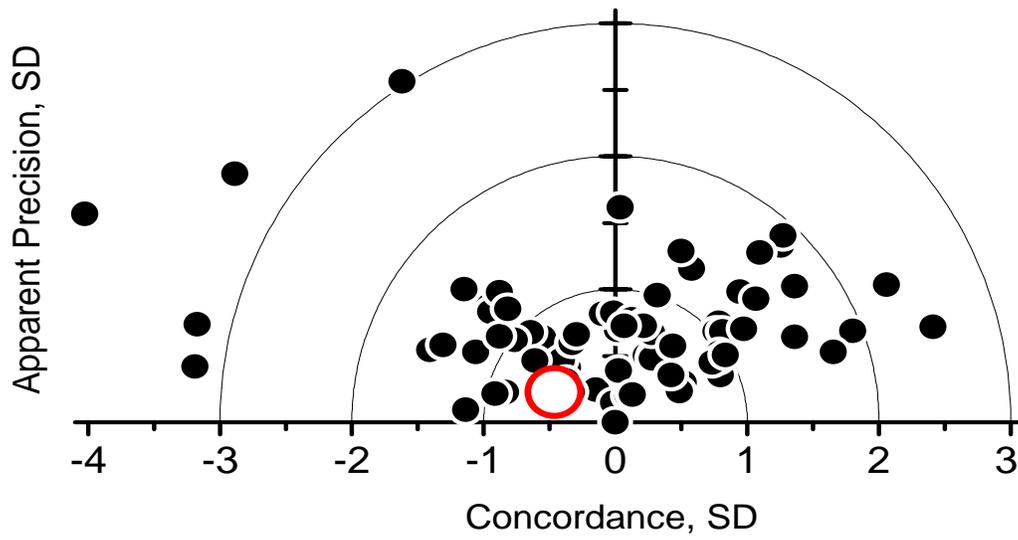
**Abstract:** For any experiment to have much utility, data and information must be transformed into knowledge and understanding. Forensic interlaboratory studies present a particular communications challenge since the audience potentially interested in the results (including the public, legal profession, participants, and Agency sponsors) have inherently diverse backgrounds. Graphs that simply but accurately summarize results help meet this communications challenge. 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. Participants reporting DNA concentrations significantly different from the consensus values typically expended more analytical effort to produce acceptable fingerprints. From anecdotal evidence, our graphical reports from earlier studies have led to improved forensic DNA measurement systems.

**Purpose:** For any experiment to have much 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) have inherently diverse backgrounds. Graphs that simply but accurately summarize results help meet this communications challenge.

**Major Accomplishments:** The third NIST-sponsored Mixed Stain Study (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. 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.

**Impact:** Interlaboratory studies have impact only to the extent that they promote their audience’s understanding of the reported measurements and help to improve the state of the measurement art. From anecdotal as well as quantitative historical evidence (documented, of course, with graphs), we appear to be making some progress toward both goals.

**Future Plans:** Development of data analytic tools is not an end in itself; rather, it is done to properly understand and effectively communicate the varied messages data are trying to tell. We will apply and modify our current tools, and likely develop new ones, as need dictates.



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

## Microfluidic Whole Cell Biosensors for Early Detection of Toxins in Water Streams

**CSTL Program:** Forensics and Homeland Security

**Authors:** *L. Locascio, E. Waddell, J. Travis, and N.G. Love (839); and A. Suggs, and B.J. Love (Virginia Polytechnic and State University)*

**Abstract:** This collaborative research effort between the NIST Microanalytical Laboratory project 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.

**Purpose:** The purpose of this work is to develop a sensitive and reliable biosensor to detect the presence of electrophilic toxins in wastewater streams. The sensor will be placed upstream of the wastewater treatment facility in order to attempt to prevent catastrophic failure of the bioreactors in these facilities.

**Major Accomplishments:** 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 this kind of failure. Recent results from Love's group have led to the hypothesis that sludge deflocculation or biofilm detachment, a mechanism by which the bioreactor can fail, occurs through the activation of the glutathione-gated  $K^+$  efflux (GGKE) system stimulated by electrophilic toxins. Therefore, prior to biofilm detachment, bacterial cells expel large amounts of potassium from inside of the cell to the outside. 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.

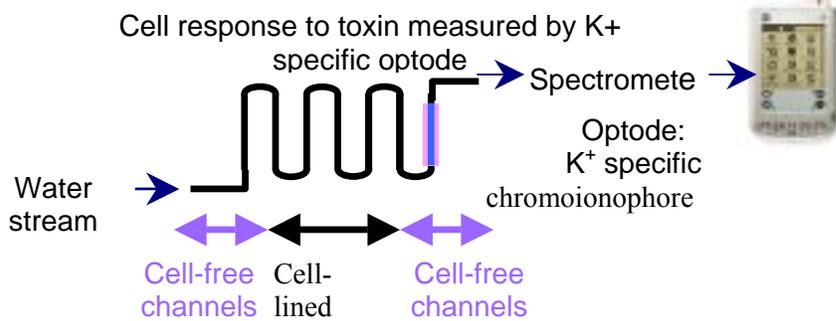


Figure 1. Schematic of cell-based microfluidic biosensor system.

The microfluidic system that we have designed for this work has 2 main components: (1) a bed of immobilized *E. coli* cells creating the micro-bioreactor; and (2) a reversible potassium sensor (Figure 1). 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 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

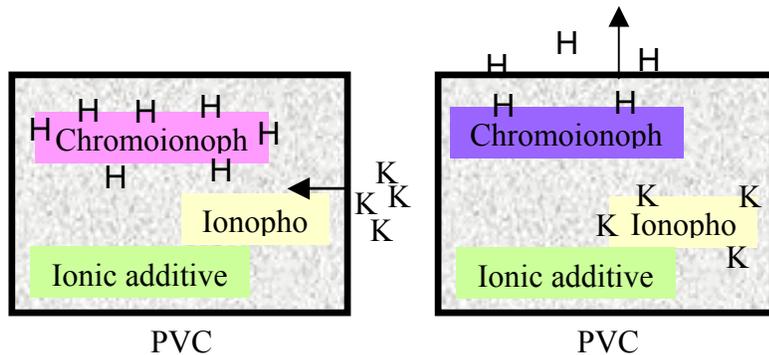


Figure 2. Left side) PVC optode membrane when no  $K^+$  is present in the membrane. Right side) Optode membrane with  $K^+$  present.

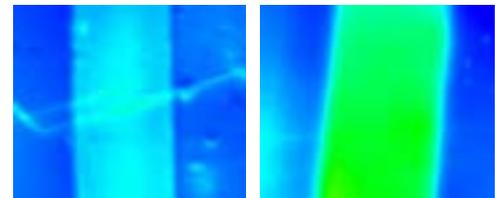


Figure 3. A) Fluorescence image of optode membrane with no  $K^+$  in solution. B) Image of membrane with 50 mM  $K^+$  in solution.

mechanisms for immobilizing cells that 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. A schematic of the optode film is depicted in Figure 2 along with fluorescence images of the film before and after exposure to potassium ions (Figure 3). 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.

**Impact:** 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. Currently, this problem is not generally addressed until after a reactor failure occurs resulting in the dumping of unprocessed sewage into streams and rivers. 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.

**Future Plans:** Future work will involve optimization of the system to improve sensitivity and reliability. If funded in the next fiscal year, we will also pursue application of the cell-based microfluidic biosensor for detection of chemical and biological toxins in drinking water.

## The Microanalytical Laboratory

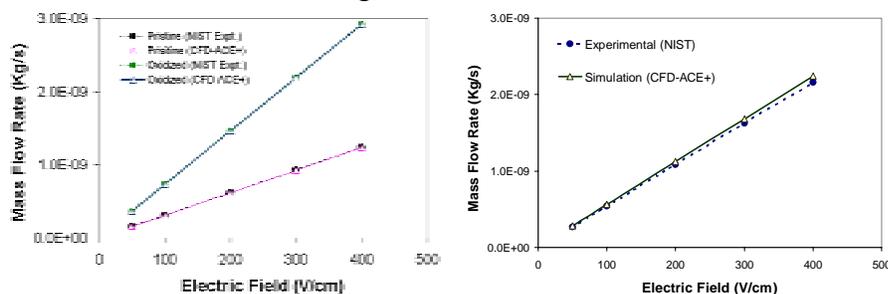
**CSTL Program:** Microfluidics

**Authors:** *L. Locascio, E. Waddell, T. Johnson, A. Henry, and P. Howell (839); D. Ross (836); and M. Gaitan (812)*

**Abstract:** The Microanalytical Laboratory competence project was established 4 years ago as a collaboration between the Analytical Chemistry Division and the Process Measurements Division (see Technical Highlight under Division 836) with the purpose of supporting and advancing an important new methodology for analytical measurements that is now commonly known as lab-on-a-chip technology. The main goals of this multi-year 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. 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 electrochromatography 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.

**Purpose:** Our focus on polymer systems is motivated by the fact that the industries involved in the development and commercialization of microfluidic technologies are interested in polymer substrates to reduce microfabrication and materials costs. An important 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. All work described here is targeted toward improving device performance and enhancing the quality and reliability of chemical measurements performed in lab-on-a-chip devices.

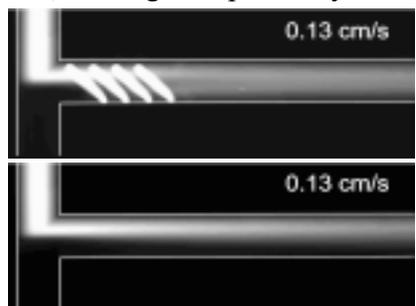
**Major Accomplishments:** In this project, we strive to produce critical fundamental data on flow control and manipulation that underpins device performance in these miniaturized flow systems. This work is comprehensive involving microflow measurement, temperature measurement, flow modeling, chemical surface modification, and materials characterization. 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. The

models will be used to predict the flow behavior in systems composed all of one material and of hybrid systems composed of 2 or more materials (Figure 1). Models will be ultimately applied to predict and optimize results of separations performed in polymer channels with a well-characterized surface chemistry.

Another aspect of our work in flow control has involved the development of micromixers. To date, mixing has primarily been accomplished by diffusion in microfluidic systems where the



**Figure 3.** Top)

Performance of micromixer (slanted wells seen as brighter lines) fabricated in polymer channels, top down fluorescence image.

Bottom) Diffusional mixing

presence of a temperature gradient (see Technical Highlight, Temperature Gradient Focusing). We have also developed expertise in the synthesis of molecularly imprinted stationary phases that can be used to impart high chemical selectivity to the walls of the microdevices for chemical separations. This method and associated advantages are detailed in a separate highlight (Molecularly Imprinted Stationary Phases for Microfluidic Chemical Separations).

Finally, our work continues to involve several active collaborations both internally (EEEL, PL, CSTL, and MSEL) and externally (Virginia Polytechnic and State University, Cornell, Stanford, Clemson, CFD Research Corporation). One of these collaborations is also highlighted (see Microfluidic Whole Cell Biosensors for Early Detection of Toxins in Water Streams).

**Impact:** In the past year, we have developed essential collaborations for the propagation of fundamental data and measurement methods. We have also developed several key collaborations for the development of systems that have application to NIST strategic focus areas including Homeland Defense, Nanotechnology and Health Care. We have continued to be very productive in information dissemination through numerous talks (12), technical publications (12) and patent applications (4).

**Future Plans:** In the next year, we will continue to develop and strengthen our role in the international microfluidics community. We will continue to develop methods and provide data, and will also begin to evaluate the need for standard reference materials and standard methods to support the growing microfluidics industry.

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

**CSTL Program:** Healthcare

**Authors:** *K. Murphy, S. Long, J. Fassett, and M. Rearick*

**Abstract:** Reference methods play a critical role in the measurement infrastructure required to diagnosis, 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).<sup>1</sup> 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 Measurement Institutes (NMI’s) 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  $\pm 0.42$  % relative. 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.

**Purpose:** The purpose of this project is to develop, critically evaluate and maintain high accuracy reference methods for electrolytes in body fluids. Such methods play a critical role in the measurement infrastructure required to diagnosis, treat, and prevent disease.

**Major Accomplishments:** Use of ICP-MS for the determination of K and Ca has been hindered by high background from the argon (Ar) plasma. Recent commercial advancements allow for operation of the ICP under “cold plasma” conditions. Under these conditions, Ar-based interferences are reduced however matrix-induced interference is increased. Cation exchange chromatography was successfully applied to remove the matrix-induced interference, allowing for accurate determination. This separation scheme permits the determination of several electrolytes in the same sample, including Ca, K, Mg, and Li.

Calcium was determined in a frozen serum material as part of the CCQM Pilot Comparison P-14 and the method was applied to the certification of K and Ca in a new oyster tissue SRM 1566b. Isotope ratios were determined using both ICP-MS and TIMS. The ICP-MS/TIMS ratio was  $1.0005 \pm 0.0017$  (1s, N=6) for K in oyster tissue, and  $0.9999 \pm 0.0016$  (1s, N=5) for Ca in serum. Comparability with the well-defined national standard, SRM 956a was achieved. Our ID ICP-

MS determination was +0.25 % relative to the certified value, which was well within the relative certified uncertainty of  $\pm 0.63$  %. On an international level, comparability within  $\pm 0.3$  % was obtained with three other NMI's that applied ID TIMS. Our results demonstrate the critical role ID ICP-MS can play in establishing traceability.

**Impact:** The clinical chemistry community has long recognized the importance of reference methods to validate the results of rapid, routine field methods. The National Committee for Laboratory Standards (NCCLS) established the National Reference System for Clinical Laboratories (NRSCL) to provide a traceability structure for clinical laboratory measurements. This structure is based on a three-level hierarchy of field, reference, and definitive methods. NIST's role in the NRSCL has been to develop and maintain the methods at the top of the hierarchy, the definitive methods. At NIST these are applied to the certification of standard reference materials and to the development of reference methods, means through which traceability is provided to field methods. Reference methods are more complex than field methods, but are generally within the capabilities of many laboratories. In the late 70's, reference methods were evaluated in conjunction with the establishment of many of the definitive methods at NIST. For example NBS Special Publication 260-36 describes an atomic absorption reference method in which Ca in serum is determined to within  $\pm 2$  % of the "true or absolute" value established by ID TIMS. 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. Improved measurement accuracy will facilitate accurate medical decision-making and lower costs due to re-tests.

**Future Plans:** NIST will participate in the CCQM Key Comparison K14, Ca in serum scheduled for completion in March 2003. Additional ICP-MS based reference methods for toxic and speciated metals are being developed. NIST will continue to work with government agencies, professional organizations, and private industry to prioritize future measurement and standards needs.

<sup>1</sup>K.E. Murphy, S.E. Long, M.S. Rearick, and O.S. Ertas, *J. Anal. At. Spectrom.*, 2002, **17**, 469-477.

## Newly Certified Trace Elements in Urine and Blood SRMs

**CSTL Program:** Healthcare

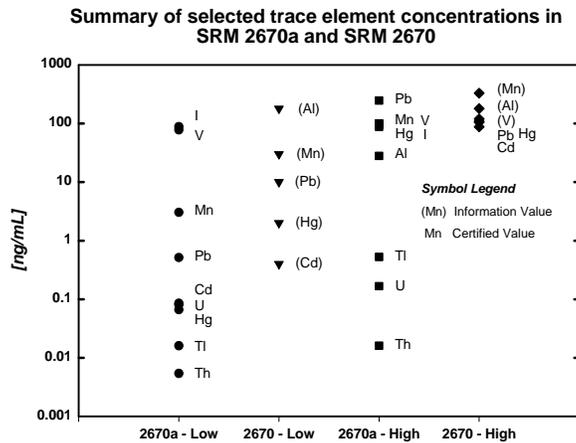
**Authors:** *R. Vocke, C. Beck, O. Ertas, W.R. Kelly, S. Long, and M. Rearick*

**Abstract:** Body fluids are the preferred way for obtaining information on human biological function. The results of clinical analyses are useful 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. Elemental analyses in urine and blood SRMs have been made at trace and ultra trace levels providing clinical and health care workers with certified values for elements at normal as well as elevated levels. The significance of this work is the increase in the number of elements that are being certified over earlier clinical SRMs and the extremely low level of the analytes in the normal level. These advances are made possible by the application of new instrumentation and analytical techniques.

**Purpose:** 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 CDC is also increasing the frequency of their health surveys in order to establish population baselines for a wide range of elements (e.g., NHANES) 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 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 focused on two reference materials, SRM 966 (Toxic elements in frozen whole bovine blood) and SRM 2670a (Trace elements in urine).

**Major Accomplishments:** 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 value of  $44.5 \pm 3.3$  ng Hg/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 value of  $31.8 \pm 18$  ng Cd/L. Both elements in this material had previously been “information values” of  $< 60$  ng Hg/L and  $< 400$  ng Cd/L.

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 set out in the figure below, comparing the values in the new material with the information values (where they existed) in SRM 2670.



**Impact:** Besides filling a need for a renewal clinical reference material for urine, SRM 2670a should become an important QC material for future CDC population surveys such as the NHANES. The addition of certified values for total Hg and Cd in the normal level of SRM 966 should help in the quantitation of the cumulative effects of these toxic elements on individuals in regions of the US with higher than normal concentrations of these elements.

**Future Plans:** This activity will be completed with the completion of the certification process.

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

**CSTL Program:** Healthcare

**Authors:** *B. Nelson, M. Satterfield, L. Sniegowski, S. Tai, and M. Welch*

**Abstract:** 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 methods are all based upon isotope dilution combined with chromatography separations and mass spectrometry. Development will begin in FY 03 for two human serum-based SRMs for these markers, one for homocysteine and folates, and the other for T3, cortisol, and several other important hormones.

**Purpose:** NIST has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. With issuance of the in vitro diagnostic (IVD) device directive by the European Union, it has become even more critical 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.

**Major Accomplishments:** New methods 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) have been developed for several health markers that are frequently measured in clinical laboratories. These include: 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. 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.

**Impact:** 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.

**Future Plans:** Development will begin in FY 03 for two human serum-based SRMs for these markers, one for homocysteine and folates, and the other for T3, cortisol, and several other important hormones including T4, thyroid-stimulating hormone (TSH), estradiol, estriol, and testosterone.

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

**CSTL Program:** Healthcare

**Authors:** *D. Bunk, and M. Welch*

**Abstract:** The clinical measurement of serum cardiac troponin I (cTnI) has become an important tool in the diagnosis of acute myocardial infarction and myocardial damage. Unfortunately, a ten-fold difference in measured concentrations among assays is common, but 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. After evaluating several candidate reference materials through two round robin studies, a human cardiac troponin complex was selected as the most suitable reference material, based upon several criteria.

**Purpose:** To reduce the large variation among results from clinical cTnI assays, NIST in cooperation with the AACC and IFCC set out to develop a cTnI reference material. A cTnI reference material will be used by the assay manufacturers to develop new assays and to validate calibration materials for their current assays.

**Major Accomplishments:** 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.

**Impact:** 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 10-15%, a substantial improvement compared to the 130% variability observed in the first round robin.

**Future Plans:** SRM 2921, purified human cardiac troponin complex, is currently being prepared. Once this material is available, a third 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.

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

**CSTL Program:** Healthcare

**Authors:** *J. Brown Thomas, D. Duewer, S. Margolis, and K. Sharpless (839); and M. Kline (831)*

**Abstract:** The Micronutrients Measurement Quality Assurance (QA) Program supports measurement technology for selected fat- and water- 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.

**Purpose:** To assess and improve the comparability and reliability of measurements of vitamins and carotenoids in human serum and plasma.

**Major Accomplishments:** Serum-based samples with assigned values for target analytes (*e.g.*, retinol, tocopherols,  $\beta$ -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.

**Impact:** Results from the interlaboratory comparison studies help the participants to make accurate clinical and health-care decisions as well as to maintain and improve their measurement comparability. Many of the participants depend on this QA program to demonstrate their compliance with Clinical Laboratory Improvement Act (CLIA) requirements, which extends federal regulation to all laboratories performing diagnostic testing.

**Future Plans:** The interlaboratory exercises for selected vitamins in serum will be continued in FY 2003. The fat-soluble program will focus on improving participants' discrimination of *trans*- and *cis*-retinol isomers. The vitamin C program will focus on further isolation and identification of systematic measurement biases. More information about the QA program is available at <http://www.cstl.nist.gov/nist839/839.02/qa.html>.

# Exploratory Research Proposal – Development of Methods for the Speciation of Selenium in Health Markers

**CSTL Program:** Healthcare

**Authors:** S. Long, D. Bunk, M. Osuna, and S. Christopher

**Abstract:** The role of selenium metallo-proteins in the body is a subject of increasing interest to the clinical community. 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.

**Purpose:** 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 which have led to conflicting information on reference intervals and estimates of toxicity and deficiency status. The development of a more accurate measurement system within CSTL may be used to provide a traceability link that would benefit the clinical community.

## Major Accomplishments:

- Defining 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
- Development of a new hydride-generation interface and flow control system for the sensitive determination of selenium by ICP-MS. The new device 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

**Impact:** 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.

**Future Plans:** 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.

## Standards for *in vitro* Diagnostics and International Trade

**CSTL Program:** Healthcare

**Authors:** *W. May, D. Bunk, J. Fassett, W.R. Kelly, S. Long, K. Murphy, B. Nelson, M. Satterfield, L. Sniegowski, S. Tai, R. Vocke, and M. Welch*

**Abstract:** Health care costs are estimated to exceed \$1.3 trillion in 2001 and currently represent over 14 % of the U.S. GDP. Estimates of the portion of these costs that are measurement related vary by which activities are included, but typically range from 10 to 15%. The Washington Post and Medical Laboratory Observer have reported that 25% - 30% of health-related measurements are performed for non-diagnostic reasons (re-test, error prevention and detection). While not providing an explicit number for the cost of non-diagnostic measurements, the Committee on Quality of Health Care in America, in a 1999 Report, "To Err Is Human: Building a Safer Health System", stated that "Dollars spent on having to repeat diagnostic tests ... are dollars not available for other purposes". The "German Health Report of 1998" states explicitly that "the costs of repeat measurement amounts to 1.5 billion US \$ per year in Germany." If normalized to the U.S. GDP for that year, these costs would be \$7.4 B. Even modest improvements in measurement accuracy and quality assurance will result in multi-billion dollar savings in health-care costs. In addition, 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). At present, reference methods and/or CRMs are available for only a limited number of the several hundred analytes that are measured in medical laboratories on a daily basis using IVDs. 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.

**Purpose:** Chemical measurements provide information that is extremely important for the prevention, diagnosis, and treatment of disease. Measurement results used by medical and other health care decision makers that are reliable and comparable over both space and time are essential for optimal patient care, most efficient use of available health care funds, and full utilization of the potential of new information technology tools. 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. This can be only accomplished if all measurement results are of known quality. NIST can contribute to increased efficiency in health care delivery by providing the measurement quality assurance tools – reference measurement methods, certified reference materials and calibrations – needed to improve measurement accuracy and reliability.

For more than 20 years, NIST has developed, maintained and refined "Definitive Methods" for health status markers to support the national reference system for clinical measurements,

including but not limited to calcium, chloride, cholesterol, creatinine, glucose, lithium, magnesium, potassium, sodium, triglycerides, urea, and uric acid. NIST methods for these health status indicators have been used to value-assign Standard Reference Materials that NIST sells to the public and reference serum pools used by the Centers for Disease Control and Prevention (CDC) as the anchor point for its reference methods and by the College American Pathologists (CAP) as its benchmark for proficiency testing more than 20,000 U.S. clinical laboratories. Improved accuracy facilitated by this program has led to better diagnosis, treatment and reduced health care costs. The provision of these accuracy-based anchor points for the clinical measurement community also facilitates the development and critical evaluation of new measurement technologies for providing cheaper and faster test results.

In addition to reliability and cost concerns, there is an increased urgency for developing internationally accepted reference methods and certified reference materials for these new diagnostic markers. On December 7, 1998, the European Directive 98/79/EC on in vitro diagnostic medical devices (IVD MD) was published in the Official Journal of the European Communities, marking the start of a transition period of five years. The stated purposes of the directive are to eliminate trade barriers within Europe by ensuring access to the entire EU market with one single product approval (CE marking), and at the same time to maintain or improve the level of health protection attained in the EU Member States. The first IVD product with a CE label may be placed on the EU market from June 2000 onwards, and by December 2003 all new IVD products that are placed on the market must be labeled with the CE mark. Alternatively, it remains possible to place new IVD products on the market under the old national regulations until December 2003, and it remains allowed to put already existing IVD products without a CE mark into service until December 2005.

Excluding home diagnostics, the overall world wide in-vitro diagnostic market (“IVD”) is approximately \$20 billion; the European market accounts for approximately \$6 billion in annual sales. Approximately 60% of the IVD products currently on the European market are imported from the USA. In order to apply the CE mark, the manufacturer must declare that his product complies with all the “essential requirements” of the Directive. One of the major components of this directive is a requirement that products be traceable to “standards of the higher order”, e.g., nationally/internationally recognized reference materials, such as NIST SRMs. At present, neither reference materials nor reference methods are available for more than 300 different chemical or biochemical species that are measured in medical laboratories using IVDs. Reference methods and/or materials exist for only about 30 worldwide.

**Major Accomplishments:** 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, 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. “Higher Order” Reference Methods and Materials” are still urgently needed for more than 100 analytes.

During the past year we have (1) critically evaluated Isotope-dilution ICPMS as a replacement to isotope dilution thermal ionization mass spectrometry for serving as the U.S. Reference method for electrolytes in blood serum, 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 urine reference material, SRM 2670a and (4) Evaluated six candidates to serve as a primary reference material for Cardiac Troponin-I (cTnI). More than 20 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.

**Impact:** The U.S. spends more than a trillion dollars per year on health care; about \$50B are spent unnecessarily because of unreliable measurements. NIST can contribute to increased efficiency in health care delivery by providing the measurement quality assurance tools – reference measurement methods, certified reference materials and calibrations – needed to improve measurement accuracy and reliability.

The U.S. IVD industry (ADVAMED) has asked NIST to work with our counterparts in Europe and the Asia-Pacific to provide the reference materials and methods of “higher order” that are urgently needed to comply with the requirements of the EU IVD Directive. Without timely completion of these standards, their access into the European market will be seriously jeopardized. Through the JCTLM, NIST, EU and AP Laboratories have agreed to share the work-load 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.

**Future Plans:** A new generation of health status markers, emerging now, show great promise from the clinical diagnostic perspective, but offers new and more difficult challenges for standardization. Many of the new markers are proteins, peptides, or other large biomolecules, usually present at very low concentrations. They are, for the most part, thermally labile, very polar, and heterogeneous, both in conformation and in what is attached to them. Because of the vast market for tests for these new markers, many different approaches have been developed and these approaches often provide different answers. These discrepancies can lead to erroneous diagnoses and/or the need for retesting -- both very costly. Plans are in place for development and disseminate of new standards for the following health status markers over the next three years.

Health Status Marker

- Bilirubin
- Cardiac Troponin-I
- C-Reactive Protein
- Folates
- HER-2
- Homocysteine

Disease State

- liver Function
- heart attack occurrence and damage
- coronary damage
- neural tube defects
- breast cancer
- heart disease risk

- Glycated Hemoglobin
- Prostate-Specific Antigen
- Thyroxine and Triiodothyronine
- Speciated Iron

diabetes status  
prostate cancer  
thyroid function  
hemochromatosis, anemia

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

**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** *J. Sieber, and A. Marlow*

**Abstract:** Research in matrix-independent XRF methods has progressed apace of metals SRM development projects. Building on the foundation laid during portland cement and zeolite SRM development work and the efforts of scientists at Corp. Scientifique Claisse, we successfully developed techniques to prepare metals and alloys for borate fusion. Once the metal (chips 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. Recent developments include new digestion procedures, applications to SRM development projects, and application to low alloy steel for the CCQM P25 Pilot Intercomparison project.

**Purpose:** 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.

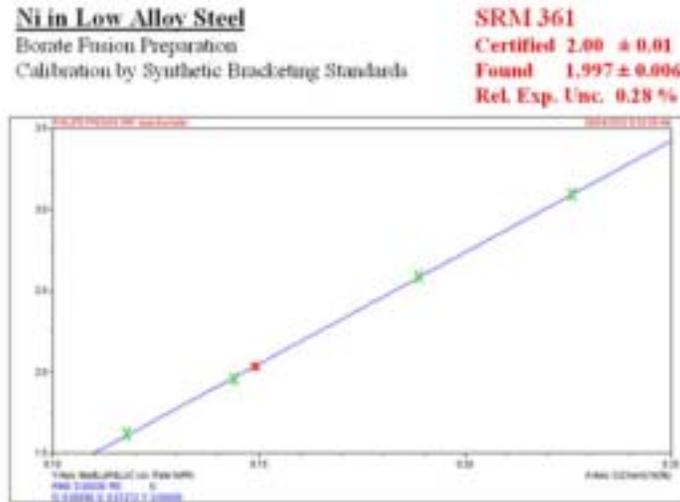
## Major Accomplishments:

- Quantitative analysis of Cr, Mn, Ni, and Mo for the CCQM P25 International Pilot Study. Chip form samples were digested using HNO<sub>3</sub> and fused with a 50/50 mix of LiBO<sub>2</sub> and Li<sub>2</sub>B<sub>4</sub>O<sub>7</sub>. Combined relative uncertainty estimates were less than 1 % for all four analytes.
- Homogeneity testing procedures and borate fusion were used to prove that two populations of Cr-Mo steel were indistinguishable. Chip form samples were digested using HBr and oxidized using HNO<sub>3</sub> followed by borate fusion. Fused specimens were used for high precision measurements of nine elements.
- A new procedure was developed to digest Zn/Al and some Al alloys using LiOH. These metals passivate when exposed to oxidizing acids and halogen acids create volatile compounds requiring extra treatment steps. This procedure will be applied to development of SRM 2426 Galvalume<sup>®</sup> and several Al alloy SRMs in progress.
- The matrix independent borate fusion XRF approach will be applied to SRM 2696 Silica Fume. Silica fume requires no acid digestion, but the material must be fused in Li<sub>2</sub>B<sub>4</sub>O<sub>7</sub> at an elevated temperature of 1100 °C. This variation on the method was shown to be effective during the development of SRM 1882a and SRM 1883a, calcium aluminate cements.
- Publication: “Matrix-Independent XRF Methods for Certification of Standard Reference Materials”, J. R. Sieber, *Advances in X-Ray Analysis*, Vol. 45, 2002.

**Impact:** Matrix independent XRF based on borate fusion dramatically increases the scope of applicability of X-ray fluorescence, especially in the metals industry. The methods increase the efficiency and the throughput for certification quality analyses in the Spectrochemical Methods

Group. If accepted by industry labs, these methods will dramatically reduce reliance on artifact certified reference materials.

**Future Plans:** Two things are of paramount importance: develop borate fusion procedures for additional materials and demonstrate the importance of matrix independence to gain acceptance by and use in industrial laboratories.



**Figure 1.** An example calibration showing the superior uncertainty characteristic of XRF/borate fusion methods. Red points are eight specimens; green points are four Synthetic calibration standards.

## Assessment of Pressurized Fluid Extraction for Enhanced Extraction of Environmental Samples

**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** *M. Schantz, B. Benner, Jr., D. Poster, and S. Wise*

**Abstract:** Organic constituents are typically removed from a sediment, soil, tissue, or particulate matrix by solvent extraction using sonication or Soxhlet methods, which are time consuming and require relatively large volumes of solvents. Recent alternative 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 was found to matrix dependent and to be less efficient for the higher molecular weight PAHs in some matrices. PFE was demonstrated to be comparable, and in the case of higher molecular weight PAHs in diesel particulate matter more efficient, than Soxhlet extraction. As a result of these studies PFE has been incorporated into the analytical scheme for the certification of organic contaminants in environmental matrix SRMs such as sediment, tissue, and particulate matter.

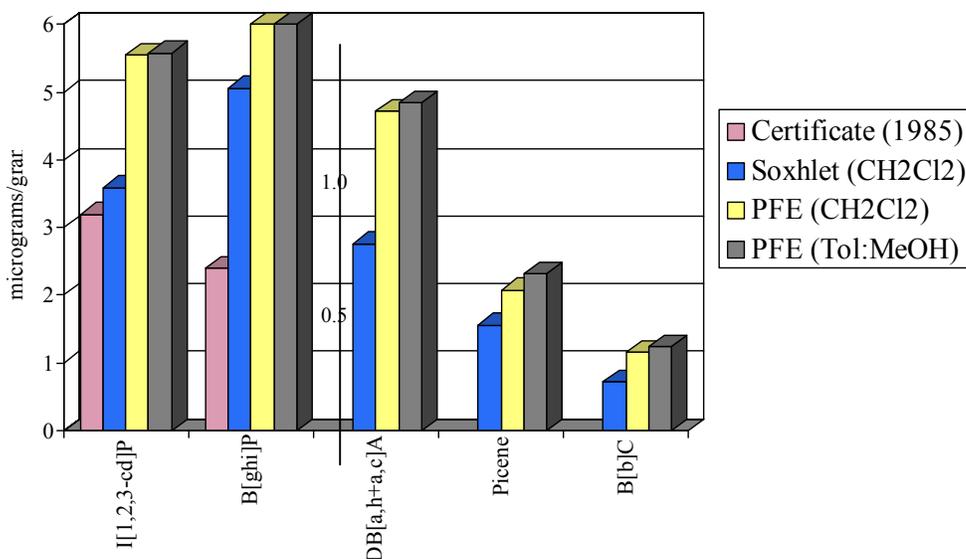
**Purpose:** Until recently Soxhlet extraction with a variety of solvents was the only extraction method employed for certification measurements of organic constituents in natural environmental matrix SRMs such as sediment, tissue, and particulate matter. The typical Soxhlet extraction requires 16 h 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 developed using supercritical fluid extraction (SFE) and pressurized fluid extraction (PFE). 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 1000 psi to 2000 psi). 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.

**Major Accomplishments:** Extensive studies comparing SFE and PFE to traditional Soxhlet extraction have been completed using several natural matrix SRMs. SFE was found to matrix dependent for the extraction of PCBs and PAHs with comparable extraction efficiencies for PCBs and PAHs in sediment but significantly lower efficiencies for higher molecular weight PAHs (> MW 252) in air and diesel particulate matter. In evaluating the applicability of PFE for use as an alternative method of solvent extraction during the certification of natural environmental matrix SRMs, the temperature and pressure conditions were optimized for the extraction of PCBs, pesticides and PAHs from sediment, fish and mussel tissue, and air and diesel particulate 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 i.e., 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 (MW278), as shown in Figure 1 for SRM 1650 (Diesel Particulate Matter).

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.

**Impact:** With the demand for increased sample capacity, faster analyses, more automation, and reduced solvent usage, environmental laboratories are constantly looking for improved extraction techniques. PFE has been demonstrated to be comparable to the traditional extraction methods, and it has now been accepted as a useful extraction technique for environmental analyses.

**Future plans:** 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.



**Figure 1.** Comparison of Soxhlet extraction and PFE for extraction of higher molecular weight PAHs in SRM 1650 Diesel Particulate Matter

## **Ruggedness Testing of HP-ICP-OES for Single and Multielement Determinations**

**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** *M. Salit, and G. Turk*

**Abstract:** 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. A recent publication documents the performance of this “High Performance Inductively Coupled Plasma Optical Emission Spectrometric Method” (HP-ICP-OES) 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 “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.

**Purpose:** To expand the applicability of HP-ICP-OES to measurement problems in optoelectronics and high purity metals, and transfer the capability to perform these measurements outside of NIST, including precious metal refiners and commercial standards producers.

**Major Accomplishments:** Aluminum, Gallium and Arsenic atom ratios were determined by HP-ICP-OES in samples of AlGaAs in a joint project with EEEL to develop composition standards for this compound semiconductor which is used in high-speed circuits for wireless communication; semiconductor lasers for short-distance communication, CD-ROM drives, bar code scanning, xerography, and laser surgery; and light-emitting diodes for remote controls, traffic lights, automotive lighting, and medical instrumentation.

Copper was determined in the series of Phosphorus Deoxidized Copper SRMs C1251a, C1252a, and C1253a. In the past this type of analysis was done using the classical method of electrogravimetry, a more difficult analysis that is no longer performed at NIST.

A collaboration to implement HP-ICP-OES was established with Metalor Technologies USA, a precious metals and advanced materials supplier. Together, methods were developed for the determination of palladium and platinum in metallic samples, and software tools were provided by NIST to perform the calculations. Less than one year later, this technique is in routine use at Metalor, complementing the gravimetric wet chemical technique used in their labs.

We are also working with commercial standards laboratories that produce elemental calibration solution CRMs that are traceable to the NIST 3100 Series of elemental solutions. In order to promote the use of HP-ICP-OES as a method of comparison to establish such traceability, we have developed a “traceability tool” spreadsheet that encapsulates a method to perform such comparisons, and performs all of the necessary data and statistical analysis. One company, VHG Labs of Manchester, NH, has incorporated this traceability tool into the value assignment procedure for their “top of the line” series of elemental calibration standards.

**Impact:** Comments received from our collaborators outside of NIST demonstrate best the impact that the implementation of HP-ICP-OES has had on their operations. Arnold Savolainen, Lab Manager at Metalor Technologies USA writes, “This technique has revolutionized our Platinum Group Metals assay lab. Assay completion times which ran 20 to 30 days, now average 4 to 6 days. Assay precisions have improved by more than an order of magnitude. Accuracy, as measured by the number of assays sent for umpire laboratory analysis, has improved to the point that we have not had to go to umpire in over three months. I am now setting up the procedure for the determination of gold at major levels.”

Susan Evans Norris, President of VHG Labs, writes “The precision of 1 to 2 parts-per-thousand that we routinely obtain are as good or better than those obtained with the wet chemical techniques. For many elements, the total analysis time has been reduced by half.”

**Future Plans:** The implementation of HP-ICP-OES is only in its infancy, and we will continue to promote use of the method in other application areas through talks, publications, participation in standards committees, and our own continued research. Tailored software tools such as spreadsheets have been found to greatly reduce the “threshold energy” to evaluate and incorporate the method in laboratories outside of NIST. A publication will appear in the “A-Pages” of *Analytical Chemistry* in 2003 that will describe our “traceability tool” for elemental solution standards, and include the software as supplemental material available on-line. We expect this to result in further use and acceptance of HP-ICP-OES.

## **New SRMS for Near Infrared and Raman Spectroscopy**

**CSTL Program:** Industrial and Analytical Instruments and Services

**Author:** *S. Choquette*

**Abstract:** SRMs 2241 and 2036 are a newly introduced Raman intensity standard and diffuse reflectance near-infrared wavelength standard. These standards were developed to correct a samples' spectrum for instrument response and to validate the performance of the spectrometer. These standards are primarily intended for use by the pharmaceutical community and other industries that require traceability. 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.

**Purpose:** To provide users of Raman and NIR instrumentation a set of performance validation standards.

**Major Accomplishment:** No standardized Raman libraries exist because of the lack of adequate calibration methods and standards. To this date, 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: Raman intensity correction standard 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.

**Impact:** The introduction of SRM 2241 and the next set of glasses for 532 nm, 488-514 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.

The introduction of SRM 2036, NIR Reflectance standard, will enable the calibration of a number of common sampling accessories currently in use by the pharmaceutical industry. As SRM 1920a is no longer available it will replace this routinely requested SRM.

**Future Plans:** We are currently developing five additional glasses that will be used for laser excitation frequencies between 488nm and 1064 nm. This suite of glasses will provide the basis for developing system independent Raman libraries.

Future plans for our NIR standards work include further work to reduce the band location uncertainties of SRM 2036, the incorporation of an internal reference for this sample, and a transition plan to help secondary standard vendors produce this SRM as well as the 2035/2065 series NIR wavelength standards.

# SpectroML—an Extensible Markup Language for Molecular Spectroscopy Data

**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** *A. Arslan, A.D. Nguyen, J.Travis, R. Schäfer, and G. Kramer*



**Abstract:** 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. 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.

**Purpose:** The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. Over the past 15 years, several different interchange formats have been developed that allow data generated by different vendor systems of a given technique to be exchanged. For example, using JCAMP-DX, users of most commercial FT-infrared and NMR systems can interchange their respective data. 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. 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. And, they don't want to have to purchase, learn, or even load proprietary programs to do this. In industrial settings, experimental data belong to and must be accessible to the entire organization, not just the laboratory personnel that generate it. Proprietary data formats and existing interchange programs simply cannot fill such corporate needs for data accessibility.

**Major Accomplishments:** Last year, we developed a "web-aware" mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectrometry data. 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.

Even before we developed SpectroML, we began to proselytize both instrument vendors and the ASTM E13 Molecular Spectrometry and Chromatography Committee about the merits of an XML-based approach to data interchange. At the 2001 Eastern Analytical Symposium, the ASTM E13 committee established a task group to support a markup language for molecular spectrometry based on SpectroML. This year at a special ASTM meeting at PittCon, we along with, a colleague from ThermoGalactic who has developed a related data exchange mark-up language called GAML—Generalized Analytical Markup Language, convinced representatives from a number of instrument companies to join in developing a unified XML-based approach for interchanging molecular spectrometry and chromatography data. The hierarchical plan, which was fleshed out at a two-day meeting in September, calls for the initial development of a core markup language containing the elements common to all applicable analytical techniques. The core schema defining the common notions of sample information, data trace, system information, quality assurance, etc. will be handled by the ASTM E13 task group. Technique specific schema will build on the core and will be the responsibilities of the respective E13 subcommittees in conjunction with other interested expert groups such as those from the IUPAC, American Society for Mass Spectrometry, etc. Data/instrument vendors are responsible for implementations of the instrument/origin specific schema that will build on both the core and the technique specific standards. Higher-level specifications could include organization –specified schema that are company specific and even end-user or application specific mark up languages. It was further agreed that the core be built as much as possible to bridge previous data exchange standards work (e.g., ANDI and JCAMP-DX), and that every effort should be made to collaborate with and include other organizations with similar interests. Encouragingly, the IUPAC group working to extend JCAMP-DX to XML has indicated their willingness to join the ASTM E13 effort. A joint meeting is planned for PittCon 2003.

We continue to develop SpectroML itself for our internal use in interchanging UV/visible data among our spectrometers, those of our NTRM vendors, and our optical filters database. 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 our 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 and hopefully much easier.

**Impact:** The development of an XML-based universal molecular spectrometry and chromatography mark up language will 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. When coupled with parallel efforts in developing XML approaches to the manipulation of other chemical data, it will pave the way for the fully electronic chemistry data archive, where any known chemical fact or information is but a simple query away on your wireless Personal Digital Assistant from anywhere in the world.

**Future Plans:** Having successfully gotten the players to the table, we are working with ThermoGalactic and the E-13 task group to extend GAML and pare down SpectroML to create the core schema. We hope to have a draft schema ready for the PittCon meeting next spring. On

the home front, we are planning to add our Agilent 8453 diode array spectrometer to our list of SpectroML-aware instruments, and to work with ThermoSpectronic (an NTRM vendor) to develop SpectroML for their instrument (our other NTRM vendor, Perkin Elmer, will be accommodated when we complete our work on the Lambda 900).

## Hydrogen Profiling in Bulk Samples via Nuclear Analytical Techniques

**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** *H. Chen-Mayer, D. Mildner, G. Lamaze, and R. Lindstrom*

**Abstract:** 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 it 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 characterizing hydrogen standards prepared by volumetric hydrogen gas reaction with titanium metal. We have some success in the area of imaging of H in bulk samples with sub-mm resolution and 100 ppm level sensitivity. PGAA has been used to verify the absolute concentration of H, whereas NIS provides rapid and position sensitive H detection.

**Purpose:** Hydrogen in metals presents a significant challenge both scientifically and economically. As the simplest impurity, even at 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 is desired. Therefore, characterization of hydrogen in materials is of great importance for the aerospace and automobile industries, the fuel-cell industry, defense agencies, and government research laboratories. Non-destructive and efficient hydrogen measurement using neutron beams can be achieved due to the unique properties of neutron-proton interactions. The Nuclear Methods Group has been pursuing hydrogen detection and characterization not only for bulk sample analysis, but also to developing methods for hydrogen mapping.

**Major Accomplishments:** As a proof of principle, we simulate varying concentration of hydrogen in the matrix by using polypropylene films of 7  $\mu\text{m}$  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 ppm levels.

**Impact:** The ability to profile hydrogen in metals and other solid materials is a much sought-after goal in the field of non-destructive testing. Currently, many industries, as well as defense agencies, are highly active in imaging of components for inspection and reverse engineering purposes. Our 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.

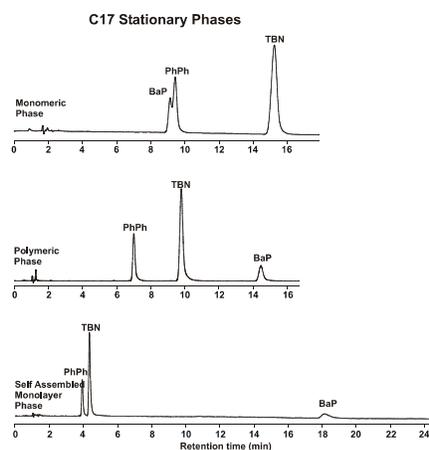
**Future Plans:** Imaging in the current mode is limited by low signal levels due to the use of a single pinhole to achieve spatial resolution. The smaller the aperture, the better the resolution but the weaker the intensity of the transmitted signal. In the future we will remove this restriction by using Coded Aperture Imaging (CAI). CAI is essentially a superposition of many pinholes in a known array. The improvement in signal intensity is directly proportional to the number of pinholes in the array. By performing a Fourier reconstruction of the projection, the image of the original object can be obtained with both good resolution and intensity. A fully developed NIS-CAI system will open up many new possibilities in the field of hydrogen mapping in industrial materials.

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

**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** *L. Sander, and C. Rimmer*

**Abstract:** The Analytical Chemistry Division (ACD) endeavors to advance chemical metrology for applications in healthcare, food and nutrition, and the environment. Chromatographic separation of organic compounds in complex sample matrixes is usually a requisite to the accurate and reproducible measurement of the targeted species. 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. Stationary phases were synthesized with alkyl chain lengths ranging from 13 to 18 carbons, by three different synthetic approaches. 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. Longer chain length columns with higher bonding 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).



**Figure 1.** Selectivity is observed to change as a function of bonding density for different synthetic approaches.

**Purpose:** To foster advances in chemical metrology by studying fundamental processes in liquid chromatography (LC), with the realization of improved analytical methods for applications in healthcare, food and nutrition, and the environment.

**Major Accomplishments:** A series of bonded phases were synthesized from consecutive length alkyl silanes ranging from C<sub>13</sub> through C<sub>18</sub>, with three different bonding chemistries (monomeric, polymeric, and self-assembled monolayer) at each phase length. The phases were characterized in terms of methylene selectivity, shape selectivity, and band broadening. It has been concluded that the performance of stationary phases in LC is influenced in different ways by the bonding chemistry, surface coverage, and alkyl chain length of the sorbent. 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 mol/m<sup>2</sup> to 8 mol/m<sup>2</sup>, a linear increase in methylene selectivity was observed with increasing bonding density. In general, retention behavior towards

nonpolar solutes is most strongly influenced by the density of the alkyl chains at the silica surface (compared with other parameters studied). Dramatic changes in shape selectivity are observed, however, as a function of temperature for alkyl phases that differ by only one carbon in length.

**Impact:** 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.

**Future Plans:** The study of sequential alkyl chain length stationary phases has been completed. The focus of related work has shifted to the study of very long chain length stationary phases, as an alternative approach for selectivity enhancement for shaped constrained solutes (especially certain classes of fat soluble vitamins). New approaches to surface modification will include the immobilization of polymer on the silica (as compared with the more conventional silanization approaches currently utilized).

# Molecularly Imprinted Stationary Phases for Microfluidic Chemical Separations

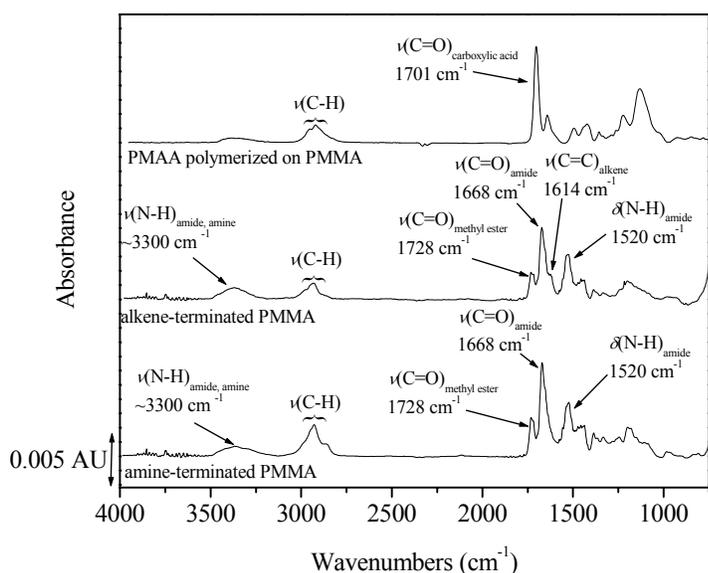
**CSTL Program:** Industrial and Analytical Instruments and Services

**Authors:** A. Henry, and L. Locascio

**Abstract:** The goal of the project is to synthesize molecular imprint polymer (MIP) stationary phases that are specific for one type of molecule. We have focused our efforts 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 incorporate 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 “pockets” that are specific in size and shape to the template molecule. To date, we have shown that the MIP thin films synthesized on plastic surfaces are selective for molecules similar to the template molecule. 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, we would like to develop a benchtop separation system that will discriminate between racemates such as those regularly synthesized in the pharmaceutical industry.

**Purpose:** The purpose of this research is to improve separations of chiral or similar molecules by synthesizing MIP stationary phases, specific for one molecule, tethered to the surface of a plastic microfluidic channel. This research is especially relevant in the pharmaceutical industry, where syntheses of pertinent pharmaceuticals are often accompanied by enantiomeric side products.

**Major Accomplishments:** The synthesis of the covalently tethered MIP films was the first major step in this project. This involved first chemically modifying PMMA surfaces such that a

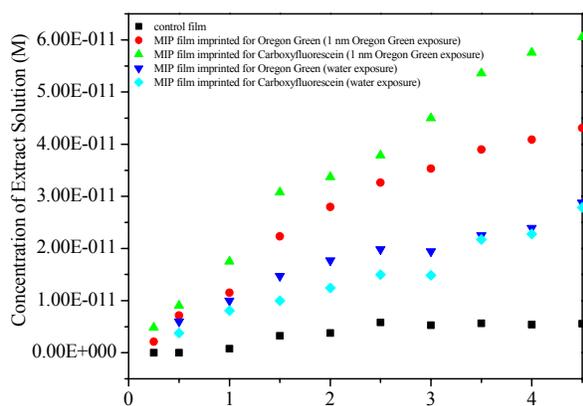


reactive functional group, primary amines, populated the surface. The primary amines were then coupled to MAA, forming amide links and terminating the surface in alkene functionalities. The alkenes were used in the polymerization of MAA in the presence of EGDMA to form a ~5 μm-thick, robust poly(methacrylic acid) (PMAA) surface. These thin films were characterized by reflection-absorption infrared spectroscopy (RAIRS) (Figure 1), water contact angle studies, and scanning electron microscopy (SEM). The RAIR spectrum of the amine-terminated

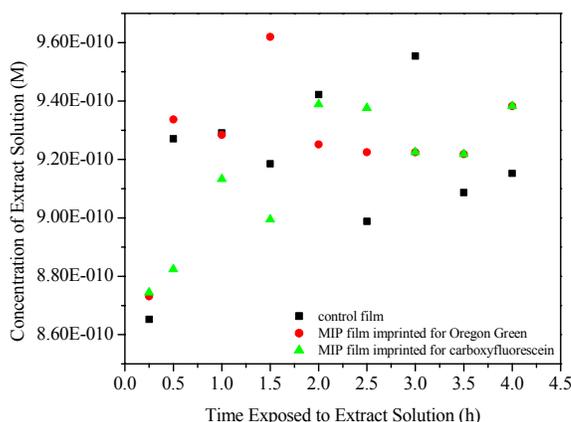
**Figure 1.** Reflection-absorption infrared spectra of amine-, alkene-, and PMAA-terminated PMMA thin films spin-coated on Au.

PMMA contains bands corresponding to an amide linkage to a primary amine on the surface of the PMMA. With alkene-terminated PMMA, many of the same bands that are found on the amine-terminated PMMA RAIR spectrum are evident; but, in addition, a band at  $1614\text{ cm}^{-1}$ , corresponding to  $\square(\text{C}=\text{C})_{\text{alkene}}$ , is noted. This band suggests terminal alkenes on the surface of the PMMA. This band is no longer evident in the RAIR spectrum of the PMAA polymerized on PMMA, suggesting that the terminal alkenes have been involved in the polymerization. A band at  $1701\text{ cm}^{-1}$ , corresponding to  $\square(\text{C}=\text{O})_{\text{carboxylic acid}}$ , is apparent.

Two fluorophores with similar structures were used as template molecules for the fabrication of MIP thin films. Three films were synthesized: (1) an MIP film that employed mixed isomers of carboxyfluorescein as the template molecules, (2) an MIP film that employed 2', 7' difluorofluorescein (Oregon Green) as the template molecule, and (3) a film that contained no template molecules. The template molecules were extracted into pH 9 carbonate buffer, and the three films were exposed to 1 nmol/L Oregon Green in pH 7 phosphate buffer for a fixed amount of time and then rinsed. The films were then extracted over time in pH 9 carbonate buffer and



**Figure 2:** Extraction of Oregon Green from MIP matrix after exposure to 1 nM Oregon Green in pH 7 phosphate buffer.



**Figure 3:** Extraction of Rhodamine B from MIP matrix after exposure to 1 nM Rhodamine B in pH 7 phosphate buffer.

analyzed by fluorescence spectroscopy, Figure 2. The carboxyfluorescein-imprinted thin film seems 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, Figure 3, no concentration trend was evident, implying only nonspecific surface interactions.

**Impact:** The focus of this work is to develop MIP stationary phases that can be used in capillary electrochromatography separations of similar molecules. This work could significantly impact characterization of chiral pharmaceuticals as the tools that we are developing would be cost-effective, small, require only minimal amounts of solvents, and would therefore be appropriate for use in high-throughput screening.

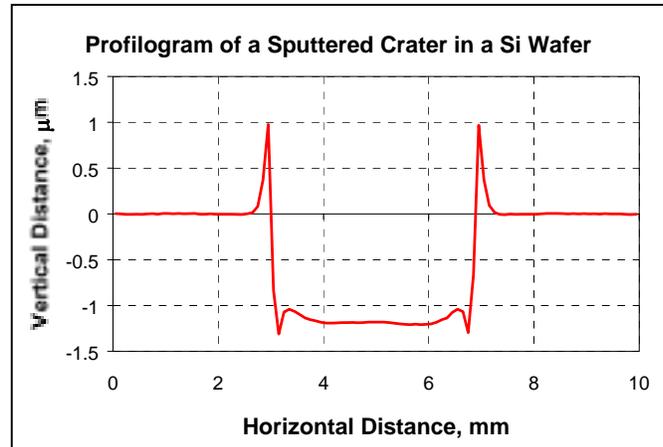
**Future Plans:** Future work will go toward improving the selectivity of the MIP thin films and using the thin films in an actual separation. We have plans to incorporate proteins and other biologically important molecules as template molecules.

# Critical Evaluation of GDOES for Depth Profiling of Layered Materials

**CSTL Program:** Microelectronics

**Author:** *M. Winchester*

**Abstract:** Glow discharge optical emission spectrometry (GDOES) is being developed for the quantitative determination of the elemental composition of surfaces as a function of depth. GDOES is inherently much faster and less expensive than most competing methods of surface analysis. A major thrust of the NIST research is on evaluating and improving the quality of data. 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

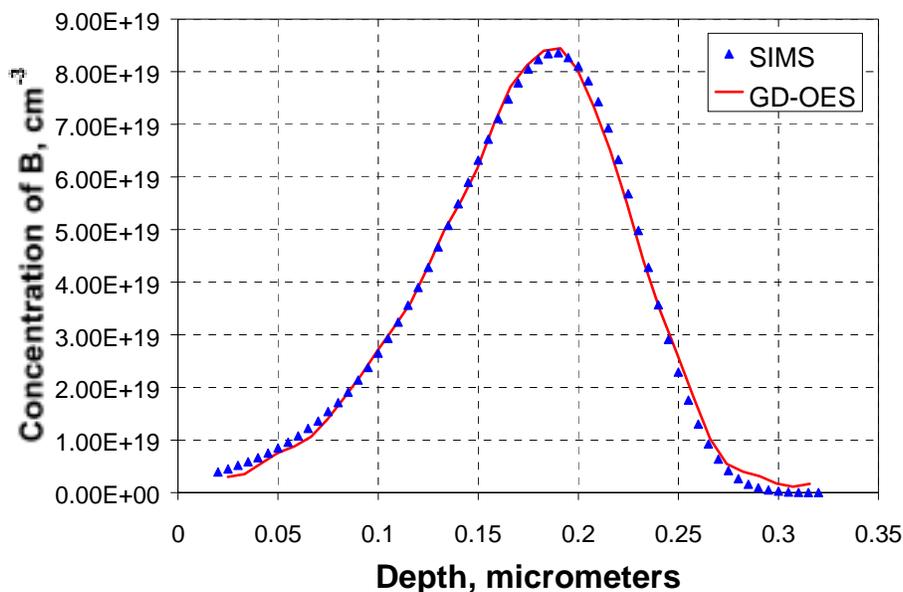


Standard for Calibration of Concentration in a Depth Profile). This sample consists of a single crystal Si substrate into which  $^{10}\text{B}$  has been implanted at a nominal energy of 50 keV. The surface was rendered disordered by Si ion implantation prior to the implantation of  $^{10}\text{B}$ . The results are encouraging, in that the GDOES depth profile matches very well previous depth profiles obtained using Secondary Ion Mass Spectrometry (SIMS). This points out the potential usefulness of GDOES depth profiling. The NIST effort to develop GDOES as a tool for quantitative depth profiling of surfaces will likely continue for at least several more years. 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.

**Purpose:** 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. Examples include the characterization of galvanneal layers on steel, the measurement of the composition and thicknesses of paint layers on various substrates, and the evaluation of the depth distributions of dopants in Si wafers. 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. A major thrust of the research is on evaluating and improving the quality of the quantitative depth profiling (QDP) data provided through GDOES.

**Major Accomplishments:** With relevance to the microelectronics industry, a GDOES method for determining the depth distribution of B in SRM 2137 (Boron Implant in Silicon Standard for Calibration of Concentration in a Depth Profile) has been developed and evaluated. This sample consists of a single crystal Si substrate into which  $^{10}\text{B}$  has been implanted at a nominal energy of 50 keV. The surface was rendered disordered by Si ion implantation prior to the implantation of

<sup>10</sup>B. The B distribution as a function of depth has been well characterized previously using Secondary Ion Mass Spectrometry (SIMS).



A GDOES depth profile obtained for SRM 2137 is shown in the figure. The corresponding 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. As shown, the agreement between the SIMS and GDOES data is good, pointing out the potential usefulness of GDOES depth profiling for this sort of application.

**Impact:** The NIST effort to develop GDOES for the quantitative determination of elemental composition as a function of surface depth is ongoing. When fully developed, GDOES QDP may provide United States industry with the ability to make these important measurements more rapidly and less expensively than is currently possible. These developments are expected to affect industry sectors that rely upon thin films, coatings, and other types of surface layers, such as the microelectronics and advanced materials industries.

**Future Plans:** GDOES QDP research at NIST will likely continue for at least several more years. Specifically, we will be working to improve the analytical capabilities of the method, 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

**CSTL Program:** Microelectronics

**Authors:** *G. Lamaze, H. Chen-Mayer, R. Lindstrom, E. Mackey, and R. Paul*

**Abstract:** 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 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 will report 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 allow 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  $5 \times 10^{15}$  at/cm<sup>3</sup> (approximately 40 ng/g in bulk samples).

**Purpose:** This evaluation is being undertaken to determine the current state of the uncertainties of the NDP and PGAA methods and to improve the techniques with the goal of lowering the uncertainties in the future. Additionally, new values of the minimum detectable amounts of boron in silicon will be determined for each technique. Because these nuclear methods are able to provide quantitation in a non-destructive measurement, they provide cornerstones for other measurement techniques.

**Major Accomplishments:** NDP-- Review of current stopping power data of alpha particles in silicon has shown discrepancies of up to 5%. Through a combination of new measurements and calculations, new values of the stopping power of alpha particles in the range of interest for boron measurements have been determined. Using an epitaxially grown silicon sample with four boron spikes at different depths, the depths were first measured with Secondary Ion Mass Spectrometry (SIMS) to high resolution. NDP measurements were then made at multiple angles yielding sets of energy loss versus depth spectra. Modeling the data to account for all sources of energy spreading, i.e., straggling, multiple scattering, geometric effects, and detector resolution, new values of the stopping power were obtained that are about 5% below those calculated by the computer program TRIM.

A new evaluation of the uncertainties involved in NDP measurements has recently been completed and will be reported at the next Washington ANS meeting. Typically for boron in silicon, the combined standard uncertainty from Type A sources is 0.2%. The significant Type B uncertainty sources include: concentration standard – 0.6%; positioning reproducibility – 1 %;

and Coulomb scattering – 1 %. Therefore, Type B uncertainties dominate. The combined standard uncertainty is 1.6 %, and the expanded uncertainty (k=2) is 3.2%.

PGAA—In order to evaluate the capability of PGAA to determine boron in doped, bulk silicon at 50 ng/g and below, we determined the detection limit of boron in silicon through analysis of a large size sample. We obtained a large piece of high purity silicon (~ 5 g), which we irradiated in the 2 cm diameter beam of the cold neutron PGAA instrument for 2 days. We then used equations derived by Currie [L. A. Currie, Anal. Chem., 1968, **40**, 586], to calculate a limit of detection of 3 ng/g for measurement of boron in silicon.

A new evaluation of the uncertainties involved for generic PGAA measurements has recently been completed and will be reported at the next Washington ANS meeting. Typically the combined standard uncertainty is about 1.3 %, and the expanded uncertainty (k=2) is 2.6 % for PGAA measurements not limited by counting statistics. However, some of the uncertainty components, specifically sample mass determination and neutron self-shielding, are considerably less for the determination of boron in silicon. An expanded uncertainty of 1.7 % should be achievable for boron in silicon.

**Impact:** The new measures of the stopping power lead to increased accuracy in the determination of film thickness and density by the NDP method. 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 our techniques to fit into the semiconductor roadmap, thereby informing our customers on how our techniques match their needs.

**Future Plans:** Determination of the sources of uncertainty has identified areas of improvement for both techniques that will lead to lower uncertainties in the future. A new curved guide cold beam line for PGAA and NDP, which will be installed in the near future, will lower backgrounds significantly and should lead to lower detection limits and lower uncertainties.

## Completion of Suite of Dopants in Silicon Standards

**CSTL Program:** Microelectronics

**Authors:** *R. Paul, R. Greenberg, R. Lindstrom, and D. Simons*

**Abstract:** 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. 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. 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  $9.55 \times 10^{14} \text{ atoms}\cdot\text{cm}^{-2} \pm 0.12 \times 10^{14} \text{ atoms}\cdot\text{cm}^{-2}$ .

**Purpose:** The purpose of this work was to provide standards to transfer the accuracy of NIST measurements to the semiconductor industry. Ion-implanted standards of known dose for B, As, and P are needed to calibrate secondary ion response for SIMS so that low systematic error can be achieved in concentration-depth profiles of these dopants in silicon. Many reference materials are commercially available, but the accuracy is not sufficient to meet all customers' needs. An accuracy-based transfer standard would enable the semiconductor industry to transfer technology from one manufacturing site to another, to compare experimental data with theoretical process models, and to provide the basis for international comparability via ISO standards.

**Major Accomplishments:** SRMs of boron and arsenic implanted silicon were prepared and certified previously. Boron was certified in implanted silicon (SRM 2137) using neutron depth profiling, while the arsenic implant (SRM 2134) was certified using instrumental neutron activation analysis. In pursuit of a phosphorus standard, an RNAA procedure for measuring phosphorus concentration in implanted silicon was developed and critically evaluated as a primary method for certification. The method is chemically specific and matrix-independent. Furthermore, because only  $^{32}\text{P}$  is measured, there is little chance of contamination during the post-irradiation chemistry, hence the blank problems encountered with most analytical methods are avoided. In order to establish RNAA as a primary method, all potential sources of uncertainty were evaluated. The method was used to measure phosphorus in pieces of an implanted silicon wafer that was previously measured in a SIMS round-robin investigation. A phosphorus concentration of  $8.30 \times 10^{14} \text{ atoms}\cdot\text{cm}^{-2} \pm 0.14 \times 10^{14} \text{ atoms}\cdot\text{cm}^{-2}$  (expanded uncertainty), was determined, in agreement with the SIMS mean of  $8.40 \times 10^{14} \text{ atoms}\cdot\text{cm}^{-2} \pm 1.55 \times 10^{14} \text{ atoms}\cdot\text{cm}^{-2}$  (1s uncertainty).

A phosphorus in silicon SRM (2133) was prepared by implantation of a 200 mm diameter silicon wafer. The wafer was cut into  $1 \text{ cm}^2$  pieces for analysis by RNAA. The phosphorus

concentration of the wafer was certified as  $(9.55 \pm 0.12) \times 10^{14}$  atoms·cm<sup>-2</sup> using RNAA as a primary method.

**Impact:** The low relative expanded uncertainties obtained for 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.

**Future Plans:** These measurements represent the completion of a suite of dopants in silicon standards. Because the presence of bulk phosphorus at native levels (< 1 ng/g) also affects the properties of silicon, the capability for RNAA to measure phosphorus at these levels will be evaluated.

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

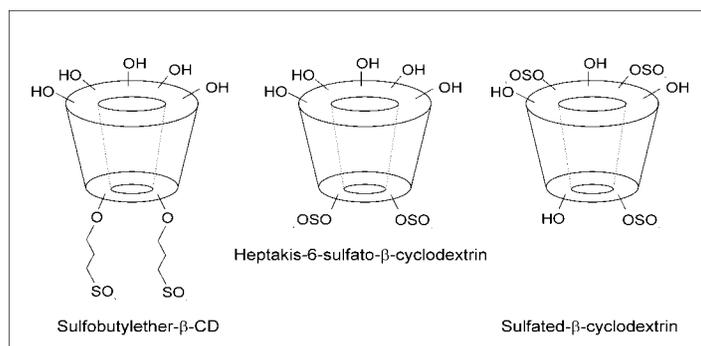
**CSTL Program:** Pharmaceuticals and Biomanufacturing

**Author:** *K. Phinney*

**Abstract:** 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. We investigated the properties of three different sulfated CDs having similar degrees of sulfate substitution, including a mixed isomer sulfated CD, a single isomer sulfated CD, and a sulfobutylether CD. The results provided important insight into successful chiral method development and optimization with different types of sulfated CD additives.

**Purpose:** The determination of enantiomeric composition is a critical quality issue in the pharmaceutical industry. Pressure to reduce both method development and analysis time has also grown in recent years. Capillary electrophoretic methods can offer advantages in speed and flexibility when compared to liquid chromatographic (LC) approaches, but successful method development depends upon an understanding of the applicability and limitations of various chiral additives to guide the selection process.

**Major Accomplishments:** Three commercially available sulfated cyclodextrins having similar numbers of sulfate substituents were selected for evaluation (see Figure 1). 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.



**Figure 1.** Simplified structures of sulfated CDs.

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

**Future Plans:** The results from this study are now being utilized to support development of chiral analytical methods for future Standard Reference Materials.

## SRMs for Hydrogen in Titanium

**CSTL Program:** Automotive and Aerospace

**Authors:** *R. Lindstrom, R. Paul, and H. Chen-Mayer*

**Abstract:** In-plant chemical analysis during the production of titanium and other metals is necessary to avoid embrittlement caused by excessive hydrogen levels. Rapid commercial hydrogen analyzers in turn require calibration standards of known composition. Methods have been developed to produce Standard Reference Materials with a known content of hydrogen, and to verify that concentration by neutron-based analyses. SRM 2453 at a level of 100 mg/kg has been completed, and bracketing materials are in preparation.

**Purpose:** Hydrogen embrittlement of many metals can be a serious problem, leading to massive structural failures in extreme cases. 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, such as SRM 2453. A combination of well-controlled preparation procedure and neutron based characterization gives assurance that these SRMs are traceable to fundamental parameters.

**Major Accomplishments:** Analytical methods for hydrogen in industry 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.

In the reversible reaction  $\text{Ti} + \text{H}_2 = \text{TiH}_2$  the equilibrium lies far toward the hydride at room temperature and far toward the elements at high temperature. Reaction is rapid at 500°C. 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 SRM (2453, 100 mg H/kg) has been prepared by this method, and its certificate has been prepared. The amount of hydrogen added to the metal was measured by the volume of gas absorbed and also by the weight gain. Prompt-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  $\approx 100$  mg/kg. SRMs 2452 and 2454 are in preparation at target mass fractions of 50 and 200 mg/kg, respectively.

**Impact:** The aerospace and, increasingly, the automotive and consumer-goods industries employ titanium alloys because of their excellent combination of high strength, lightweight, and high-temperature properties. These SRMs will help ensure that fabricated components in fact have these desired properties.

**Future Plans:** The set of three Standard Reference Materials will be completed in FY 2003. The measurement of hydrogen in titanium and other materials by PGAA and NIS remains an analytical capability and research interest.

## **Fourier-Transform Microwave Spectroscopy Critically Evaluated for Real-time Measurements of Automotive Exhaust Emissions**

**CSTL Program:** Automotive and Aerospace

**Authors:** *R. Bousquet, R. DaBell, and P. Chu*

**Abstract:** Improved analytical tools that provide real-time measurements of trace-level vapors are critical for addressing pressing industrial issues such as global climate change and the detection of hazardous emissions. Towards this end, the use of Fourier-transform microwave (FTMW) spectroscopy as a quantitative analytical technique is being investigated. The high spectral resolution and high sensitivity of 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. Success of this work will also impact many other critical applications including the detection of chemical warfare agents.

**Purpose:** There is a continuing need for improved analytical techniques to measure trace concentrations of a broad range of species including hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. This project focuses on evaluating FTMW spectroscopy as a potential tool for these critical measurements. Specific problems currently being addressed include the measurement of oxygenates in emissions for the automobile industry and chemical warfare agents in ambient air for Department of Defense Agencies.

**Major Accomplishments:** 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 require rare gas based matrices.

**Impact:** This current work is complementing research by the U.S. Army at Edgewood Chemical and Biological Center.

**Future Plans:** 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, which more closely match the specific applications, e.g. systems with high concentrations of carbon dioxide and water.

## SRMs to Support Lower Automotive Tailpipe Emissions

**CSTL Program:** Automotive and Aerospace

**Authors:** *W. Thorn, III*

**Abstract:** 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. 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 ppm and 10 ppm) and to produce new stable nitric oxide standards between 0.5 ppm and 1.25 ppm. 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 ppm and 1.0 ppm. With the assistance of AIGER member funding, NIST and Scott Specialty Gases 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.

**Purpose:** 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. NIST continues to support AIGER members by maintaining a reasonable inventory of these gaseous Standard Reference Materials (SRMs), which contain dilute mixtures of hydrocarbons, carbon oxides and nitrogen oxides in nitrogen or air. New more efficient catalysts and computer optimized combustion parameters have reduced tailpipe pollutants to very low 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. Developing lower concentration nitric oxide SRMs have been given the highest priority by AIGER members and specialty gas suppliers.

**Major Accomplishment:** Fifty-four verified mixtures at 0.5 ppm and 1.0 ppm are being certified by NIST; to be released as SRMs in December 2003. This year, twenty of these verified gravimetric mixtures were shipped to AIGER members who ordered them. The California Air Resources Board contracted to have NIST perform additional analyses on their six standards, and was released to CARB in June 2002.

**Impact:** The project has technically resolved low NO stability problems, provided AIGER members with interim standards and will provide NIST certified SRMS in the near future; to facilitate meeting EPA and CARB's lower emission regulations.

**Future Plans:** NIST will perform a full one-year analysis in early FY03, and a follow-up analysis in August-September 2003. The plan is to release 25 cylinders each of SRM 2737 and

SRM 2738 by 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 ppm methane.

## Development of Critical Benchmark Standards for the Metals Industry

**CSTL Program:** Chemical and Allied Products

**Authors:** *J. Sieber, A. Marlow, J. Fassett, G. Turk, R. Lindstrom, M. Salit, M. Winchester (839); and J. Rumble (230)*

**Abstract:** The Analytical Chemistry Division and the Standard Reference Materials Program improve the chemical metrology infrastructure of the US metals industry through method development, training, representation, and SRM development. To develop 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. This fiscal year, five SRMs for metals, alloys and related materials were completed. These SRMs benefit the copper, steel and aluminum segments of the industry and their customers in aerospace, automotive, electrical, semiconductor, and construction industries.

**Purpose:** 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.

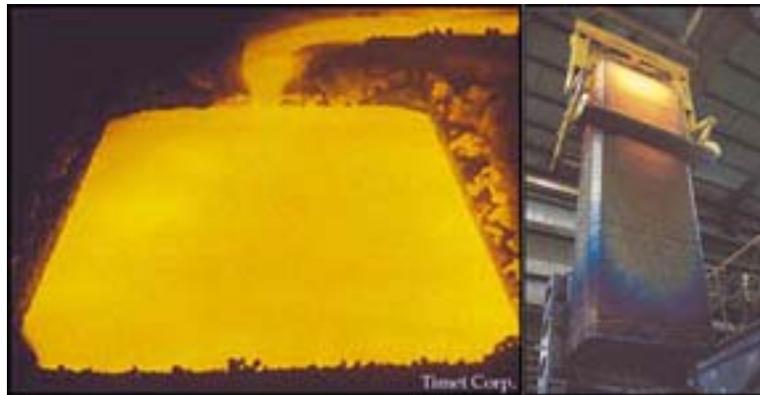
**Major Accomplishments:** Achieved through close cooperation with industry:

- » SRMs C1251a, C1252a, C1253a, Phosphorus Deoxidized Copper (renewals, completed)
- » SRM 699 Alumina (Reduction Grade) (revision of SiO<sub>2</sub> 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.
- » Co-sponsored the CCQM P25 Pilot Comparison on Analysis of Low Alloy Steel.

**Impact:** Efforts to establish benchmarks for the metals industry resulted in a clearer understanding of the difficulties and the need for close cooperation. Renewed activity in SRM development will stabilize the supply of important SRMs and introduce new compositions for value-added processes. Development of matrix-independent methods and assistance to commercial RM producers will decrease the demand for SRMs. Industry representatives are aware that NIST has more to offer than just SRMs. Presentations at key industry meetings carried the message that NIST has new analytical methods and statistical procedures for

evaluation of analytical results and that the Mutual Recognition Arrangement and the Key Comparison Database are crucial elements of international traceability and comparability.

**Future Plans:** 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.



**Figure 1.** Electron Beam Cold Hearth Refining of specialty metals. In this case, Ti bars are created in a continuous refining process.

## Development of Critical Benchmark Standards for the Cement Industry

CSTL Program: Chemical and Allied Products

**Authors:** *J. Sieber, and A. Marlow (839); B. MacDonald, and C. Fales (232); S. Leigh, and J. Yen (898); D. Broton (Construction Technology Laboratories); and S.A. Wilson (USGS)*

**Abstract:** For several years, the Spectrochemical Methods Group has been working with Construction Technology Laboratories and the United States Geological Survey on SRM projects to benefit the cement industry. The final four of 10 portland cement SRM renewals were completed this year. These materials represent cement compositions produced around the world with materials obtained in 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. 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.

**Purpose:** 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. ASTM C 114 requires at least six SRMs; therefore, it was necessary to replace the SRM 1880 series.

**Major Accomplishments:** 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 versions. 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.

**Impact:** All cement producers must qualify for ASTM C 114. Therefore, NIST cement SRMs are used by hundreds of cement labs around the world. Contacts developed through these projects will work together to develop an ASTM method for XRF analysis of cements. It is critical to establish a method because an overwhelming majority of cement labs use XRF for elemental analysis and qualification for C 114. However, C 114 does not contain any instrumental methods other than atomic absorption.

**Future Plans:** NIST analysis methods developed during the portland cement projects will be applied to other materials to support the cement industry and related industries such as mining, steel making, and environmental protection. Current related SRM projects include argillaceous limestone and silica fume. Argillaceous limestone is a key ingredient of portland cement. In addition, it is used by the lime industry and steel industry. Silica fume is SiO<sub>2</sub> of small particle size (~5 μm) and relatively high purity (> 85 %). Due to its small particle size, silica fume has

become an important admix ingredient for densification and strengthening of concrete. The Spectrochemical Methods Group will work with ASTM C01 to develop standard test methods for XRF including both traditional calibration methods and matrix-independent methods.



**Figure 1:** A modern cement plant in Chanute, Kansas.

## International Benchmarking of CSTL Analytical Measurement Capabilities

**CSTL Program:** International Standards

**Authors:** *W.E. May, R.M. Parris, and Analytical Division Staff*

**Abstract:** International agreements and decisions concerning trade and our social well-being are increasingly calling upon mutual recognition of measurements and tests between nations. The absence of such mutual recognition is considered to be a technical barrier to trade and environmental and health-related decision-making. In recent years, mutual recognition agreements have been established related to testing and calibration services and in respect of the bodies accrediting such activities. All of these rest upon the assumption of equivalence of national measurement standards and reliability of the link between national measurement standards and the relevant testing services in each country. ACD is extensively involved in efforts to establish intercomparability of chemical measurements with National Measurement Institutes both globally and regionally in the Americas. ACD staff participate and provide leadership 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) working groups, in comparison studies, and in the critical review of chemistry Calibration and Measurement Capability (CMC) claims of NMIs submitted for publication in the BIPM Key Comparison Database for Appendix C of the CIPM MRA. NIST claims comprise about 1000 of approximately 3000 NMI CMC claims for chemistry that will be included in the BIPM Database by the beginning of 2003. Since beginning in the late 1990's, 53 CCQM comparison studies have been completed or are underway; NIST has participated in 46 of these—serving as the pilot or copilot laboratory for 18 studies. Published Key Comparison results can be viewed at: [http://www.bipm.fr/enus/8\\_Key\\_Comparisons/database.html](http://www.bipm.fr/enus/8_Key_Comparisons/database.html), <http://icdb.nist.gov/>.

**Purpose:** International agreements and decisions concerning trade and our social well-being are increasingly calling upon mutual recognition of measurements and tests between nations. The absence of such mutual recognition is considered to be a technical barrier to trade and environmental and health-related decision-making. In recent years, mutual recognition agreements have been established related to testing and calibration services and in respect of the bodies accrediting such activities. All of these rest upon the assumption of equivalence of national measurement standards and reliability of the link between national measurement standards and the relevant testing services in each country. In October 1999, the Directors of National Metrology Institutes (NMI) for the thirty-eight member states of the Meter Convention signed the mutual recognition arrangement (CIPM MRA) on national measurement standards and calibration and measurement certificates issued by national metrology institutes. This MRA provides a framework for assessing the degree of equivalence between the measurement capabilities and standards issued by NMIs or NMI-designated labs throughout the world. It requires: 1) demonstration of a system for assuring quality of each NMI's measurement services, 2) evidence of successful participation in formal, relevant international comparisons, and 3) declaring and document calibration and measurement capabilities (CMCs).

In support of US chemical measurement infrastructure, NIST successful participation in relevant comparisons is used to:

- Compare NIST Definitive and Reference Methods for Chemical Analysis with other methods used to establish national measurement standards around the world
- Assist in the verification of NIST primary standards
  - primary gas mixture suites
  - elemental standard solutions
  - primary spectrometer
- Assess and document NIST (US) chemical calibration and measurement capabilities (CMCs) with those of other countries around the world
  - CIPM MRA

**Major Accomplishments:** ACD is extensively involved in efforts to establish intercomparability of chemical measurements with National Measurement Institutes both globally and regionally in the Americas. 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. CCQM working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues. ACD staff participate and provide leadership in working groups, in comparison studies), and in the critical review of chemistry Calibration and Measurement Capability (CMC) claims of NMIs submitted for publication in the BIPM Key Comparison Database for Appendix C of the CIPM MRA. NIST claims comprise about 1000 of approximately 3000 NMI CMC claims for chemistry that will be included in the BIPM Database by the beginning of 2003.

During FY01-02, ACD participated in 25 CCQM comparison studies; serving as the pilot or copilot laboratory in 13 of these. Since beginning in the late 1990's, 53 CCQM comparison studies have been completed or are underway; NIST has participated in 46 of these—serving as the pilot or copilot laboratory for 18 studies. Published Key Comparison results can be viewed at: [http://www.bipm.fr/enus/8\\_Key\\_Comparisons/database.html](http://www.bipm.fr/enus/8_Key_Comparisons/database.html), <http://icdb.nist.gov/>).

The field of chemical metrology is so diverse, broad and multidimensional (measurands, concentrations, matrices, etc.) that an immense number of key comparison studies can be envisioned as needed to cover this field. As illustrations of strategies being used to expand leverage of these studies, recent CCQM comparisons in the organic analytical area include:

- the continuation of a series of studies to evaluate NMIs' purity assessment of organic materials for use as primary standards
- in the healthcare area, a series of studies (piloted by NIST ACD) to provide a measure of the capabilities of NMIs for measuring well-defined organic analytes of clinical interest in serum. NIST piloted initial studies and subsequent key comparisons for the determinations of serum cholesterol, glucose and creatinine.
  - Cholesterol is lipophilic and present in serum primarily as fatty acid esters.
  - Glucose is highly water-soluble and also associates strongly with proteins.
  - Creatinine is very polar, present at much lower levels than cholesterol, and its determination requires considerable care to assure separation from creatine, without interconversion between creatinine and creatine.

***NIST Activities in FY 01-02 within the  
Framework for CCQM Key Comparisons and Pilot Studies***

<b><i>Health</i></b>	<b>9 KCs completed or underway; NIST leads 7</b>
<b><i>Food</i></b>	<b>7 KCs underway; NIST leads 1</b>
<b><i>Food &amp; Env. Environment</i></b>	<b>Pesticide Residues, 7 completed or underway Water, 1 underway Atmospheric Pollutants, 3 underway, NIST leads 2 Point Source Emissions, 1 underway Primary Gas Standards, 16 underway, CSTL leads 3 Contaminants in soils/sediment, 6 underway, NIST leads 2</b>
<b><i>Advanced Materials</i></b>	<b>Semiconductors, 1 underway Metal Alloys, 2 underway, NIST leads 1</b>
<b><i>Commodities</i></b>	<b>1 underway, NIST lead</b>
<b><i>Forensics</i></b>	<b>4 underway</b>
<b><i>Pharmaceuticals</i></b>	<b>None planned</b>
<b><i>Biotechnology</i></b>	<b>Five areas identified</b>
<b><i>General Analytical</i></b>	<b>Calibration Solutions, 4 underway, NIST leads 1 pH Standards, 4 underway</b>

- In the environmental area, an ensemble of comparisons to provide the framework for assessing the capabilities of participating laboratories to measure chlorinated pesticides in lipid samples in the range of 70 ng/g to 6000 ng/g was completed; studies to assess measurement of chlorinated hydrocarbons in sediment are in progress; and, studies for Organic Calibration Solutions (PCBs, PAHs, Pesticides) and Organic Contaminants in Tissue are being developed.
- A pilot and subsequent key comparison of Ethanol in Aqueous Matrix comprised materials suitable for the forensic community (ethanol in water at two levels) and a stabilized wine material for the commodity sector.

The NIST results for these comparisons were in good agreement with the key comparison reference values or other indicators of the “best” values for these studies. Examples of NIST performance in these organic comparisons are shown in the figures below. (See separate FY02 ACD Technical Activity Reports for figures of comparisons illustrating results of inorganic, pH, conductivity and gas mixture studies.)

In five pilot and key comparisons in the inorganic area in which NIST participated 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, it is noteworthy that in these intercomparisons we have applied the four of the main inorganic analytical techniques of the Division: Optical Spectrometry, Mass Spectrometry, Instrumental Neutron Activation Analysis, and X-ray Fluorescence Spectrometry. The NIST results for these comparisons were in good agreement with the key comparison reference values or other indicators of the “best” values for these studies.

In FY 2002, NIST participated in 4 pilot studies connected with international benchmarking of classical (assay) techniques, pH metrology, and anion solutions. 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.

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 VOCs Key Comparison. The results of each study indicated that the NIST measurements are at the state of the art for the respective measurement.

The Systema Interamericano de Metrologia (SIM) is the metrological regional organization (RMO) that includes the United States. RMOs have the responsibility for carrying out supplementary comparisons and other actions within their regions 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 review of calibration and measurement capabilities (CMCs) of their member NMIs. In order to most effectively address the unique needs of the 34 countries within SIM whose capabilities in chemical metrology span a very broad range, the SIM program initially has focused on training and capability assessment rather than participation in MRA- driven Key and Supplemental Comparisons. During the past year, six intercomparison exercises were completed to assess the proficiency of SIM NMI's and/or their designated collaborators for addressing chemical measurement problems within their regions and the Americas. ACD staff participate and provide leadership in SIM working groups, have piloted and participated in all eleven SIM performance assessment (comparison) studies, have conducted workshops and laboratory training, and have led the critical review of SIM Calibration and Measurement Capability (CMC) claims. SIM has approximately 1140 CMC entries scheduled for inclusion in the BIPM database by the start of FY03.

In addition to these global and regional activities, 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 (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 /Standards laboratories are being discussed.

ACD 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.

**Impact:** To serve US interests, it is important that NIST demonstrates and documents the equivalence of its chemical measurements with those of other NMIs. This is essential for NIST (for benchmarking and establishing veracity of NIST standards), for NIST partners (e.g., other NMIs), and for NIST customers (industry, regulatory agencies, end users of NIST services).

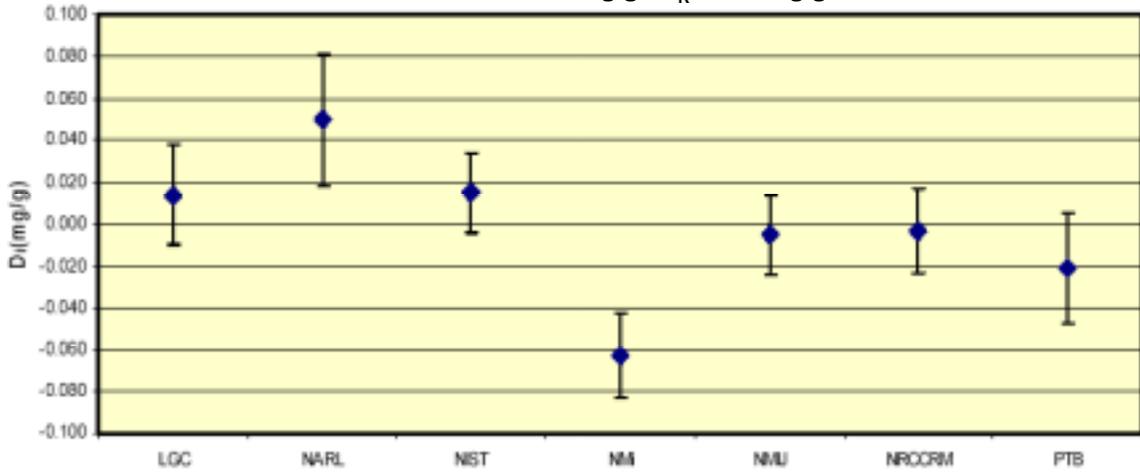
**Future Plans:** In ACD, we continue to assess needs of the US measurement community and strategically prioritize our international benchmarking activities to support these needs. ACD has increased its participation and leadership in relevant international organizations and in activities to promote acceptance and confidence in US measurements. For chemical measurements, the number of comparisons is increasing and the application areas widening. Of 36 CCQM comparisons now in the planning stages, ACD has agreed to pilot at least 9 of these.

In further support of recognition and acceptance of claims for chemical measurements made within the Analytical Chemistry Division, ACD is planning for a second international peer review of our chemical measurement and standards programs by a group of international chemical metrology experts (the first was conducted in October of 1999) and a review of ACD's System for Assuring Quality and its implementation.

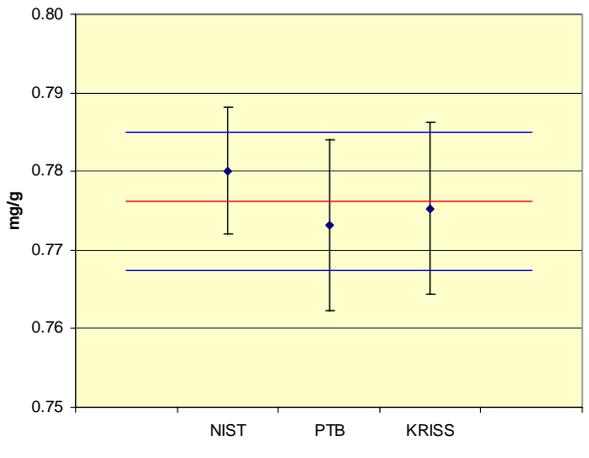
## CCQM-K6 Cholesterol in Human Serum

CCQM-K6 Material A  
Degrees of Equivalence

Material A KCRV: 2.200 mg/g  $U_R$  0.019 mg/g

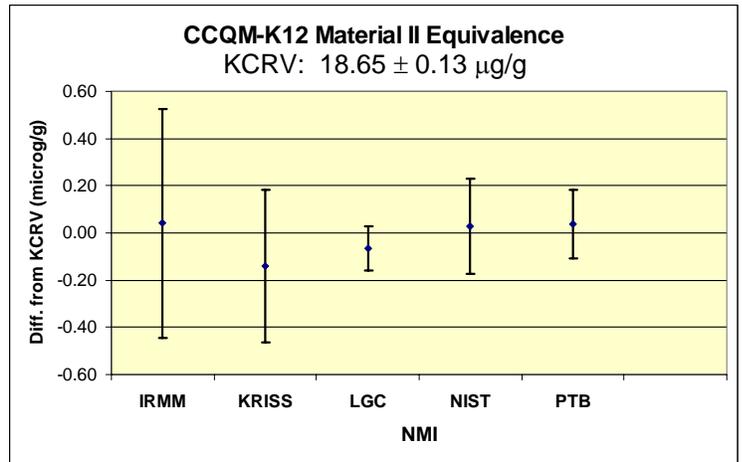


## CCQM-K11 Glucose in Human Serum Material I

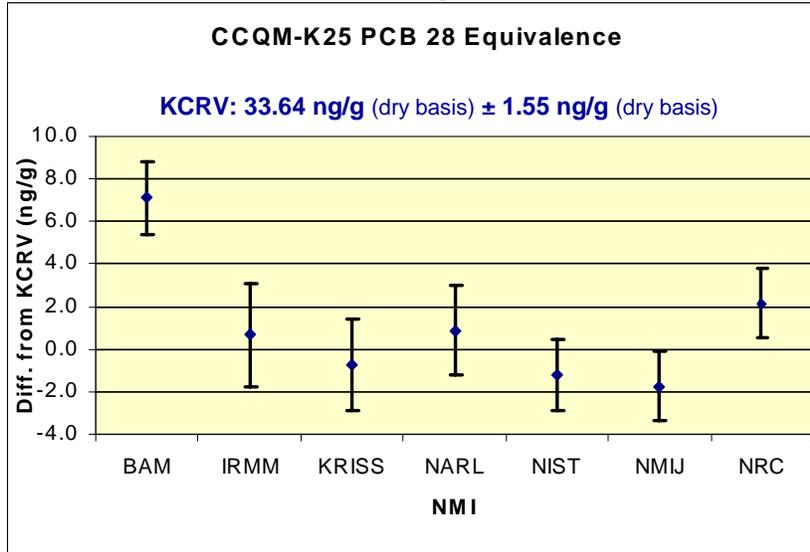


## Creatine in Human Serum

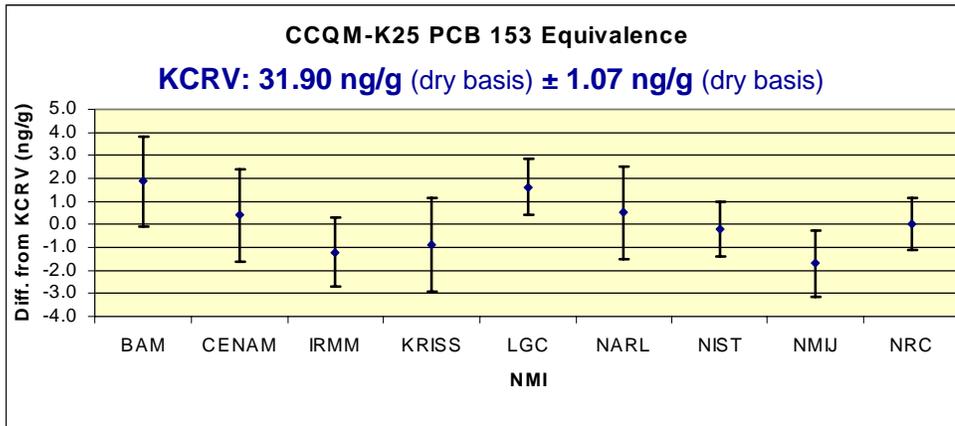
CCQM-K12 Material II Equivalence  
KCRV:  $18.65 \pm 0.13 \mu\text{g/g}$



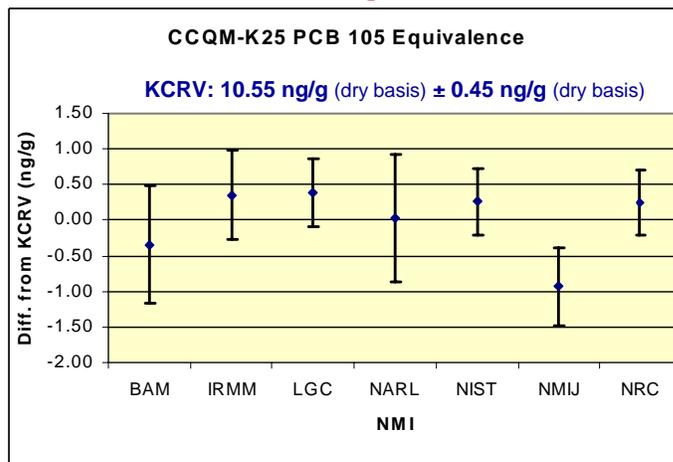
### CCQM-K25: PCB Congeners in Sediment



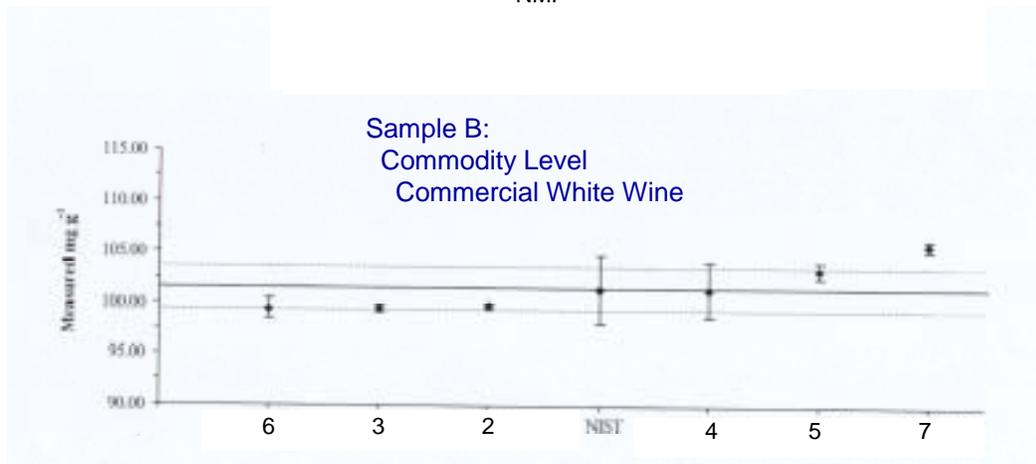
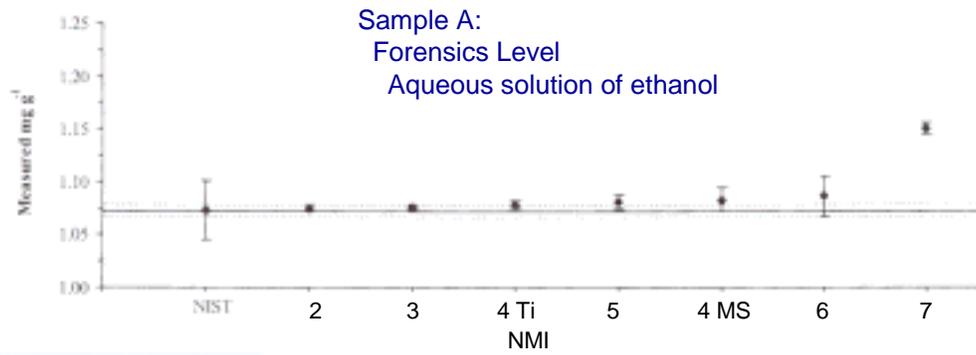
### CCQM-K25: PCB Congeners in Sediment



### CCQM-K25: PCB Congeners in Sediment



## CCQM-P35: Ethanol in Aqueous Matrix



## International Benchmarking of CSTL Inorganic Analytical Measurement Capabilities

**CSTL Program:** International Standards

**Authors:** *R. Greenberg, J. Fassett, K. Murphy, R. Zeisler, E. Mackey, L. Yu, L. Wood, J. Sieber, and A. Marlow*

**Abstract:** ACD 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. The NIST results for these comparisons were in good agreement with the key comparison reference values or other indicators of the “best” values for these studies. It is noteworthy that in these intercomparisons we have applied the four of the main inorganic analytical techniques of the Division: Optical Spectrometry, Mass Spectrometry, Instrumental Neutron Activation Analysis, and X-ray Fluorescence Spectrometry.

**Purpose:** 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.

**Major Accomplishments:** Analytical Chemistry Division (ACD) successfully participated this year in four pilot and one key comparisons of the inorganic working group of the CCQM: 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 (Figure 1). 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, which 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:  $(14.36 \pm 0.23) \text{ nmol/g}$ ; CCQM:  $(14.40 \pm 0.09) \text{ nmol/g}$  (Figure 2)]. 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:  $(0.3554 \pm 0.0067) \mu\text{mol/g}$ ; NIST INAA:  $(0.3559 \pm 0.0095) \mu\text{mol/g}$ ; CCQM:  $(0.3527 \pm 0.0024) \mu\text{mol/g}$ ]. 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.

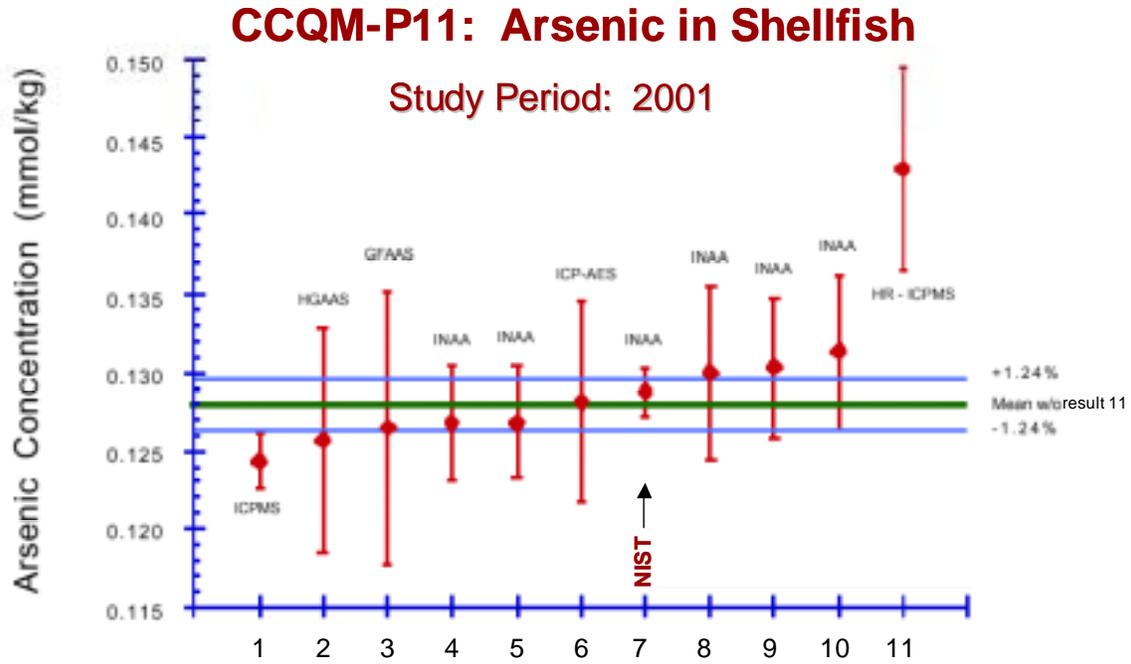
The Pilot Study CCQM-P13 Metals in Artificial Food Digest was proposed by LGC to benchmark measurement of nutritional, essential and toxic elements in foods. They proposed to avoid the difficulty in assigning a consensus key comparison reference value by spiking a synthetic matrix solution with gravimetrically 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 standards addition 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 deviation 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 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. The first 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.

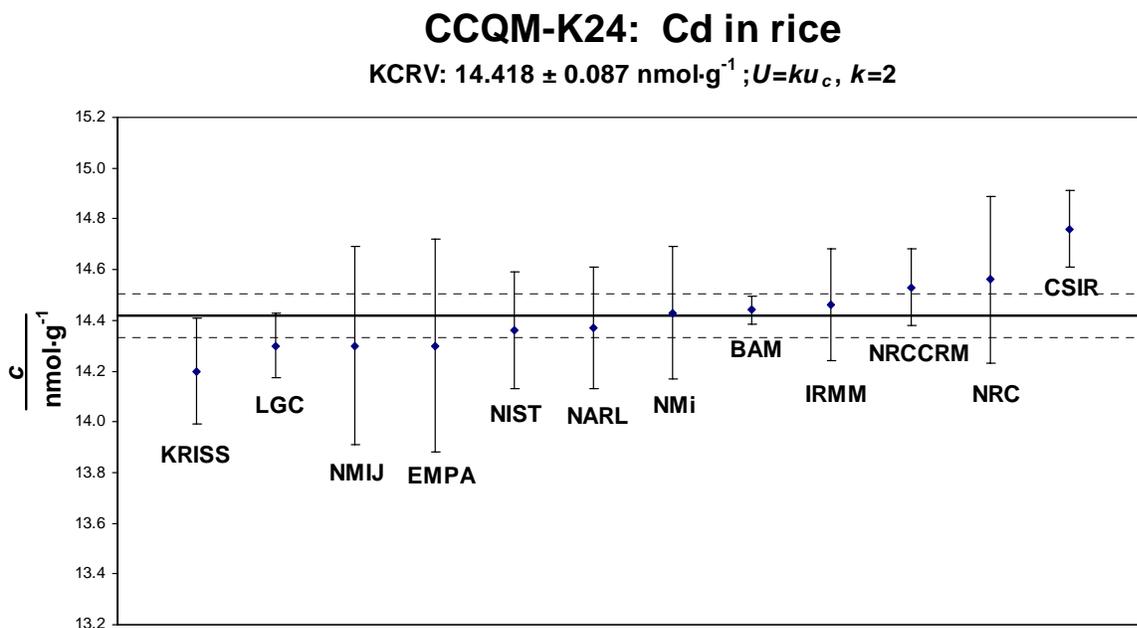
**Impact:** We have successfully demonstrated the accuracy of four of our main inorganic analytical techniques of the Division: Optical Spectrometry, Mass Spectrometry, Instrumental Neutron Activation Analysis, and X-ray Fluorescence Spectrometry, as well as our comparability with other NMIs. In addition, we have applied these methods to a number of quite different matrices thus providing an indication of how far the light shines.

**Future Plans:** We will continue to participate in CCQM comparison as appropriate to demonstrate and document our measurement capabilities in support of support US national interests.

**Figure 1.** Final results for CCQM-P11



**Figure 2.** Reported results for CCQM-K24



## International Benchmarking of CSTL Classical and Electroanalytical Measurement Capabilities

**CSTL Program:** International Standards

**Author:** *K. Pratt*

**Abstract:** In FY 2002, NIST participated in 4 pilot studies connected with international benchmarking of classical (assay) techniques, pH metrology, and anion solutions. Each study was performed under the auspices of the Comité Consultatif pour le Quantité de Matière (CCQM) and 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.

**Purpose:** 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 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 which 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.

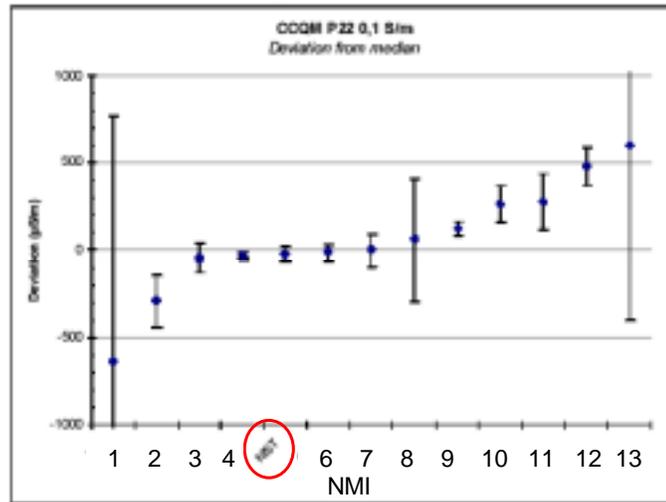
**Major Accomplishments:** CCQM-P36 [potassium acid phthalate (KHP) assay] provided information on the importance of  $CO_2$  interference in assay of this widely-used acidimetric standard and exposed a ca. +0.04% bias at NIST that had arisen since the latest KHP SRM certification. CCQM-P19.1 (HCl assay) 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 SRMs. The CCQM-P32 results ( $PO_4^{3-}$  and  $Cl^-$  anion solutions) supported the NIST claims for measurement capability for the respective measurements.

**Future Plans:** 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).

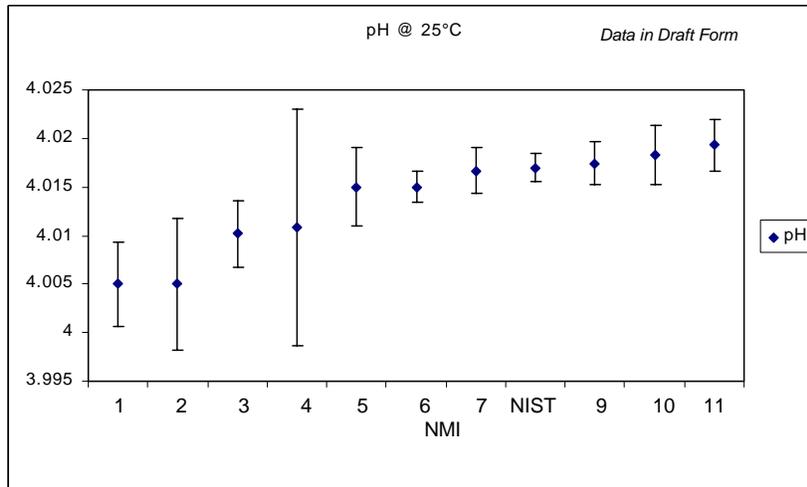
The results of the HCl assay and fundamental pH studies provided essential information regarding primary pH measurements and systematic sources of uncertainty that impact all primary pH measurements. These results directly impact details of the methodology used by the NMIs participating in already planned future pH KCs.

### CCQM-P22: Conductivity (primary and secondary measurements) Study Period: 2001



Nominal 0.1 S/m (1000 µS/cm)

### 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]



## International Benchmarking of CSTL Capabilities in Gas Metrology

**CSTL Program:** International Standards

**Author:** *F. Guenther*

**Abstract:** 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 VOCs Key Comparison. Each study is performed under the auspices of the Comité Consultatif pour le Quantité de Matière (CCQM) and 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 C13 in the pure carbon monoxide used to prepare the gas standards. Once this difference was factored into the results, all the NMIs were in good agreement.

**Purpose:** 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

**Major Accomplishments:** 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 straight forward to determine small uncertainty sources. The results showed major biases between the NMIs, especially NIST and KRISS (Korea). After much work at NIST and several other NMIs, the source of the bias was traced to differences in the C13 abundance of the pure carbon monoxide (CO). It turns out that gas suppliers strip out the C13 CO from the pure gas to sell to producers of isotopically pure organic compounds. The result is that primary standards produced with the depleted C13 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.

**Future Plans:** 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 hopefully 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.

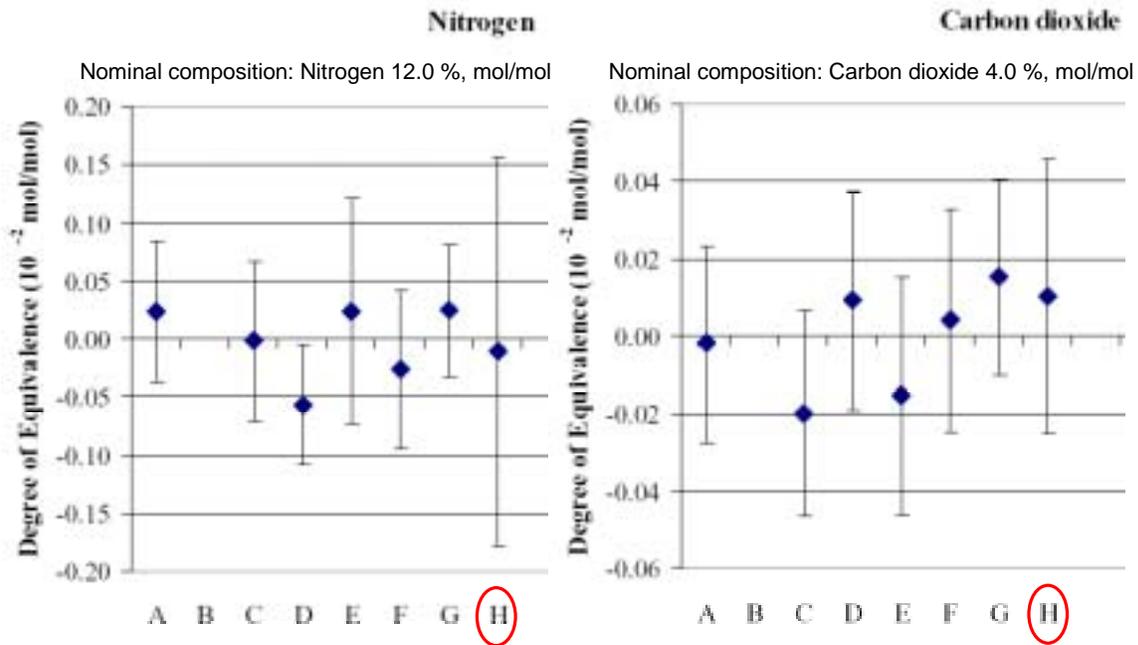
NIST is working with NMIJ in Japan on the proposed CCQM-K22 Volatile Organic Compounds (VOC) in Air. During the past year NIST has analyzed a prototype VOC standard for NMIJ. In the coming year, NIST will host two guest scientists from NMIJ. The scientists will be working here on VOC primary gas standards. Also in the spring George Rhoderick will be visiting NMIJ to view their capabilities in VOC gas standard preparation.

**CCQM-K16a: Gas mixture - Natural Gas Type IV (low calorific mixture)**

Period of Study: 2001

12 measurands

NIST is Lab "H"



## **Collaboration with BIPM to Develop an Advanced Primary Ozone Photometer**

**CSTL Program:** International Standards

**Authors:** *P. Chu, J. Norris, and F. Guenther*

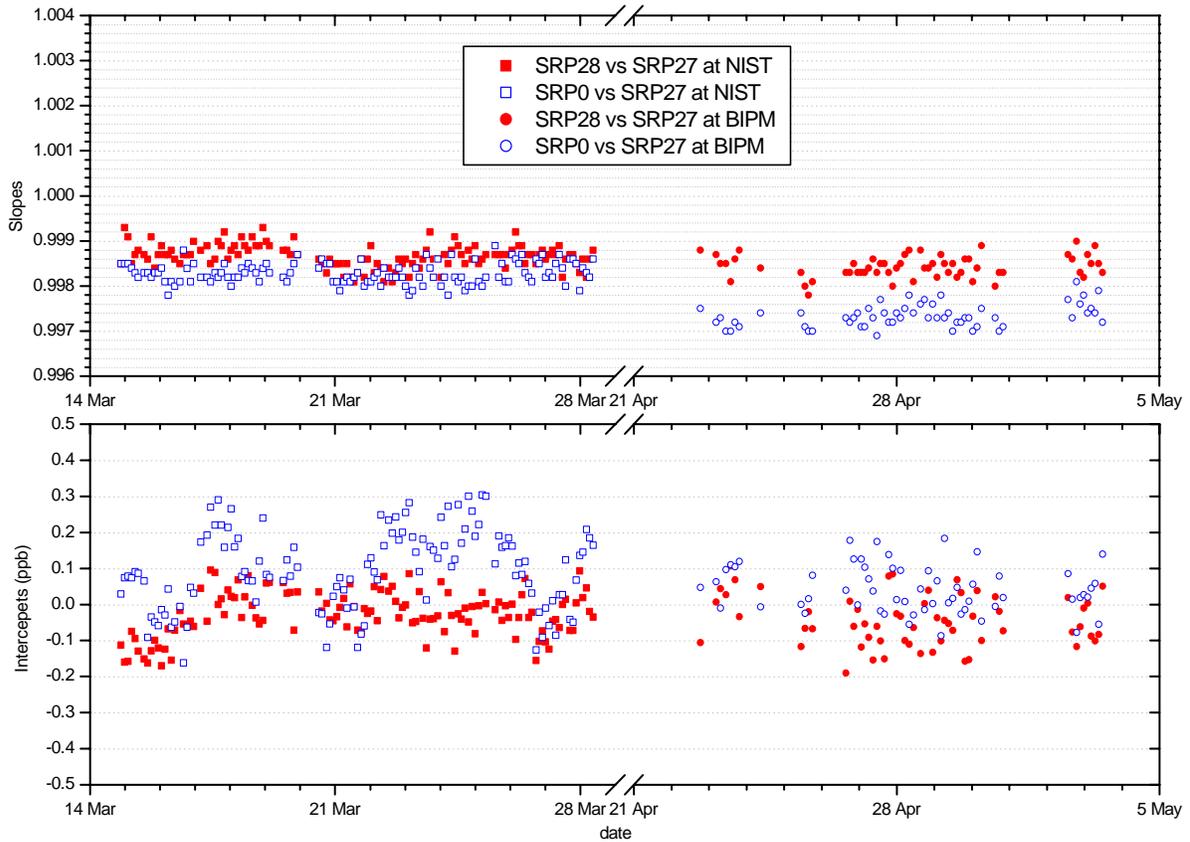
**Abstract:** The concentration of ozone in the atmosphere remains a significant issue from both scientific and political perspectives. Ozone, at tropospheric levels, is a health concern and contributes to global climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. 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. While the SRP has nobly served monitoring communities for many years, 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.

**Purpose:** To support the growing national and international need of improved ambient ozone measurements, NIST is partnering with BIPM to develop advanced primary ozone reference standards and to share the dissemination of secondary or transfer standards. The basic project plan includes 1) 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) Develop a primary reference standard with capabilities of SI-traceability verification.

**Major Accomplishments:** 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 on going effort is dedicated towards identifying and eliminating this bias. To assess the current comparability of global ozone measurements, a pilot ozone key comparison will be initiated fall of 2002. An advanced photometer demands instrumentation with high sensitivity, accuracy, precision, and 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 NO<sub>2</sub>, NO, and O<sub>3</sub> at trace levels have been established.

**Impact:** 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.

**Future Plans:** 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 be initiated during FY 2003.



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