

Analytical Chemistry Division (839)

Willie E. May, Chief

Division Overview and Selected Technical Reports

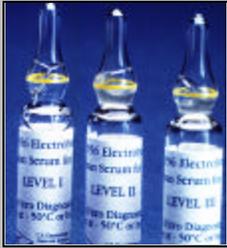
The Analytical Chemistry Division is one of five Divisions in the Chemical Science and Technology Laboratory, National Institute of Standards and Technology. The Division has approximately 100 scientists, technicians, and administrative/clerical support staff and an annual budget of about \$15M of which 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: Spectrochemical Methods, Organic Analytical Methods Gas Metrology and Classical Methods Molecular Spectrometry and Microfluidic Methods and 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



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- Spectrochemical Methods
- Organic Analytical Methods
- Gas Metrology & Classical Methods
- Molecular Spectroscopy & Microfluidic Methods
- Nuclear Methods

evaluation of measurement methods of known accuracy, sensitivity, and uncertainty. The Division is currently focusing its research and service activities to address measurement problems in the following areas:

- Analytical Instrument Performance and Calibration
- Chemical Characterization of Materials
- Environmental Monitoring and Technology
- Forensics/Defense/Security
- Healthcare and Clinical Chemistry
- Nutrition, Contamination and Adulteration of Foods

The Division has established and is maintaining the infrastructure for providing national traceability and assessing international comparability of chemical measurements for

these and future high priority program areas with infrastructural tools and activities such as:

- 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.

Further discussion regarding the Division's core competencies, focused program areas, and products/services provided to customers will be provided later in the Group Sections of this Overview and the Selected Technical Activity Reports.

SRMs as Tools for Providing Traceability for Chemical Measurements

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 1300 different types of SRMs; in 2000 NIST sold over 34,000 SRM units to more than 6,500 unique customers. Approximately 18,000 of the units sold were from the 850 different types of materials that are certified for chemical composition.

During the past year, fifteen high-priority SRM renewals were completed along with twelve completely new SRMs. Special attention was placed on addressing out-of-stock and completing

in-progress standards development projects. 97% (based on \$ value) of pre-FY99 in-progress projects (worth \$1.8M) were completed. The remaining 3% is attributable to two SRMs; one where technical problems are being addressed another where rebottled materials are not yet available for certification.

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, this document was referenced in Plenary Lectures at two European Reference Materials conferences this past spring. 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.

NTRM Programs for Leveraging NIST 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, 11 specialty gas companies have worked with NIST to certify over 8500 NTRM cylinders of gas mixtures that have been used to produce more than 500,000 NIST-traceable gas standards, with a market value of approximately \$110M, for end-users. 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 [SO₂ emissions trading] of the 1990 Clean Air Act." The NTRM model for the commercial production of reference materials is being extended to other mature and high volume areas to both more effectively deliver the increasing number of NIST-traceable standards to end users and allow more of our internal resources to be diverted to address new and/or more difficult measurement problems. Immediate plans are to provide NTRMs for optical filter and metal alloy standards. We had previously planned to also develop NTRMs in the Elemental Standards area. Those plans are being re-evaluated as explained in Technical Activity Report 21. NIST now owns the NTRM trademark and has applied for an NTRM certification mark for use by NIST-designated commercial standards producers.

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 21-25.

International 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 a framework for assessing the degree of equivalence between the measurement capabilities and standards issued by NMIs or NMI-designated labs throughout the world. 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 five working groups: (1) gas analysis, (2) organic analysis, (3) inorganic analysis, (4) pH and Conductivity, and (5) 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. NIST is leading various activities within all five working groups; participating in 13 Comparison Studies; leading 7. A draft report for a recently completed key comparison for cholesterol in human serum (CCQM-K6) is provided in the Technical Activities Reports section as Report #26. A similar report is required for each key comparison upon its completion.

Participation in CCQM 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 the 34 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 FY99, we developed and conducted 5 one-week basic level courses in Chemical Metrology (Organic Analytical, Inorganic Analytical, Nuclear Analytical, Classical, and Gas Metrology) for approximately 65 scientists and technicians within the Americas. At the beginning of FY00, we decided to link any further training to unique needs of the SIM countries. During the past year, six intercomparison exercises were developed to assess the proficiency of SIM NMI's and/or their designated collaborators for addressing chemical measurement problems within their regions and the Americas. While SIM is focusing 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.

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. Additional details concerning these activities can be found in Technical Activity Report 22

As part of our plan for supporting our MRA Appendix C "Calibration and Measurement Capability" claims for chemical measurements made within the Analytical Chemistry Division, we developed the format for and underwent an international peer review of our chemical measurement and standards programs. The Peer Review was conducted by a group of international metrology experts from October 25 to October 28, 1999. Robert Kaarls, Chairman of the Consultative Committee of the Amount of Substance (CCQM), which operates under the auspices of the International Treaty of the Meter, served as Chair of the peer Review Team. The remainder of the panel, all leaders of chemical metrology programs within their respective organizations, were: Steve Ellison LGC UK, Ed deLeer NMI the Netherlands, Helene Felber EMPA Switzerland, James McLaren NRC Canada, Martin Milton NPL UK, Yoshito Mitani CENAM Mexico, Wolfgang Richter PTB Germany, Hun Young So KRISS Korea. At our request, the team evaluated both our vertical traceability and horizontal comparability activities including, but not limited to, the following:

- Adequacy of Division Approach for Providing National Traceability and International Comparability of our Chemical Measurement Services
- Overall Assessment of NIST SRM Program for Support of Chemical Measurements (Technical and Business)
- NIST Approach for Value Assignment of its SRMs for Chemical Measurements
- Adequacy of Approach for Providing Evidence to Support Measurement Capability Claims
- Impressions Concerning Quality Systems Approach for NIST Chemical Measurement Services
- Quality and Focus of Chemical Measurement Research Activities in Spectrochemical Metrology, Nuclear Analytical Metrology, Organic Analytical Metrology, Gas Metrology, Classical Analytical Metrology, and Molecular Spectrometry.

The Panel concluded “the Analytical Chemistry Division of NIST - CSTL has competent staff, does excellent and well documented work and delivers excellent services to industry and society.” Moreover the contribution of the Analytical Chemistry Division to the development of a system of reliable and world-wide recognized measurements in chemistry is well appreciated and recognized around the world”. The Panel also stated, “by this peer-review exercise the panel also gathered experience in how future peer-reviews in the scope of the CIPM-Arrangement can be best conducted. With some refinements, this type of peer-review and the results obtained are well acceptable in underpinning best measurement capability claims as will be published in the Annex C of the CIPM-Arrangement.”

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. Details concerning this activity can be found later in the Gas Metrology and Classical Methods section of this overview and in Technical Activity Report 22. 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.

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 more than 25 projects with eleven federal and state government agencies. We also had technical interactions that led to laboratory research activities with more than 20 professional organizations and societies including the American Association for Clinical Chemistry, American Society for Testing and Materials, the College of American Pathologists, National Food Processors Association, the National Council on Clinical Chemistry, and the National Environmental Laboratory Accreditation Council. Details concerning four programs provided in Technical Report 23 give a flavor of the nature of these interactions. Additional illustrations are provided in the Groups Section of this overview.

Impact of Division Research and Services Programs

Formal Economic Impact studies on two Division standards programs were completed during the past year (Sulfur and Cholesterol) to assess the effectiveness of Division processes for project/program selection. The report “Economic Impact of Standard Reference Materials for Sulfur in Fossil Fuels,” was prepared for NIST by the Research Triangle Institute’s Center for Economics Research. This report confirmed the key role in the National Metrology System that SRMs have and for this particular group of SRMs, the large economic impact that results. The study quantifies a portion of the economic benefits, including improvements in product quality, production efficiency, and reductions in transaction costs for fossil fuels. Many companies and individuals benefit from the measurement improvements provided by NIST sulfur in fossil fuel SRMs, including: the sulfur measurement industry (instrument manufacturers, contract laboratories, secondary standards producers); fossil fuel extraction and processing industry (coal processors, petroleum refiners, coke producers); and primary users of fossil fuels

(electric utilities, steel industry, etc.). Of the economic measures assessed, this activity had the largest social rate of return (1,056 %) of any of the NIST activities studied to date. The \$400 million net present value (a time-normalized net benefit estimate) and benefit-cost ratio of 113 are also very positive statements about the impact that standards have. This very large economic impact results directly from the accuracy that the NIST primary method, isotope dilution thermal ionization mass spectrometry, provides. This method is uniquely applied at NIST and has been at the center of SRM certification activities for over 15 years.

Like the sulfur, the impact of our cholesterol standards worked is also directly linked to our development and use of a very specialized method. NIST developed its isotope dilution gas chromatography/mass spectrometry method for cholesterol in 1980 and has used this technology for value assignment of cholesterol in eight SRMs. The Economic Impacts of NIST's Cholesterol Standards Program was focused on four of the eight: SRMs 911, 909, 1951 and 1952 and considered only the economic impact resulting from the first 3 levels of supply chain were a social rate of return of 154%, a benefit-cost ratio of 4.5 and a net present value of \$3.5M. However, with cholesterol and other health status markers, the primary economic impact is associated with the costs of retests and consequences of inaccurate measurement of patient samples. The Government Accounting Office has stated that improvement in measurement accuracy for cholesterol over the past 20 years may save ~\$100M/year in unnecessary treatment costs.

In another program whose true impact cannot be measured in economic terms, our Gas Metrology Group has worked with the National Institute for Child Health and Development to facilitate the implementation of Inhaled Nitric Oxide (INO) Therapy which could help save the lives of 2,000 U.S. newborns annually. During the coming year, plans are to conduct formal impact studies for our Gas Mixtures NTRM and Optical Filters Standards Programs and further develop our analysis of the impact of INO activities. A study on the Division's Gas NTRM Program has been initiated and a study on the Division's Optical Filter Standards Program is in the planning stage.

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. We will also increase our effort to have formal studies undertaken for measuring the economic of selected Division programs.

During the coming year, we will redirect a significant portion of our efforts to expand our program in new health-status markers. Recent reports on medical errors highlight the need for quality, traceability and standards. Inaccuracy in health-related measurements raises overall health care costs, results in misdiagnoses, and leads to inaccurate conclusions in clinical studies. 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. By December 2003 all new IVD products that are placed on the market must be labeled with the CE mark.

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 highest order", e.g., nationally/internationally recognized certified reference materials (CRMs). At present, neither CRMs nor reference methods are available for most of the several hundred analytes that are measured in medical laboratories. These analytes fall into two classes: "A list" analytes that are well-defined chemical species of which there are approximately 80 and "B list" analytes that are less well-defined and number in the hundreds.

Excluding home diagnostics, the overall world wide in-vitro diagnostic market ("IVD") is approximately \$20 billion. The total IVD market in Europe was about \$5.6 billion in 1998.

Approximately 60% of the IVD products on the European market are imported from the USA. The U.S. IVD industry has requested that NIST provide them with commutable, internationally recognized CRMs as their basis for establishing "traceability to standards of the highest order". In response to this request, we have commenced a major effort to develop new reference methods and materials for the following new health-status markers:

- **Cardiac Troponin I** (*new definitive biomarker of heart attack not being effectively utilized due to measurement problems*)
- **Folates** (*important antioxidants; neural tube defects and blood homocysteine levels*)
- **Homocysteine** (*marker for heart attack risk*)
- **Glycated Hemoglobin** (*diabetes diagnostic marker*)
- **Ionized Calcium** (*important marker for diagnosis of various disease states such as skeletal resorption and stone formation in the urinary tract*)
- **Thyroid Stimulating Hormone** (*marker of thyroid function*)
- **Speciated Iron** (*marker for anemia and hemochromatosis; to address clinical need to measure iron associated with various iron-containing proteins*)
- **Bilirubin** (*marker for liver function*)
- **Total and Speciated Selenium** (*to support clinical monitoring in the relatively narrow range of beneficial effect*)

While we will focus increased attention to the health-care/clinical measurements area over the next 3-5 years, we will continue to address high-priority standards needs in other areas. The following standards are targeted for completion during the coming year:

- **Liquid Baking Chocolate** (*to complement suite of SRMs developed in response to new nutritional labeling laws*)
- **Frozen Spinach** (*to complement suite of SRMs developed in response to new nutritional labeling laws*)
- **Drinking/Wastewater Standards** (*to provide accuracy benchmark and measurement traceability for proficiency*)

testing of >5000 environmental laboratories conducted by commercial providers)

- **Isotopically-depleted Protein Mass Standards** (*with the Biotechnology Division; for use in calibrating mass scale in 1,5000-50,000 dalton range*)
- **NIR Transflectance** (*wavelength standards suitable in form for calibration of single- and double-pass transmission in both wavenumber and wavelength*)
- **Toxic Elements in Freeze Dried Urine** (*to provide QA for toxic metal exposure measurements*)

Projects that are in progress with expected completion dates in FY 2002 include:

- **Low Sulfur in Gasoline** (*to support proposed US regulation of sulfur content in gasoline for EPA Tier 2 Clean Air Act Amendments*)
- **Low Nitric Oxide Standards** (*to support measurements needed for development of very low emission vehicles and future automobile emissions regulations*)
- **Anion Solutions** (*nitrate, bromate, chlorate, chlorite, and cyanide*)
- **Gravimetric Elemental Primary Standards** (*"Mole standards for metals"*)
- **Very Volatile Organic Compounds in Methanol** (*to provide accuracy benchmark for determination of very volatile compounds in drinking water*)
- **Organics in Household Dust** (*for providing QA for measurement of toxic organic contaminants such as pesticides and PAHs in indoor air residues*)
- **Fluorescein Fluorescence Intensity** (*in collaboration with the Biotechnology Division for calibration of instrumentation widely used by clinical and biotechnology communities*)
- **Trans Fatty Acids in Foods** (*to support labeling requirements for trans fatty acids in foods*)
- **Anode Tin** (*to support lead and material purity measurements for this important steel coating material manufactured, used, and recycled worldwide; lead content of the recycled high-value tin is*)

of particular importance for food safety, product liability, and environmental concerns)

- **Gunpowder Composition** *(to validate forensic methods used to identify gunpowder residues)*

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 25 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.

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.

A major milestone for the Group was the completion of the report "Economic Impact of Standard Reference Materials for Sulfur in Fossil Fuels," as mentioned earlier in this Overview. The economic impacts result directly from the accuracy that the NIST primary method, isotope dilution thermal ionization mass spectrometry, provides. This method is uniquely applied at NIST and has been at the center of SRM certification activities for over 15 years. Last year we have reported on pushing the technique to measure sulfur at state-of-the-art levels in an aerospace alloy. In the coming year, we will be collaborating with Institute for Reference Measurements and Materials (IRMM), a European Community standards laboratory to certify S in several European reference materials. This activity supports the Division's goal to promote global comparability of chemical measurements through strategic collaborations with other National Measurement Institutes.

The X-Ray Fluorescence (XRF) team has added a number of accomplishments to its credit this year, in both the measurement and research areas. The collaborative effort with a guest researcher from the Naval Research Laboratory to study and compile XRF fundamental parameters has resulted in a completed database that is now available. In collaboration with Claisse Scientific, a new oxidation procedure has been developed for the matrix-independent analysis of metal alloys and has been tested on a range of metal types: Al, Pb/Sn, ferroalloys, copper alloys, and Galvalume, among others. A significant amount of SRM work was also completed. Homogeneity testing was done for about a dozen materials in preparation. Quantitative XRF measurements were included in the certification of Wear Metals

in Oil, Hard Rock Mine Waste, Air Particulate on Filters, and Zeolite SRMs. SRM 1848, Lubricant Additive Package, was completed this year. This SRM is the first certified reference material of this type and will benchmark accurate measurements of this important industrial commodity (the value of the U.S. finished lubricants market was \$2.85 billion in 1999). The ASTM Committee on Elemental Analysis of Petroleum Products awarded John Sieber a Certificate of Appreciation for his leadership of this project.

The Group made a major breakthrough in the accurate measurement of Hg this year with the development of a cold vapor isotope dilution inductively coupled plasma mass spectrometric method of measurement (CV-ID-ICP-MS). A key aspect of this development was the application of the Carius tube dissolution technique that is used intensively for sulfur IDMS determinations. In fact, the parallels to the sulfur program are strong. Emissions of both elements from coal combustion are of environmental concern, with EPA only recently singling out mercury emissions for monitoring/regulation. During isotope dilution analysis, equilibration is an important issue for both elements since they have volatile gas phases; closed Carius tube dissolution addresses this issue. In addition, both sulfur and mercury can be separated by a simple and efficient reduction step for subsequent isotope ratio measurement. As opposed to sulfur, the blank level for mercury measurement is sub-ng; thus, the ng/g levels of mercury in coal are readily determined. This technique (combined with improved RNAA measurements) allowed us to upgrade the Hg value assignment in SRM 1630a Trace Mercury in Coal from reference to certified and meet the accuracy needs of the electric power industry. The mercury content in a range of samples (7 SRM coals, 4 BCR and a SARM coal, coal fly ash SRM, hard rock mine waste SRM, oyster tissue SRM, and 12 fuel SRMs) was also surveyed using this technique.

The high performance (HP-) ICP-OES research and measurement effort continues to impact a wide range of activities in the Group. It is an established part of the Spectrometric Solution SRM program. In the past year, we have also combined it with high performance ICP-MS to demonstrate a calibration transfer mechanism

between SRMs, discussed below. On the international level, we have used it in the CCQM K8 Key Comparison of standard solutions (Al, Mg, Cu, Fe), completed in December. The NIST results compared favorably with classical methods applied by other NMIs (titrimetry, coulometry, gravimetry, and IDMS were the competitive techniques) in terms of both its precision and accuracy. These results again support our claim that the improved ICP-OES method can be placed in the context of "replacing classical analysis." It matches the precision and approaches the understanding of sources of error of classical analysis, especially for cases where the sample can be reduced to a simple dilute solution. This claim was submitted to the CIPM/CCQM during its Primary Methods Symposium. We will continue to promote its broader dissemination. We have established a CRADA with a commercial ICP-OES manufacturer and in the coming year will explore the software/hardware modifications necessary to make it more generally available. We also plan to develop and apply the HP-ICP-OES methodology to several challenging problems. For instance, we have asked to characterize the stoichiometry of aluminum gallium arsenide, an important semiconductor material. Absolute measurements are needed to benchmark the calibration-dependent, indirect measurement methods used by industry.

One of the challenges of the Division is the support and maintenance of existing Standard Reference Materials while identifying and certifying new materials. Resources are often insufficient for renewal of the SRMs that go out-of-stock, forcing difficult choices to be made—limiting data collected during a recertification, or even abandoning SRMs that may still benchmark important measurements. This year we have explored a new mode of certification that is applicable to recertification projects and is both efficient and practical. This new mode of certification relies on the direct transfer of value assignment between lots of a reference material through comparative measurements under carefully controlled conditions. When precise methods are used, the expansion of uncertainty in the value assignment transfer will be negligible. This method was applied to three coal SRMs for which sulfur is certified. It was also applied to the multielement recertification of two environmental SRMs, Buffalo River Sediment

and Trace Elements in Coal. The results of both examples were presented at the BERM-8 Conference in September 2000 and will be published.

In the first example, the three coal renewal materials (coded as "b") were directly compared to the corresponding previous generation "a" material by means of a high temperature combustion method with infrared detection. The certified S mass fraction for each "b" material was then assigned based on the known S mass fraction for the "a" material. All of the "a" materials had been certified previously using ID-TIMS. In the comparative measurement protocol, neither the "a" nor "b" material were identified, the analytical measurements were blind. The comparison of the S contents of the "a" and "b" materials via the comparative method effectively establishes a direct traceability link to the ID-TIMS measurements. Importantly, the propagation of uncertainty did not result in inflated expanded uncertainties. The experimental design provided more degrees of freedom than the ID-TIMS certification. Thus, in each case, the additional uncertainty components were almost totally offset by a smaller coverage factor.

The second example used both HP-ICP-OES and HP-ICP-MS methods cited above. The HP methodology is based on three interacting procedural tools: an internal standard that allows both short-term and long-term noise to be minimized, an experimental design that includes many measurements (15 hours), and drift correction using the method of Salit and Turk. In the Buffalo River Sediment example, ICP-MS measurements were made on 28 isotopes of 17 elements and ICP-OES measurements were made on 15 elements. For the coals, ICP-MS was used to measure 13 isotopes of 10 elements. The results were consistent among elements, and agreement with other techniques was excellent. However, this method does have its limitations. The accuracy of any result is tied to the accuracy of the original certification, and the stability of certified values versus time. In addition, any new developments in measurement capabilities that might have occurred since the older SRM was certified will not be taken advantage of. Nonetheless, this work clearly demonstrates that this comparative mode of measurement can be used for re-certifications, for establishing

traceability of secondary reference materials to higher level reference materials, and for showing equivalence of different materials.

One of the unique facilities that the Group now has is its chemical clean rooms. The rebuilding of the two clean room suites was completed this year and their operational characteristics assessed. The new clean rooms now possess state-of-the-art cleanliness and a far-improved laboratory environment (lighting and working space). Testing showed that the laboratory bench areas to be at least Class zero and this level is maintained during room use. The aisles and sink areas are better than Class 100. The certification program for SRM 2783 Air Particulate Matter on Filter was the first opportunity to put the new clean rooms to the test. Each unit of this SRM consists of an air filter with only 480 μg of particulate matter upon it. We have determined the total mass for a number of elements in the APM using ID-ICP-MS and these measurements were possible only because of the low chemical blanks. For instance, the chemical blanks for Pb, Ba, Cd, and U were all sub-nanogram, ranging from 410 pg for Pb to 1.1 pg for uranium. The amounts of Pb, Ba, Cd, and U on the filters were 330 ng, 333 ng, 3 ng, and 1.2 ng, respectively. The Cd proved to be inhomogeneous, but the U at this level showed only a 2.3 % relative standard deviation for 8 samples at this level. These measurements were made using the high resolution ICP-MS, operating in low-resolution mode with its remarkable sensitivity.

The results of the re-engineering of the single element spectrometric solution program became evident this year. All but 3 of the 69 standards were either in stock or nearly completed at the end of the year. The work for one of the three remaining standards (Th) is completed. The remaining two, Rh and Re, are stalled due to technical problems. 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, and will support the planned NTRM Program in this area.

The summary above shows that the full range of optical, x-ray, and mass spectrometric techniques available in the group were applied in the past year to the certification of inorganic constituents in reference material matrices. Our role is

expanding, however, including not only the development and certification of reference materials but also an increasing number of international activities and interactions. These interactions—comparative measurement programs, metrology workshops, studying uncertainty and traceability guidelines—have been with both the International Bureau of Weights and Measures (CIPM/CCQM) and with regional organizations (SIM, NORAMET). They promote the development of a global infrastructure for chemical measurement, an infrastructure where NIST must represent the highest U.S. interest.

Organic Analytical Methods

Research 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 organic mass spectrometry and analytical separations techniques. These separation techniques include gas chromatography (GC), liquid chromatography (LC), supercritical fluid chromatography (SFC) and extraction (SFE), capillary electrophoresis (CE), and capillary electrochromatography (CEC).

Recent research activities in organic mass spectrometry have focused on the development of techniques for characterization and quantitative determination of proteins in biological matrices. Levels of specific proteins in blood and serum are indicative of certain disease states. These so called Ahealth status markers≡ often permit more rapid diagnosis of disease with greater certainty, than is possible by other methods. Twelve health status markers have been identified as highest priority for immediate studies. This year efforts have been directed toward the development of primary and reference methods for troponin I (a new marker of myocardial infarction), glycated-hemoglobin (an important new marker for diabetes), serum thyroxin (a marker for thyroid function), speciated iron (elevated or low iron levels), and homocysteine (a risk factor for myocardial infarction). Because protein biomarkers occur naturally with considerable heterogeneity (i.e., with glycosylation, acetylation, phosphorylation) we have developed techniques for chemical characterization of the target species. For example, techniques have been developed for the characterization of

purified troponin I preparations, thus providing a means for comparing the molecular structure with results from field studies involving immunoassays. An important aspect of the health status marker program is commutability. To be useful to the clinical community, biomarker levels in reference materials certified by primary and/or reference methods must be commutable to field methods. To this end, an interlaboratory comparison exercise of candidate Troponin I reference materials was carried out in collaboration with the Troponin Subcommittee of the American Association of Clinical Chemists. Ten Troponin I materials were evaluated by 13 manufacturers of immunoassays. The most suitable troponin I preparation will be identified for issuance as an SRM.

Two new LC/MS instruments are providing new capabilities for the determination of analytes of health, nutritional, forensic, and environmental importance, as well as for structural studies of natural products. LC/MS methods have been developed for PAHs and nitro-PAHs, and measurements of various nitro-PAHs have been made in two environmental SRMs (i.e., SRM 1596 Dinitropyrene Isomers and 1-Nitropyrene in Methylene Chloride, and SRM 1597 Complex Mixture of Polycyclic Aromatic Hydrocarbons from Coal Tar) for stability assessment. Acquisition of LC/MS/MS instrumentation is anticipated for FY01, to further increase group capabilities for the determination of protein biomarkers. Our new high-resolution mass spectrometer is being used in the development of methods for complex environmental contaminant mixtures such as dioxins, furans, and toxaphene as a prelude to future certification measurements of these substances in natural matrix SRMs. GC/high resolution MS measurements of coplanar PCBs and dioxins have been made on SRM 1945 Organics in Whale Blubber, SRM 1944 New York New Jersey Waterway Sediment, SRM 1588a Organics in Cod Liver Oil, and SRM 2974 Organics in Freeze Dried Mussel Tissue. Ion-trap mass spectrometry is being combined with chemical ionization (CI) to improve the measurement of polyunsaturated fatty acids. Fatty acids such as these are found in fish oils and are believed to provide health benefits; however, their determination by mass spectrometry is hindered by their extensive fragmentation under electron ionization conditions, but with CI and acetonitrile as the

source of reagent ions, it is possible to obtain strong signals for characteristic ions from these compounds. Mass calibration of mass spectrometers used for high molecular weight species is difficult and few appropriate materials are available. Research is underway to develop a set of proteins produced by bacteria grown in a media depleted in ^{13}C and ^{18}O . Such proteins will have much simpler isotope clusters which will be useful for mass calibration in matrix assisted laser desorption ionization (MALDI) and electrospray mass spectrometry of biomolecules.

Research activities in separation science continue 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. 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. Method development is in progress for the determination of amphetamine and methamphetamine enantiomers in hair extracts. In other studies, functionalized cyclodextrins have been evaluated as chiral selectors in CE. The use of dimethyl, hydroxypropyl, and sulfated forms of β -cyclodextrin improve aqueous solubility and alter the chiral recognition properties of this compound. Finally, chiral GC methods have been developed for the determination of DDT and DDE in environmental samples where enantiomeric resolution required coupling of chiral and achiral columns.

Insights gained from our research in separation mechanisms have provided us the knowledge to develop two new LC column performance test mixture SRMs. SRM 870, Column Performance Test Mixture, will be used to characterize LC column properties such as silica silanol activity, trace metal activity, and separation efficiency. SRM 877, Chiral Selectivity Test Mixture for Liquid Chromatography, will be useful in predicting the performance of columns for resolving enantiomers. Production of these SRMs has been completed this year.

Our research in organometal speciation has continued with improvements in the GC-atomic emission-based method for methylmercury and alkyl tin species. The new approach involves derivatization and a solid phase microextraction (SPME) step to concentrate the analytes and 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 tributyl tin in several of the marine matrix SRMs (i.e., SRM 1941a Organics in Marine Sediment, SRM 1944 New York/New Jersey Waterway Sediment, and SRM 1646 Estuarine Sediment). Other research has involved development of multidimensional chromatographic approaches for quantification of non-ortho polychlorinated biphenyl congeners (considered to be the most toxic congeners) in some of our existing cod liver oil, sediment, and whale blubber SRMs, and nitro-substituted polycyclic aromatic hydrocarbons in diesel-related SRMs.

In FY 00, measurements have been completed on a number of SRMs of importance to the clinical, environmental, and food and nutritional communities:

Clinical Reference Materials

SRM 968c Fat Soluble Vitamins Carotenoids and Cholesterol in Human Serum, is the fourth issue of a serum based SRM intended for use in validating methods for the determination of fat-soluble vitamins, carotenoids and cholesterol. This material has certified values for 7 fat-soluble vitamins and cholesterol, reference values for 9 additional vitamins, and information values for 7 additional constituents.

Environmental Reference Materials

SRM 1589a PCBs Pesticides and Dioxins/Furans in Human Serum, has PCBs at naturally occurring levels in contrast to its predecessor where high levels of Aroclor mixtures were spiked into serum. This replacement material has been analyzed at NIST and the Centers for Disease Control to provide certified and reference values for PCBs, chlorinated pesticides, and PCDDs/PCDFs.

Three diesel particulate materials were completed this year. SRM 1975 Diesel Particulate Extract is a complex solution prepared from an extraction of the particulate material also available as SRM 2975 Diesel Particulate Matter (Industrial Forklift). These materials were developed at the request of EPA to provide reference materials representative of emissions from modern diesel engines. Certified values are provided for 8 PAHs, and reference values are provided for 23 additional PAHs and 18 nitro-PAHs. In addition, mutagenicity data is provided for SRM 1975. A complementary material based on an alternate source of diesel particles has been reissued as SRM 1650a Diesel Particulate Matter.

Two mussel tissue materials were completed this year. SRM 2977 Mussel Tissue Organic Contaminants and Trace Elements, and SRM 2978 Mussel Tissue Organic Contaminants Raritan Bay New Jersey were developed in response to the recommendations of the Group of Experts on Standards and Reference Materials (GESREM). The collection, preparation, and value assignment of these materials resulted from a collaboration between NIST and National Research Council of Canada (NRCC). Certified values are provided for 14 PAHs, 24 PCBs, 7 chlorinated pesticides, 6 elements, and methylmercury. Reference values are provided for an additional 16 PAHs and 9 elements.

Work continues to progress on calibration solution SRMs to support the recently externalized Water Performance Evaluation (PE) Studies Program orchestrated by the United States Environmental Protection Agency (USEPA) in conjunction with NIST. Twenty-six candidate solution SRMs have been prepared and certification measurements have been completed on all but 9 solutions. These SRMs are intended to support the PE program; however, it is

anticipated that widespread usage will result after issuance. Several additional calibration solution SRMs have been completed this year, including SRM 2269 Perdeuterated PAH-I Solution in Hexane/Toluene, SRM 2273 DDTs and Metabolites in Iso-octane, SRM 2275 Chlorinated Pesticide Solution-II in Iso-octane, SRM 2276 Three Planar PCB Congeners in Iso-octane, and SRM 2274 PCB Congener Solution-II in Iso-octane.

Food-Related Reference Materials

Several food matrix reference materials were updated with reference concentration values for proximates, total dietary fiber, selected fatty acids (as triglycerides), and calories. These materials are a joint material of Agriculture Canada and NIST, and are distributed by NIST. Materials updated include RM 8436 Durum Wheat Flour, RM 8433 Corn Bran, RM 8432 Corn Starch, and RM 8418 Wheat Gluten. SRM 1846 Infant formula was also issued this year. This material is intended for validation of methodology for determining vitamins, minerals, and trace elements in infant formula and similar matrices. Certified values are provided for 7 vitamins, and reference values are provided for proximates and calories.

Other Standard Reference Materials

Two new standards have been completed this year for the characterization of liquid chromatographic column performance. SRM 870 Column Performance Test Mixture for Liquid Chromatography is intended to complement SRM 869a Column Selectivity Test Mixture. SRM 870 can be used to characterize silanol activity, trace metal activity, retentiveness, and column efficiency (SRM 869a, which was initially issued in 1990, can be used to evaluate column selectivity). Also completed this year is SRM 877 Chiral Selectivity Test Mixture for Liquid Chromatography. This SRM consists of five separate solutions, each containing a mixture of single component enantiomers. Both materials are useful in method development, as control materials to assess column performance over time, and to aid quality control of column manufacturing. In addition, two oil matrix reference materials have been issued with reference values for moisture: RM 8507

Moisture in Mineral Oil, and RM 8506 Moisture in Transformer Oil.

The research and standards development activities within this Group have resulted in 53 manuscripts either submitted, in press, or published in refereed journals during the past year. Seven papers were published or accepted by the journal *Analytical Chemistry*, one of which was featured in the "A" pages, which are authoritative, high visibility reviews of specific topics written for a broad audience.

Gas Metrology and Classical Methods

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

During FY00 the following SRMs were completed; 12 gas cylinder, 2 anion solution, 8 conductivity, 1 ion activity, 9 Volatile Organic Compound solutions and a zeolite material. A total of 99 gas cylinder SRMs were recertified for 15 companies. While these certified reference materials are critically needed, their continuous production consumes considerable fiscal and human resources and limits our ability to address new standards needs. The gas NIST Traceable Reference Materials (NTRM) Program was created to address this concern. In FY00, we worked with 6 specialty gas vendors to certify 63 NTRM batches. The over 1000 individual gas cylinders in these certified batches will be used to produce approximately 100,000 NIST-traceable gas standards for end-users. Over the next year we will be defining a new approach for gas NTRM certification that will allow Specialty Gas Companies to expand their production while still maintaining the strong linkage to National Standards maintained at NIST.

In the area of electrolytic conductivity, we continued our research into new packaging technology. The problem we face is that the packaging used in the past was not suitable for storage of conductivity solutions for more than one year. This has caused us problems in that each solution must be remade every year and uncertainties could not be lowered below 1 %. During the past year we decided to package all

conductivity solutions in 50 mL ampoules. In sealed ampoules the transpiration problem, which has plagued us in the past, is solved and the solutions have multiyear self-lives. This should help us in the electrolytic conductivity program by reducing the SRM reissue rate so we can devote more time to low conductivity research, which is desperately needed to address needs in industry driven by regulations. Collaboration with the Netherlands (NMI) and the England (NPL) will help in this research. We have also benchmarked and compared our capabilities in the area of electrolytic conductivity measurements with the National Metrology Institutes for Denmark and Hungary. The data from this benchmarking activity will appear in the journal of the BIPM, *Metrologia*, in the very near future.

In the area of pH we continue to be very active in the international arena. In FY00 we participated in a pH Key Comparison (CCQM-K9) and directed a Pilot Study (CCQM-P19) on the assay of hydrochloric acid, which is required for accurate primary pH measurements. We also completed a SIM pilot study on pH (SIM QM-P4) with 16 participating laboratories in South America, Mexico and the Caribbean. We also continue to be active in IUPAC commission V.5, completing revisions to the pH document that will define the traceability of pH to the Bates-Guggenheim convention, thus assuring continued traceability of pH to sound thermodynamic principals.

Other activities in the classical methods program include research to produce 6 new anion solution SRMs for the EPA PE program. We also had a very interesting interaction with the fertilizer industry. The EPA came out with a paper critical of the fertilizer industry and NIST fertilizer SRMs for including excessive amounts of perchlorate. We went to great lengths to investigate these charges and found that the EPA was in error in that the SRMs did not include perchlorate. This put into question the EPA procedures and demonstrated that their analytical method may be giving artificially high results. This interaction saved the fertilizer industry from expending resources into defending itself against bad data.

In collaboration with EPA and the remote sensing community, we have developed a quantitative

database of infrared spectra, which is required for establishing FTIR-based technology as a reliable tool for real-time monitoring for airborne chemical contaminants along plant boundaries and within plant facilities. Because the spectra are being prepared using NIST primary gas standards, well-defined traceability to NIST can be established for any subsequent field measurements. These spectra will be required to be used in the new update of EPA method TO-16. At the present time we have released 30 compounds and are evaluating data for 10 more. We are working in collaboration with PNNL on an error affecting the higher wavenumbers, which deals with a fundamental problem with commercial instruments. This will result in an interesting paper that will detail needed changes in commercial instruments to resolve this systematic bias.

We continue to be very busy in the Ozone Program with upgrading the EPA Standard Reference Photometer (SRP) network with new electronics. This upgrade will continue into the new year, and will bring most of the SRPs in the world up to the newest version. Many more countries have expressed interest in receiving an SRP to provide traceability in ozone measurements. This presents a problem to NIST, in that we expend many resources to provide traceability to most of the world. It would be in NIST's interest to transfer this responsibility to another organization so we could shift those resources to areas of concern to U.S. industry. In the past year we have concluded talks that will transfer the responsibility for world traceability for ozone to the BIPM in Paris, France. Over the next 5 years NIST will be working with BIPM to build an SRP to act as a traveling standards, and a Primary Reference Photometer (PRP) to act as the stable international standard for ozone. Two PRPs will be constructed, one at NIST to serve as a backup and to provide primary traceability to the EPA network, and the PRP at BIPM to act as the primary international standard. Work will begin in FY01 on construction of the SRP, and in design of the PRP.

We continue to promote U.S. industry through development of high priority standards, and through standards organizations such as ASTM. Over the past two years we have developed the capability to produce low concentration nitric oxide gas standards. These standards are need 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. This research will result in two new nitric oxide gas SRMs in the coming years, one at 0.5 $\mu\text{mol/mol}$ and one at 1.0 $\mu\text{mol/mol}$. We also are continuing to assist the health industry with the task of safely implementing the inhaled nitric oxide therapy for newborns. Over the past year we have held another workshop to test clinical instruments, which measure nitric oxide and nitrogen dioxide. We have also assisted ASTM and industry draft a standard procedure to test these clinical instruments effectively before introduction into the hospitals and in continued operation. The European Union has taken the rare step of adopting this standard procedure, as the U.S. was very much ahead of Europe in this activity.

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; 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 FY00, 144 sets of solid absorbance filter SRMs were certified, and 203 optical filter sets were recertified. In addition, 49 units of new SRM 1935 (Potassium Dichromate Solution for Use as an Ultraviolet Absorbance Standard) were certified. Pre-certification measurements were

conducted on a reflectance version of SRM 2035 that will be designated SRM 2036. Development continued on SRM 2037, a standard for calibrating the amounts of the red dye used to mark off-road diesel fuels for tax purposes. Purification of the Solvent Red 26 dye originally proposed as the standard proved impossible on a preparatory scale, and therefore, the material for the standard was shifted to Solvent Red 24, which is commercially obtainable in high purity. After further purification, the material will be certified for purity and molar absorbance at two wavelengths in representative solvents. The new high accuracy reference uv-vis spectrophotometer (HAS-II) was validated and certification measurements were shifted to it. The original HAS-I instrument was moved to the ACSL and is being renovated for research use. An intercomparison of uv-vis absorbance filters (both gray glass on metal-on-fused-silica filters) was conducted between NIST-PL and NIST-CSTL. All of the measurement results were within the uncertainty limits over the ranges that we support; we plan to make this an annual event. A NORAMET/SIM interlaboratory wavelength intercomparison study using holmium oxide in perchloric acid solutions was begun. At this point, the NORAMET portion of the study is complete, and the SIM portion is about to begin.

In conjunction with coworkers in the Process Measurements and Surface and Microanalysis Science Divisions, we have identified three glasses (a chromium glass for 785 nm, a uranium glass for 515 nm, and an europium glass for 1064 nm) as promising candidate source intensity correction standards for Raman systems with 785-nm diode, argon-ion, or neodymium-YAG lasers. These glasses provide fluorescence spectra that, when calibrated against a standard white light integrating sphere source (calibrated by the NIST Optical Technology Division), can be used to correct Raman spectra for laser color and intensity variations. This in turn will allow Raman spectra to be made essentially instrument independent and will facilitate the development of Raman spectra libraries. At present, the chromium glass has been characterized sufficiently well to permit its being sent out to ASTM Raman subcommittee members and instrument manufacturers for evaluation.

In collaboration with the Biotechnology Division, we are developing a fluorescein solution standard

(SRM 1932) that will be certified for purity and for concentration. The material is to be used to peg the MESF (moles of equivalent soluble fluorophore) scale used in flow cytometry. We have acquired a state-of-the-art fluorometer (SPEX JY Fluorolog 3) and are currently validating its performance.

The NIST Special Publication (SP 260-140) and a supplement to NVLAP Handbook 150-21 that define the NIST Traceable Reference Material (NTRM) program for visible, gray-glass optical filters were published and sent to interested parties with an invitation to enroll in the initial class of NTRM producers. Three companies, Andover Corporation, Starna/Optiglass, and Unicam submitted documentation to initiate the NVLAP certification process necessary to become NTRM vendors for optical filters. Andover was assessed in August, and the laboratory assessments for Starna/Optiglass and Unicam are scheduled. All three companies hope to have their NTRM gray glass filters ready for sale by PittCon 2001. We have been developing automated schemes to "control chart" the certification and calibration data that will be submitted by the NTRM vendors as part of our quality assurance oversight of the NTRM program.

We have initiated a project through the Office of Law Enforcement Standards (OLES) at NIST to advance and support the concept of quantitative measurements for forensic analysis. Specifically, we have been developing a quantitative extraction and analysis method for the recovery of gunpowder additives based on ultrasonic liquid extraction and micellar capillary electrophoresis. In FY99, the technique was used to compare quantitatively the chemical composition of gunpowder residues to unfired gunpowder for identification. Last year we embarked on effort to develop a standard reference material "Additives in Smokeless Gunpowder" (SRM 1928) that will help law enforcement agencies such as ATF and FBI to validate measurement methods for the composition of gunpowder and residue samples that are submitted for forensic identification. We carried out an interlaboratory comparison exercise with international, national, and state forensic laboratories and with US military laboratories to access the state-of-the-practice in identifying and quantitating gunpowder additives

and stabilizers. Round robin participants included: NSWC Indian Head MD, Geo-Centers/ARDEC Picatinny Arsenal NJ, US Army Aviation and Missile Command, Washington State Patrol Crime Laboratory, South Carolina Law Enforcement Division, Israel Police Division of Identification and Forensic Science, Forensic Science Agency of Northern Ireland, Forensic Explosives Laboratory, DERA Fort Halstead Centre of Forensic Sciences (UK), San Diego PD, US Army Crime Laboratory, NYC Police Dept Lab, ATF, Ohio University, Virginia Division of Forensic Science, US Army. With sponsorship from OLES, we have created a graduate fellowship in quantitative forensic studies. Our initial "fellow" was recently hired by the U.S. Bureau of Alcohol, Tobacco, and Firearms as a forensic analyst.

We have partnered with EEEL on an ATP project for integration of silicon active components into plastic microfluid chips to design and build silicon chips with microheating elements combined with channel interconnects in silicon, to devise methods for linking silicon chips to plastic microfluid devices, and to test heater operation in fluids for coupling to chemical reactions. We acquired a uv-laser micromachining system and are studying its use in the fabrication of microfluidic channels and devices. In addition to its microfabrication uses, we are examining the physical and chemical characteristics of the micromachined surfaces produced with it. We are particularly interested in the characterization of the surface charges in the machine channels, since these determine the flow characteristics in electroosmotic and electrophoretic flows that are commonly used to move material through the channels. In conjunction with colleagues in Division 836, we have developed a technique for coating the microchannel walls with polyelectrolytes that produces very stable, predictable, and reproducible coatings. This allows us to tailor the flow characteristics of the channels by altering the particular polyelectrolyte coating used.

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 they depend upon characteristics of the nucleus of the element rather than the electron shells, and therefore are insensitive to the chemical state. In addition, the nuclear analytical methods are generally nondestructive and do not require sample dissolution, thus providing an independent assay.

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 it to address new measurement needs. 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 minimal 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, currently under certification for "normal" sample sizes (SRM 2102) was selected for further processing as an SRM for microanalytical techniques.

Evaluation of the Ingamells model describing homogeneity has been initiated with the Statistical Engineering Division.

Instrumental neutron activation analysis has been used as a primary method for certification of the arsenic implanted dose in SRM 2134, Arsenic Implant in Silicon. This material has been a high-priority need of the semiconductor industry for a number of years. It was 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. The INAA results used for certification include 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.

Significant advances have been made this year in the use of state-of-the-art signal processing techniques in our gamma-ray spectrometry systems that are used for INAA. Losses through pulse processing dead time and pile-up are best assayed with an external pulse technique. In this work, the virtual pulse generator technique as implemented commercially with the Westphal loss free counting (LFC) module has been set up and tested with four high-resolution gamma-ray spectrometers. Dual source calibration and decaying source techniques were used in the evaluation of the accuracy of the correction technique. Results demonstrated the reliability of the LFC with a standardized conventional pulse processing system. The accurate correction during high rate counting, including during rapid decay of short-lived activities, has become the basis for highly precise determinations in reference materials studies.

A radiochemical separation procedure has been developed to determine phosphorus in metals using RNAA with beta counting. The method has been used to value assign phosphorus in SRMs 861 (Aircraft Superalloy) and 2175 (Refractory Alloy), both containing phosphorus at lower mg/kg levels. The method is currently being adapted to determine phosphorus in ion-implanted silicon, with the goal of producing a phosphorus-implanted silicon SRM for the semiconductor industry.

Pioneering research is conducted by this 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.

We are currently developing and critically evaluating a second nondestructive technique for the quantitative determination of hydrogen based upon neutron incoherent scattering. This will serve as a complement to PGAA measurements for this element. The incoherent scattering cross section of neutrons by the H nucleus is much greater than that of any other nuclei is much greater (by about two orders of magnitude) than the absorption cross section. These two properties accentuate the potential of NIS for rapid H determination. In addition, NIS has very limited overlap of error sources with PGAA, and agreement of results between the two techniques provides a high degree of confidence.

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. Homogeneity is checked by neutron incoherent scattering, and concentration is verified by cold-neutron prompt-gamma activation analysis and gravimetry. The first SRM material has been prepared near the critical level of 100 mg/kg. In addition, new materials will be prepared at two bracketing levels. The apparatus has also been used to prepare standards for neutron-tomographic nondestructive analysis of turbine blades at McClellan Air Force Base.

We have begun measurements of nitrogen in chemical vapor deposited diamond films by NDP. Several problem areas have been identified such as the elevated background from gamma ray

induced electrons. While the initial results are encouraging, more effort needs to be made to improve the signal-to-background levels. These improvements will be useful in future measurements of SiON, which is of importance to the next generation of semiconductors. We are continuing our measurements of lithium migration in thin films, as applied both to electrochromic multilayers and to lithium batteries. NDP is well suited to these studies as lithium mobility can be observed in real time.

The cold neutron PGAA and NDP spectrometers, which are available to outside researchers as part of the NIST Center for Neutron Research national users' facility, continue to provide high-quality measurements for outside scientists. Current experiments of interest at the NDP instrument include the measurement of shallow-doped boron content in silicon in conjunction with Advanced Micro Devices, calibration of in-house boron standards for Lucent Technologies, and the characterization of boron and nitrogen thin films in a wide variety of materials for high tech applications.

Nondestructive, matrix-independent measurements of hydrogen by the cold neutron PGAA technique have become particularly valuable in view of the difficulties of accurate determination of this element by other techniques. Many of our current PGAA collaborations involve determining hydrogen in a wide variety of materials for different applications. These include metal embrittlement studies, studies of materials for new types of lithium batteries and fuel cells, and the determination of H concentrations for materials prior to neutron scattering measurements. PGAA has also been used together with NIS to map the hydrogen content of wet concrete samples as a function of position in an effort to create a moisture profile of the material and hence monitor the uniformity of drying. Other measurements made at the PGAA facility this year include the determination of H, S, Ca, and K in Nafions, derivatives of Teflon which have potential use as membranes in electrochemical separations and in fuel cells; the characterization of antiferromagnetic materials which are of interest to the superconductor industry; and the analysis of a Martian meteorite, to serve as a prototype spectrum in the design of a PGAA instrument for analysis of the Martian surface.

PGAA has also been used with focused neutrons in the determination of chromium in a chromite layer deposited on steel casings that are used in Kraft recovery boilers by the pulp and paper industries for process chemical recovery.

A novel approach is under investigation to alleviate some persistent problems in PGAA. Detection sensitivities of this technique are often restricted by the following factors: poor signal to noise ratios, interferences from background signals, and, in some cases, overlapping energy lines from different origins, namely ultra-short lived decay lines interfering with prompt decay. Discrimination between prompt and delayed emissions from a sample source, as well as against background events, can be achieved by examining the timing between the gamma-ray acquisition with the actual capture events using a pulsed beam of cold neutrons. Coincidence gating is used to select the prompt gamma-ray emissions. Signals from background capture gamma rays are suppressed because of different flight times of neutrons to the sources of background radiation, providing a reduction in direct gamma-ray interferences. Anti-coincidence gating allows measurement of only decay radiation that originates from short-lived activated states of the nuclides after capture. Spectra of decaying nuclides are free of interfering prompt activities, and also have lower continuum background from Compton scattering of high-energy prompt gamma rays in the detector. The measurements provide the opportunity to use ultra-short half-life nuclides for analytical purposes; no sample decay occurs during transfer times, and repetitive activation and counting cycles are achieved with the use of pulsed neutron beams.

An upgrade of the thermal neutron PGAA facility is now in progress. The newly designed facility will provide better detection limits, lower gamma-ray background, and reduce or eliminate background count-rates for H, B, C, N and Fe. The first phase of the redesign involved inserting a sapphire filter in the shutter assembly to reduce the number of fast neutrons and low energy gamma rays in the beam. The filter de-created the fast neutron component of the beam by a factor of five and the thermal neutrons by 15%. This filter also reduced the low energy gamma-ray background, greatly improving the peak-to-background ratios for gamma-rays of energies <500 keV. The second phase of the upgrade

involves replacement of all external components of the PGAA facility. The design has been completed in collaboration with the Reactor Radiation Division, and construction of the new components is underway.

Efforts continue within the group to develop the techniques and methodology of focusing cold neutron beams for analytical applications of PGAA and NDP. A monolithic lens consisting of a fused tapered bundle of polycapillaries that provides a smaller focus of about 0.15 mm (FWHM at the focal spot) has been installed in the NDP chamber. The chamber has also been modified to accept a separate lid through which the lens can be mounted for remote-controlled alignment. A new alignment system containing a CID camera with vacuum capability has also been installed. It provides a spatial resolution of 12 μm (pixel size) and an 8 bit dynamic range for the neutron intensity, and provides the possibility for charged particle imaging. Together with the new vacuum-compatible motion stages, the alignment system permits measurements without breaking the vacuum. Furthermore, the lens can be retracted out of the beam for measurements on the same sample with normal NDP geometry. The new lens and focusing system now gives us the capability of measuring samples as small as 0.1 mm in diameter or, together with our current scanning system, of measuring concentrations in three dimensions with much improved spatial resolution. Previously, our three dimensional profiles were performed with beam spot sizes of 1 cm.

More recently, a prototype monolithic capillary lens for focusing neutrons produced by thermally drawing straight multicapillary bundles has been characterized with cold neutrons. This lens gives an intensity gain of a factor of 25 over the focal spot area (width of 87 μm at focal distance of 8 mm). This reduced spot size is an order of magnitude smaller in area than those for multifiber capillary lens. The spatial resolution available with the lens has been tested with prompt gamma measurements on slivers of dysprosium. In addition, the boron glass of the tapered monolithic lens provides better shielding from unfocused neutrons near the lens focus.

A "neutron thermometer" technique has been employed to characterize neutron beam spectra at various instruments. The idea is to measure the

prompt gamma signals from a "thin" (negligible absorption) and a "thick" (total absorption) sample, and to deduce the effective absorption cross section with the average neutron wavelength. This information is very useful in "white beams" such as those used for NDP and PGAA, as well as when additional components are introduced in the beam (such a filter or a neutron lens), which alters the neutron beam spectrum.

Analytical Chemistry Division Selected Technical Reports

1. The Microanalytical Laboratory

L. Locascio, E. Waddell, T. Johnson, G. Kramer, M. Gaitan (EEEL), D. Ross, S. Barker (836), M. Tarlov (836)

Objective: To promote the development of plastic microfluidic devices by (1) developing methods for characterization of plastic microchannels fabricated by imprinting or laser ablation; (2) modulating surfaces to influence microflow; (3) integrating active components into microfluidic systems.

Problem: Interest in the development of microfluidic lab-on-a-chip devices continues to thrive with the introduction two new commercial microfluidic devices this year from Hitachi and Shimadzu. Both IBM and Intel also announced recently that they will compete in the DNA chip arena, and it is likely that their efforts will involve microfluidics technologies. The latest commercial devices are microchannel arrays fabricated in glass with no integrated active components. These devices are essentially treated as disposables and are sold with associated commercial instrumentation. Because they are disposable reagents, there is a tremendous industrial incentive to lower the cost by fabricating devices in plastics. Our group has pioneered microfabrication methods for the development of microfluidic devices in several commercially available plastic materials. We are very active in the development and characterization of plastic microfluidic devices with particular interest in bioanalytical application of these devices. In the second year of our competence effort, we have continued to

focus on the understanding and characterization of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods (see also Microfluidic Measurement Technology for a discussion of other aspects of our program, Division 836). We have also recently acquired new instrumentation that allows us to fabricate plastic devices by laser ablation. The critical evaluation of plastic microfluidic devices will provide the knowledge-base to promote the development of plastic lab-on-a-chip devices for industrial application.

Approach: The surface charge and charge density on microchannel walls are critical in microfluidics since both the rate and direction of electroosmotic flow are a function of wall charge. Wall charge also controls the adsorption of chemical and biochemical species. We have developed methods to probe the surface charge and surface charge density in plastic devices using fluorescent labeling and microscopy to identify the presence of active charged moieties. We have determined that the process used to fabricate plastic microfluidic devices, as well as the plastic material itself, can have a profound influence on the surface charge on the microchannel walls. We have implemented flow-imaging techniques to evaluate the effect of charge distribution on sample dispersion.

Results and Future Plans: Figure 1 shows the density and distribution of surface charge in plastic microchannels fabricated by three different techniques. It can be seen that the surface charge varies significantly with the fabrication method with the lowest surface charge associated with the hot-imprinted channel and the highest associated with the laser ablated microchannel. We have determined that the

surface charge density in ablated channels can also be altered by changing conditions such as process gas and the ablation power. The fabrication method also has a great effect on surface morphology of the microchannel as shown in Figure 2. By understanding the influence of our fabrication techniques on the surface of the plastic microchannels, we can begin to develop microfluidic systems specifically designed to meet the needs of our application. The idea of tailoring the microchannel surface by varying the fabrication method is pioneered by our group and is very

unique to plastic microfluidic devices indicating that there are advantages other than cost to utilizing plastics as the preferred material.

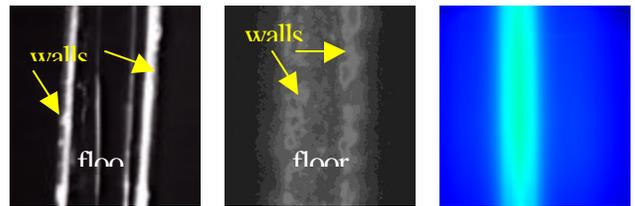


Figure 1. Fluorescence image showing localization of channel charge (bright spots on images). Channels approximately 50 μm wide. (A) Room temperature imprinted; (B) Hot imprinted; (C) Laser ablated channel.

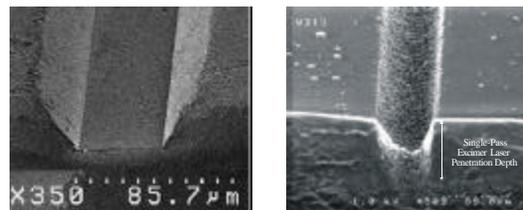


Figure 2. SEM images of (A) hot imprinted channels; (B) laser ablated channels.

2. Validation of a New Fully Automated National Reference Instrument for Optical Filter Certification

J.C. Travis, M.V. Smith, D.F.R. Mildner, G.W. Kramer, and M.J. Burns (Hypertek)

After nearly thirty years of service, the national reference spectrophotometer maintained by the NIST Analytical Chemistry Division was decommissioned and moved to a new building on December 17, 1999. The instrument had been used to certify and re-certify some 3000 sets of Standard Reference Material (SRM) neutral density filters for verifying the accuracy of chemical spectrophotometers. Certification measurements were resumed on January 3, 2000 using a new instrument based on the same operational principles as the original, but with modernized hardware and software. The use of a high-speed linear motor with micrometer positional reproducibility has increased the sample capacity from seven to twenty-six, providing simultaneous higher throughput and the capacity for control filters at all levels.

The new instrument was put through months of qualification testing before being commissioned, and comparative measurements with other

instruments are ongoing. As with the prior generation instrument, the principle of light additivity has been implemented with an automated double aperture device coupled to a variable attenuator to establish the transmittance accuracy of the instrument. Provision has been made to direct-couple atomic lamps to the input slit of the prism pre-disperser, and a set of five lamps has been employed to provide wavelength calibration to within 0.1 nm. Wavelength and transmittance corrections are both accommodated in software and are updated at least quarterly.

Improved signal levels resulting from modern integrating sphere materials, boosted lamp levels, and automated dark current correction have permitted the extension of the single step dynamic range of the new instrument to two decades of transmittance, compared to the single decade for the prior version. Similarly, the improved UV sensitivity along with lamp selection automation has permitted a simplified protocol for certifying materials with measured values in both the UV and visible. The improved throughput from these characteristics, as well as the increase in sample capacity, is critical to the expanded use of the instrument to support the new NIST Traceable Reference Material (NTRM) collaboration with commercial suppliers of NTRM filters.

3. *Critical Evaluation of Instrumental Neutron Activation Analysis as a Primary Method*

R.R. Greenberg and R. M. Lindstrom

Objective: To evaluate the potential of INAA as a primary method of measurement as defined by the CCQM.

Problem: NIST certifies elemental concentrations in SRMs through either use of a single "primary method" with confirmation, or through the use of two independent, critically-evaluated analytical methods. There are often cases when two totally independent methods are not available and so a primary method is required. Because the only currently available primary method for trace element analysis in complex matrices, isotope dilution mass spectrometry (IDMS), cannot be used for

monoisotopic elements, another primary method that can be used to determine such elements is desirable. IDMS also requires chemical dissolution/decomposition of solid samples. There are a variety of matrices for which complete sample decomposition is both difficult to achieve as well as to demonstrate. Therefore a primary method which is non-destructive, such as INAA would be valuable. In addition, there has been a recent increased importance of international comparisons for national traceability and international comparability. Such intercomparisons typically involve primary methods.

Approach: The CCQM has described a primary method as "... a method having the highest metrological properties, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units. A primary direct method: measures the value of an unknown without reference to a standard of the same quantity. A primary ratio method: measures the value of a ratio of an unknown to a standard of the same quantity; its operation must be completely described by a measurement equation." Although instrumental neutron activation analysis (INAA) has the potential to meet all aspects of this definition of a primary ratio technique, a complete uncertainty budget containing all individual uncertainty sources has been difficult to achieve. We have therefore decided to completely evaluate all sources of error for an important INAA determination (Ion-Implanted Arsenic in Silicon, SRM 2134), and to evaluate how well the methods developed for such an evaluation could be transferred to other INAA measurements.

Results and Future Plans: Approximately 30 individual uncertainty sources were identified for INAA determinations; the exact number depends upon the specific procedures used. Most of these uncertainty sources are matrix and analyte independent, and result from parameters such as assay of materials for use as standards, sample size and shape, position during irradiation and counting, as well as factors related to determining the various times needed to correct for radioactive decay. More or less standard methods were used for evaluating such uncertainties. Other uncertainty sources are related to the nuclear properties of the matrix under investigation such as matrix transparency

to neutrons and gamma rays, and the amount and energies of gamma radiation emitted by matrix constituents. The dependence of these uncertainties on the nuclear properties of the matrix must be stressed. Although the chemical properties of Cd and Zn are very similar, their nuclear properties are entirely different. Accurate determination of trace constituents in Zn metal can be made, but such measurements would be impossible in Cd metal due to this element's extremely high cross section (probability) of absorbing neutrons. For most elements, the magnitude of matrix related effects is usually very small in samples of a few hundred milligrams, and so any uncertainties due to corrections for these effects are typically insignificant. However, when such corrections become large enough their associated uncertainties may limit measurement accuracy.

Other uncertainty sources depend upon the specific analyte such as half life, natural isotopic variability, actual concentration, counting statistics (also depends upon matrix), gamma-ray energy, and potential interferences. Although the effects of these factors may be difficult to predict prior to analysis, they can readily be evaluated afterwards.

INAA can meet all the requirements of the CCQM definition of a primary method. Whether a specific analysis meets these requirements will depend on the experimental design, the level of effort expended (i.e. have all uncertainty sources been evaluated), and the magnitude of the actual measurement uncertainty of the analysis in question. While the measurement uncertainty may be difficult to predict in some cases prior to measurement without knowing the actual composition of the material to be analyzed, it will be clear after the measurement and uncertainty evaluation are completed whether the measurement is "of the highest metrological properties".

4. *High Performance ICP-OES: A Primary Method for Elemental Composition*

M. Salit and G.C. Turk

Objective: To develop a commercially-viable

primary method for elemental analysis, permitting traceable measurements with small uncertainty in the wider chemical analysis community.

Problem: Elemental analysis with so-called *classical* chemical analysis has been the benchmark for traceability to SI and quality. This capability has been expensive and difficult to deploy commercially. A primary method for elemental analysis that can be commercially adopted will have strong impact on measurement quality.

Approach: At the end of the 20th century plasma spectrochemical analysis had matured such that sources of dispersion and bias in measurements were well understood, and well-engineered measurement instruments were commercially available. This technology was coupled with a ratio-based measurement and quantitation scheme, a novel drift correction procedure, gravimetric sample handling, and an experiment design that permits complete evaluation of uncertainty. The result, *High Performance Inductively Coupled Plasma Optical Emission Spectroscopy* (HP-ICP-OES), is a method of the highest metrologic order that uses unmodified, widely deployed (~2000 instruments), commercially available equipment.

Results and Impact: HP-ICP-OES has been applied in the certification of the major constituents of a high temperature alloy, the characterization of a LiAlO₂ ceramic material,¹ and in the certification of 64 single element SRMs. In the three year period over which HP-ICP-OES has evolved, its application in the spectrometric Solution SRM program has become routine.

¹M.L. Salit, R.D. Vocke, and W.R. Kelly "An ICP-OES Method with 0.2% Expanded Uncertainties for the Characterization of LiAlO₂," *Anal. Chem.* **2000**, 3504-3511.

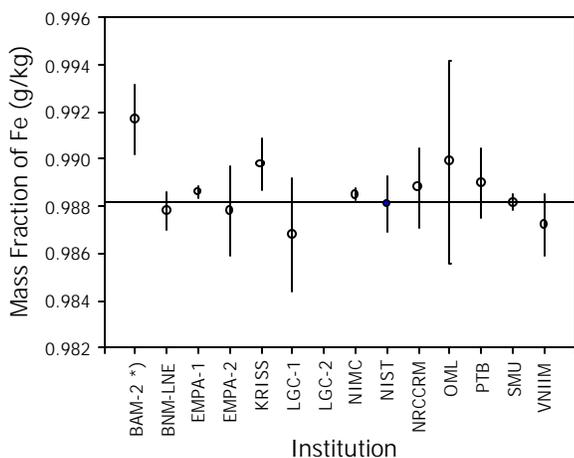


Figure 0. CCQM-K8 for Cu. NIST result is solid circle.

NIST used HP-ICP-OES for CCQM K-8, an international comparison of capability to analyze 4 single element solutions. These results had the smallest average deviation from the reference value of any participant, with uncertainties well below the target of 0.5%. These results were obtained with relative ease, with the routine protocol developed for our SRM program. Other participating laboratories employed classical analysis and isotope dilution mass spectrometry. Demonstration of comparability of this relatively prosaic method was completely satisfactory.

Future Plans: Two programs are underway to transfer this method to the commercial sector. We are collaborating with commercial reference material producers to implement this method, with a focus on HP-ICP-OES as a tool for traceability; and together with a manufacturer of ICP-OES equipment, we are refining the spectroscopic tools to simplify the practice of the method, and extend it to minor and trace constituents in samples.

5. A Matrix-Independent XRF Method of Analysis for Metals

J.R. Sieber, and A.F. Marlow, J. Blanchette and F. Claisse (Corporation Scientifique Claisse)

Objective: To develop a matrix-independent method of XRF analysis for a wide variety of metals and alloys in disk or chip forms and with calibration using primary reference materials.

Problem: X-Ray fluorescence (XRF) is used for non-destructive analysis of metals. However, methods must include corrections for matrix effects thereby increasing the uncertainty of a determination. This is true even when type standards are available because the ranges of concentration are wide. XRF cannot be applied to chip form samples due to particle size effects. It is only applicable to homogeneous disks and thin films, which are often difficult to prepare.

Approach: A useful method would employ a sample preparation technique that works for most, if not all, metals in chip form using the same ingredients in all cases. The preparation method would allow creation of calibration standards from ultrahigh purity metals and compounds. The resulting specimens would be solid, homogeneous, and ready to measure by X-ray fluorescence spectrometry.

The borate fusion method was investigated because it satisfies these requirements except that most metals and alloys cannot be fused. Those that can be fused are done with difficulty and a high probability of ruining the platinum ware through contact with metal particles at high temperature.

Results and Future Plans: Analysts who dissolve metals for classical or atomic emission methods must get all material into aqueous solution. With borate fusion, dissolution is not necessary because the fusion process dissolves solids so long as everything is oxidized. Therefore, it is possible to use acid reaction techniques deemed unsuccessful by metals laboratories. So, when the knowledge of those skilled in borate fusion is combined with the extensive knowledge of metals chemists, the result is a new approach to metals analysis by X-ray fluorescence.

Method development has been successful to the point where a single procedure works for a wide variety of metals and alloys. Nitric acid (HNO₃) dissolves or oxidizes most metals and alloys without forming volatile compounds. It can be used directly in platinum ware used for fusion. Al, Ti and Zr alloys dissolve when hydrofluoric acid (HF) is added in small amounts. Treatment with hydrogen peroxide (H₂O₂) ensures complete oxidation and releases the halogen in gaseous form.

High temperature alloys remained a problem because the best way to dissolve them is with hydrochloric acid (HCl), which attacks platinum. It was discovered that boiling HF dissolves these alloys slowly and, adding HNO₃, without quenching the boiling, accelerates the process. H₂O₂ treatment is still required to remove excess fluoride. However, fluoride is tolerable because fluoride compounds are not volatile as are chlorides and bromides, plus fluorine does not interfere with XRF analysis.

It has been demonstrated that nearly all metals and alloys can be fused at a dilution of about 12



Metal chips



Glass disk

to 1 compared to 40 to 1 or greater for typical acid dissolution. It remains to optimize the techniques to minimize dilution factors then

apply the procedures to a certification project to demonstrate the utility of this approach from chips to homogenous solid in a single vessel without grinding.

6. *New Analytical Methodologies for Environmentally Significant Organic Species*

M.M. Schantz, D. Bezabeh, W.W. Brubaker, Jr., J. R. Kucklick, S. Tutschku, and S.A. Wise

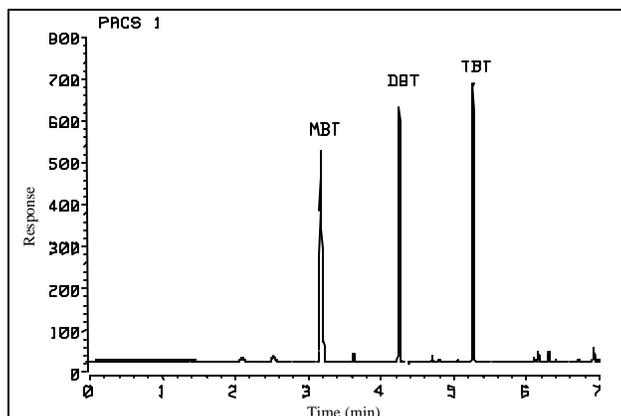
Objective: To develop analytical methodologies for the quantification of organotins, dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) congeners, polychlorinated naphthalenes (PCN), and nitro-substituted polycyclic aromatic hydrocarbons (nitro-PAHs) in natural matrices.

Problem: Analytical methodologies were developed last year for the measurement of non-ortho polychlorinated biphenyl (PCB) congeners and methylmercury in natural matrices. These methodologies have been extended to the measurement of additional groups of compounds: PCDD, PCDF, and PCN congeners and organotin species. Concentrations of PCDD and PCDF congeners hit peak levels in the 1970s; however, the US EPA, in a draft report released in May 2000, suggests that PCDD may pose a significant health risk to some people who ingest the congeners through foods in a normal diet. PCNs are a class of halogenated aromatic compounds that had applications similar to PCBs. Some of the PCN congeners have comparable toxicity to the non-ortho PCBs. Tin compounds, used in anti-fouling coatings and as stabilizing agents in polymers, show a very high toxicity and are subject to restrictions on their use in a number of countries. The organic forms of tin are the most readily available to living organisms. Nitro-PAHs, although typically found at lower concentrations than their analogous PAHs, are believed to be more toxic than PAHs.

Approach: The method developed for the determination of non-ortho PCBs has been modified for the determination of the PCDD, PCDF, and PCN congeners while the method developed for the determination of methylmercury has been applied to the measurement of organotin compounds. Using a 2-(1-pyrenyl)ethyl-dimethyl-silylated silica (PYE)

liquid chromatographic (LC) column, the non-ortho PCBs and PCNs (planar compounds) are separated from the ortho PCBs. The PCDDs and PCDFs are retained on the PYE column until the solvent is changed to toluene. The use of an LC column for these fractionations reduces the amount of sample handling and makes automation of the analyses possible. The method has been applied to the determination of PCDD and PCDF congeners in SRM 1649a and SRM 1944 and the determination of PCN congeners in SRM 1941a, SRM 1941b, SRM 1945, and SRM 1946.

The microwave digestion-solid phase microextraction (SPME)-gas chromatography with atomic emission detection (GC-AED) method, developed previously for methylmercury, has been applied to the analysis of organotin (monobutyltin, MBT; dibutyltin, DBT; and tributyltin, TBT) in SRM 1646, SRM 1941a, SRM 1941b, and SRM 1944. A typical chromatogram is shown below.



A normal-phase liquid chromatographic step using an amino/cyano column has been utilized to isolate the nitro-PAH fraction. The fractionation method that has been developed for the nitro-PAHs has been applied to the analysis of three diesel materials, SRM 1975, SRM 2975, and SRM 1650a. In some cases, data were available from Environment Canada and Battelle Columbus for comparison. Also, for SRM 1650a, measurements had been done for 1-nitropyrene during the original certification in 1985.

Results and Future Plans: Data for the PCDDs and PCDFs have been compared to that obtained from an interlaboratory study coordinated jointly by NIST and Environment Canada. The data

compare very well indicating that the LC cleanup is satisfactory for these measurements. The method will be applied to additional SRMs. Of particular interest will be the fish tissue SRM, SRM 1946.

For the PCNs, there are no data for comparison. The next step is an interlaboratory study again coordinated jointly by NIST and Environment Canada that first concentrates on the quantitation of the congeners using a technical mixture, since not all of the individual congeners are available in a pure form, and then on the analysis of natural matrix materials. The organotin method will be used to value assign existing SRMs and to participate in a future CCQM exercise.

7. *Critical Evaluation of Gravimetric Determination of Na in Serum as a Primary Method*

T.W. Vetter

Objective: To develop definitive gravimetric methods and critically evaluate them to determine whether they can be applied by National Metrology Institutes, NMIs, to meet the requirements of a primary method. Specifically, the gravimetric determination of sodium, Na, in serum by its precipitation as sodium sulfate, Na_2SO_4 , is evaluated.

Problem: NMIs are interested in determining the amount of substance using primary methods. A primary method has the highest metrological qualities, its operations can be completely described and understood, and a complete uncertainty statement can be written down in terms of SI units. Gravimetry is commonly considered an example of a primary method because, in its simplest form, it is the determination of the amount of substance by determining its mass. However, in order to be considered primary, any gravimetric method needs to be critically evaluated to determine if analyte is lost in the procedure and if the mass is stoichiometric and free of contaminants. A critical evaluation of the gravimetric method for the determination of Na in serum can serve as a basis for the evaluation of gravimetric methods, in general.

Approach: Each step in the gravimetric method for Na in serum was critically evaluated to determine if it contributes to the loss of analyte or contamination of the precipitate. Errors could be incurred from mechanical losses in sampling and transfers between vessels. Additional losses could result from incomplete recovery from the ion-exchange column and volatility of the heated precipitate. Errors could result from contaminants in the Na₂SO₄ precipitate or the platinum crucibles used to weigh the precipitate. The presence of sodium bisulfate, NaHSO₄, would cause an error in calculations based on the stoichiometry of Na₂SO₄. Mass error could be incurred in weighing. Measurements were made and controls were analyzed to determine the magnitude of any errors and the uncertainty associated with any errors. The magnitude of any undetected losses was estimated. Instrumental techniques, capable of determining trace amounts, were used to determine Na in the fractions collected from the ion-exchange column, before and after the Na fraction, and contaminants in the precipitate.

Results/Future Plans: This critical evaluation demonstrated that gravimetry can be applied by NMIs as a primary method. Errors and uncertainty about any errors were found to be minimal. Errors and uncertainties were lowest for the larger sample sizes and serum with higher concentrations of Na. All uncertainties mentioned are relative standard uncertainties, unless otherwise noted. A relative correction of 0.025 % to 0.07 % was applied to correct for possible undetected losses. The uncertainties of these corrections were 0.02 % to 0.05 %. The largest uncertainty source was for the mass determination and the possible volatilization of Na₂SO₄ and ranged from 0.03 % to 0.10 %. The uncertainties of Na retained on the ion-exchange column and the sample volume were less than 0.03 % and 0.04 % respectively. Corrections and uncertainties for losses or contamination based on the instrumental determinations were less than 0.01 %. The measurement replication ranged from 0.03 % - 0.08 % and the uncertainty of the recovery of standards was 0.075 %. Total uncertainty was less than 0.3 %. As determined

by this method, the value determined for Na in high-purity NaCl agreed with an indirect determination of Na by coulometry, another primary method, within 0.02 %, relative. This method can be applied to the determination of Na in high-purity salts and, with some modifications, can be applied to the determination of K in high purity salts, as was recently done in the determination of K in SRM 200a, potassium dihydrogen phosphate. The results for Na have been published and the results for K will be published also.

8. *Characterization of Liquid Chromatography Column Performance*

L. C. Sander, K. W. Phinney, and S. A. Wise

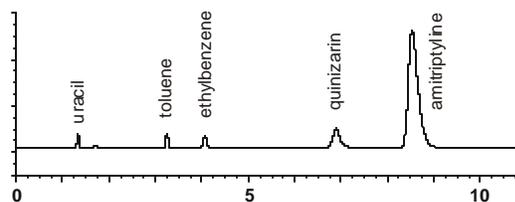
Objective: To develop uniform approaches for characterizing the chromatographic performance of columns used in liquid chromatography.

Problem: Central to any discussion of the theory and practice of liquid chromatography are the physical and chemical properties of the stationary phase, and column selection is one of the first and most important decisions made in method development. A variety of properties of the sorbent are known to affect chromatographic performance. Variations among columns from different manufacturers are typical even though such columns are prepared by similar approaches. For example, a common class of LC columns are described as "C18 Columns"; however, performance criteria measured as retention, selectivity, and/or efficiency vary widely among commercially available products. As a consequence, LC methods developed with specific columns are unlikely to be directly transferable to columns of the same type from different manufacturers. This variability is also observed for different bonding lots from the same manufacturer. Method development is relegated to the level of trial-and-error, with unpredictable results.

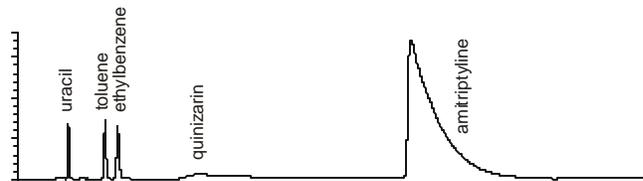
Approach: The Organic Analytical Methods Group maintains an on-going research effort to investigate retention processes and relate them to physical and chemical properties of the chromatographic system. These investigations have taken the form of spectroscopic characterization of stationary phases, preparation of new types of LC columns, and chromatographic evaluation of existing commercial columns. In past years, spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR), solid state nuclear magnet resonance spectroscopy (NMR), fluorescence spectroscopy, and small angle neutron scattering (SANS) have been used to characterize stationary phase morphology. These properties have been correlated with chromatographic retention behavior to provide an understanding of cause and effect relationships affecting column performance. Based on these fundamental studies, chromatographic tests have been developed as broad indicators of column performance and expected retention behavior.

Results and Future Plans. This year two new Standard Reference Materials (SRMs) were completed to facilitate LC column characterization and classification. SRM 870 Column Performance Test Mixture for Liquid Chromatography, consists of a mixture of five compounds selected as sensitive indicators of silanol activity, trace metal activity, retentiveness, methylene selectivity, and column efficiency. SRM 877 Chiral Selectivity Test Mixture consists of a suite of five individual solutions of single component enantiomers (i.e., nonsuperimposable mirror-image species). This SRM is intended for the evaluation of column selectivity toward chiral species. Both materials are useful 1) for column evaluation and classification, as an aid in method development, 2) as an objective indicator of changes in column performance over time with use, and 3) for control of column manufacturing processes. These materials supplement a third column evaluation SRM 869a Column Selectivity Test Mixture. This popular SRM was first issued in 1990 and reissued in 1998, and is widely used by analysts and column manufacturers to assess aspects of column performance related to molecular shape. Differences also exist among columns used in gas chromatograph (GC), and an SRM for assessing GC column performance is under study.

low silanol activity
low metal activity



high silanol activity
high metal activity



Comparison of SRM 870 separated on dissimilar C₁₈ columns

9. Development of a Primary Reference Photometer for Ozone Measurements

P.M. Chu, J.E. Norris, and F.R. Guenther

Objective: To develop an advanced primary ozone photometer with improved SI-traceability. This project is in partnership with researchers at the Bureau International des Poids et Mesures (BIPM) to assure the comparability and SI-traceability of global ozone measurements.

Problem: 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 climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. These concerns have led to the establishment of air quality standards and international protocols to reduce the emissions of pollutants that either contribute to tropospheric ozone formation or deplete stratospheric ozone. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to ten EPA facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. The international interest in ozone measurements has

prompted eleven national laboratories to acquire NIST SRPs.

During the initial development of the SRP, the calibration of the NIST instrument was verified using gas-phase titration with nitric oxide using chemiluminescence and diode laser spectroscopy to quantify the nitrogen dioxide formed. Since then, the performance of the NIST SRP has been monitored solely through instrumental diagnostics and comparison with another NIST SRP. The continued concern about global ozone concentrations and the need for comparability in these measurements emphasizes the importance of frequently validating the NIST standard. To this end, an advanced UV-based, SI-traceable ozone standard that is designed to simultaneously accommodate secondary methods of measuring the ozone concentration will be developed.

Approach: The major challenge of this project is to incorporate a secondary method that can accurately measure the ozone concentration simultaneously with the UV measurements and eliminate any uncertainties due to ozone transport. Several approaches are being considered. Gas phase titration with nitric oxide is an attractive method, since the ozone concentration can be directly linked to primary gravimetric NO and NO₂ standards. An additional advantage is that the ozone assay can be run under conditions similar to those used for the calibration of other ozone analyzers (a mixture of ozone, oxygen, and nitrogen).

A major drawback of the gas phase titration approach developed in 1982 was that it does not directly measure the ozone concentration where the UV absorption is measured. The advanced primary ozone photometer will be more versatile by allowing for optical probes at multiple wavelengths rather than a single Hg line, so that the ozone, nitrogen dioxide, nitric oxide concentrations and other contaminants can be analyzed simultaneously. To complement the gas phase titration measurements, the new standard will be developed with a sample cell that can be prepared with a known amount of "pure" ozone. Subsequently, the UV absorption, sample pressure, and ozone impurities (e.g. oxygen) can be measured simultaneously. This approach has recently been used for improved measurements of the ozone absorption cross-section.

Results and Future Plans: As a collaborative project between NIST and BIPM, 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. The accuracy of the NIST SRP would be checked for consistency with the NIST SRM gas program and primary standards developed by BIPM. This new photometer will be designed to include advanced spectroscopic methods with measurements approaching the shot-noise limit, clearly creating the ultimate ozone standard. Furthermore, knowledge gained from this project will be used to improve the performance of the existing SRPs.

10. *Exploiting Traceability Between Reference Materials: A New Mode for the Value Assignment of Reference Materials*

M.R. Winchester, W.R. Kelly, J.L. Mann and G.C. Turk

Objective: To explore the implementation of a new approach to the value assignment of reference materials.

Problem: NIST in general and CSTL in particular are under ever increasing pressure to do more with less. As a result, it is imperative to search continually for ways to be more efficient and to leverage limited resources to realize greater impact on the US economy. An important technical activity of CSTL is the production and value assignment of reference materials. Because this activity requires a major fraction of available time and resources, it is a logical area to target for improvement. Benefits might be gained through the invention of quicker and less expensive approaches to the production and value assignment of reference materials within NIST. Additional benefits might be realized through the development of suitable approaches for non-NIST entities to produce reference materials that are fit-for-purpose, yet tightly linked to NIST measurements.

Approach: A new approach to the value assignment of reference materials both within and outside of NIST has been developed. Briefly, the candidate reference material of interest is

directly and rigorously compared to a single existing NIST SRM of an appropriate matrix using a high-precision analytical method. The measurand for the candidate reference material is then computed from the certified value for the calibrant and any observed differences between the two materials. In essence, this approach establishes a direct, robust, traceability link between the unknown and previous NIST measurements.

This approach is founded upon several assumptions: (1) The certified value for the calibrant is assumed to have been measured accurately at the time of the original certification. For this reason, it is desirable that the previous NIST measurements be made with a primary analytical method. (2) It is assumed that the calibrant has not changed since certification in any way that would significantly affect the certified value or its usefulness for calibration. (3) Because the direct comparison between the two materials represents a single-point calibration, it is assumed that the high-precision analytical method used for the comparison is linear with negligible blanks. This assumption is not so important, if the calibrant and unknown are extremely similar. (4) Finally, it is assumed that differences between matrix effects and interferences for the calibrant and unknown are minimal. Here again, it is helpful if the calibrant and unknown are similar.

Results and Future Plans: The utility of the alternative approach to value assignment has been assessed through the recent certification of S mass fraction in coal SRMs 2682b, 2684b and 2685b. Each of these candidate materials was compared to the corresponding “a” lot SRM by means of the high temperature combustion method with IR absorption detection. The assumptions discussed above are all valid in this case. Specifically, the certified S mass fractions for the “a” materials were determined using isotope dilution thermal ionization mass spectrometry (ID-TIMS), a primary NIST method, and there is substantial evidence that the materials have not changed over the years (e.g., see Figure 1). Further, the combustion /IR absorption technique is sufficiently linear with negligible blanks. Finally, each “b” coal is known to be extremely similar to the corresponding “a” material, because in each case the “a” and “b” lot

SRMs were produced from the same bulk, homogenized coal.

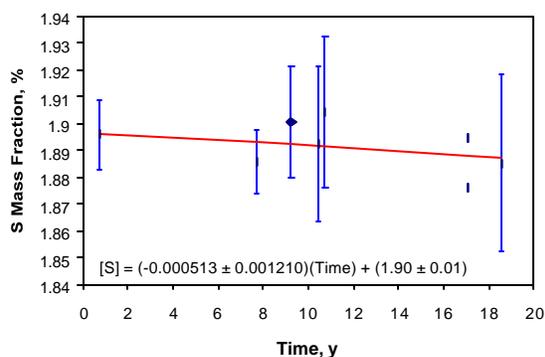


Figure 1: Stability of the bulk, homogenized coal used to produce SRMs 2683, 2683a and 1632b, as measured by ID-TIMS over an 18-year period.

The certified S mass fractions of SRMs 2682b, 2684b and 2685b determined using the alternative approach are $0.4917\% \pm 0.0079\%$, $3.076\% \pm 0.031\%$ and $4.730\% \pm 0.068\%$, respectively. Notably, the expanded uncertainties are only very slightly larger than those associated with the certified values for the “a” calibrants. In every respect, the renewal SRMs are suitable for use in the affected US industries (e.g., coal, electric power, and steel producers). This work shows that the alternative approach can be used effectively for the certification of at least some NIST SRMs. Further work is needed to assess better the scope of applicability.

Possibly more importantly, this work demonstrates that the alternative approach might be used by reference material producers outside of NIST (e.g., secondary RM suppliers) as an effective means of benchmarking their measurements to NIST measurements. By exploiting traceability between reference materials, NIST work might be leveraged for greater overall impact. This application should be explored more fully in the future.

11. *Quantitative Infrared Database Developed to Support Remote Sensing Applications*

P.M. Chu, F.R. Guenther, G.C. Rhoderick, and P.A. Johnson

Objective: To develop the NIST Standard

Reference Database 79 (SRD 79) "Quantitative Infrared Database" to support optical-based measurements of chemical emissions and hazardous air pollutants (HAP).

Problem: Over the last decade, growing concerns about the environment in general and air quality in particular have stimulated the development of improved, cost-effective field monitoring methods. With the recent advances in optical-based technologies, methods such as open-path Fourier transform infrared (FT-IR) spectroscopy are now becoming viable tools for field measurements. The *in-situ* and real-time nature of this approach offers several advantages over traditional point source monitoring techniques for applications such as determining fugitive emissions and chemical contaminants from industrial processing plants, hazardous waste and municipal landfills, water treatment plants, oil refineries, and chemical plants. Following successful testing of FT infrared methods during remediation of several Superfund sites, the US Environmental Protection Agency (EPA) has issued a protocol (TO-16) for FT infrared open-path remote sensing. Since molar absorptivity data in the literature widely differ, a validated quantitative database traceable to national measurement standards is a critical part of the infrastructure required for establishing emerging infrared-based monitoring technologies. New infrared-based technologies coupled with the NIST spectral database provides both industry and EPA with a tool for assessing regulatory compliance that is both cost effective and less invasive.

Approach: SRD 79 data are based on NIST primary gravimetric standards prepared with starting materials of assessed purity and procedures that minimize contamination. For each compound, the absorption coefficient spectrum was calculated using nine transmittance spectra and the Beer's law relationship. The uncertainties in the absorption coefficient data are estimated from the data analysis and considerations of other error sources such as the non-linear detector response.

Recently, data comparisons with Battelle Pacific Northwest laboratories have helped us characterize two additional FT-IR intensity artifacts. Both of these artifacts are associated with the single limiting aperture design in most

commercial FT-IRs. The first artifact is attributed to the detector viewing the warm annulus of the aperture in higher resolution measurements (0.2 cm^{-1} or higher). The off-axis rays distort the line shapes and increase the observed band intensities. Adding an additional aperture system or a multipass cell after the interferometer minimizes the off-axis rays that reach the detector and in turn the contributions from these artifacts. For the SRD 79 data, we currently estimate a 1.5 % bias in the integrated band intensities in the finger print region and no biases are expected at higher frequencies. An effort is underway, to further reduce this bias. The second artifact is due to the light exiting the interferometer, which is directed towards the source, can be reflected off the aperture back into the interferometer. This double modulated light produces a $2f$ alias throughout the spectrum. Reducing the reflectivity of the aperture wheel minimizes this artifact.

Results and Future Plans: Currently, Absorption coefficient data for 30 HAPs on a US EPA priority list is available as the NIST Standard Reference Database (SRD) 79, version 1.1. The data are stored in the standard JCAMP-DX format to enable universal access to the data. The 0.12 cm^{-1} resolution data were processed to generate data at a number of different resolutions and apodizations to provide users with data that closely match their experimental parameters. A digital signature accompanies each data file, allowing users to ensure the integrity and source of the data file and traceability to NIST.

This quantitative infrared database is an ongoing project at NIST. Additional spectra will be added to the database as they are acquired and updates will be available over the internet. Plans include continued data acquisition for the compounds listed in the 1990 US EPA Clean Air Act Amendment, as well as for those compounds that are of concern in global warming and emissions trading. Additionally, intercomparisons of NIST primary standards and molar absorptivity data with National Metrology Institutes will be expanded to facilitate the use of this database in issues of global interest and impact.

Publications:

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12. *Lithium Profiles for Thin Film Battery Applications*

G.P. Lamaze and H. Chen-Mayer

Objective: To measure lithium concentration and mobility in thin multilayer films.

Problem: Lightweight, less toxic lithium-based batteries now dominate the market for laptop computer, cell phone and other consumer electronics. Market demand for all types of rechargeable cells is expected to grow at a compound rate of 11 per cent per year, reaching a total of 2 billion cells by 2001 of which the lithium ion cell share is expected to be 440 million cells. Thus the market for lithium ion cells will soon reach about \$4 billion. In spite of this rapid commercialization, much of the underlying materials science is not completely understood. As part of a continuing collaboration between NIST and Tufts University, we have employed cold neutron depth profiling (NDP) to measure elemental concentrations versus depth for different lithium ion battery materials. One goal is to rationally design the thermo-mechanical properties of amorphous lithium phosphorous oxynitride (lipon) (a solid ionic conductor), particularly the thermal expansion coefficient. One part of this study is to relate the resulting thermal stress to starting composition and temperature of evaporant, and of the composition and pressure of the background gas during deposition.

Approach: The technique of neutron depth profiling (NDP) permits the analysis of depth profiles in thin films up to a few micrometers for several light elements. The most readily

analyzed elements are lithium, nitrogen and boron. We have previously reported measurement of lithium mobility in electrochromic devices. The lithium depth profiles are based on the measurement of the energy of alpha particles and/or tritons from the ${}^6\text{Li}(n,\alpha){}^3\text{H}$ reaction. Nitrogen depth profiles are based on the measurement of the energy of protons from the ${}^{14}\text{N}(n,p){}^{14}\text{C}$ reaction. Samples are placed in a beam of "cold" neutrons, and surface barrier detectors, which record both the number and energy of the particles, are used to measure the outgoing charged particles. Comparing the emission intensity with that of a known standard leads to quantitative determination of the lithium and nitrogen concentrations. Moreover, the emitted charged particles lose energy as they exit the film; this energy loss provides a direct measurement of the depth of the originating lithium nucleus. A great advantage of the NDP technique is that it is non-destructive, which allows repeated observations of the concentrations under different conditions. When combined with other techniques, e.g., activation analysis, ratios to other constituents can be determined.

Results and Future Plans: Figure 1 gives an example of profiling results using the NDP technique. Shown here are profiles for two lipon samples manufactured under different conditions. The elemental concentrations in at/cm^3 are presented as a function of depth. Because the alpha particle loses energy at a greater rate than the proton, the resolution for the lithium profile is better than that for nitrogen. One observes that the sample Y245 has a much more uniform distribution of lithium than sample Y239. By integrating the areas under the curves, one obtains the total concentration of lithium and nitrogen, respectively, and therefore the ratio of the two in the sample. The table gives measured lithium/nitrogen ratios thus obtained and the corresponding thickness of four lipon samples. This data will be correlated with mechanical studies to see if thermal expansion coefficient decreases with increasing nitrogen content. Our future plans include the study of LiCoO_2 films (a battery electrode material) to determine 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 of the Li/Co ratio.

Sample	Atom ratio - Li/N	Thickness, µm
Y243	4.46	0.7
Y232	4.39	1.5
Y239	1.63	0.4
Y245	3.1	0.6

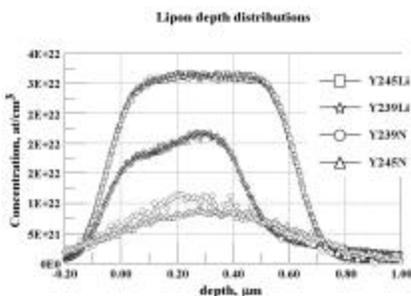


Figure 0 Depth profiles of lithium and nitrogen for two different lipon samples.

13. Standards to Assist Perfluorocarbon Reductions in Aluminum Production

G.C. Rhoderick, P.M. Chu, and F.R. Guenther

Objective: To support the Voluntary Aluminum Industrial Partnership (VAIP) in reducing emissions of perfluorinated carbon compounds (PFCs) in order to become more efficient in producing aluminum and emitting less greenhouse gases into the atmosphere.

Problem: It has been well established that the age-old method for production of aluminum, the Hall-Heroult process ($\text{Al}_2\text{O}_3 + 3/2 \text{C} \Rightarrow 2\text{Al} + 3/2 \text{CO}_2$), periodically produces varying amounts of two PFCs: tetrafluoromethane (CF_4) and hexafluoroethane (C_2F_6). Molten cryolite (sodium aluminum fluoride) is placed in cathode blocks. Immersing baked carbon anodes in the cryolite completes the electric path. The amount of these PFCs produced is related to the frequency and duration of “anode effects”. An anode effect occurs when the concentration of the alumina, dissolved in molten cryolite, falls below the level necessary to support the current flow at the nominal voltage of 4.2- 4.5 volts (up to 280,000 amperes) for aluminum production. During these anode effects, the voltage rises to levels that initiate reactions resulting in the production of

PFCs. These PFCs are extremely potent greenhouse gases and are believed to contribute to global climate change. CF_4 has an atmospheric lifetime of 50,000 years and one metric ton has a global warming potential (GWP) equivalent to 6,500 metric tons of carbon dioxide (CO_2) over a 100-year period; C_2F_6 has an atmospheric lifetime of 10,000 years and a GWP equivalent to 9,200 metric tons of CO_2 . In 1995, the Voluntary Aluminum Industrial Partnership (VAIP) was created by the Environmental Protection Agency (EPA) and twelve of the nation’s primary aluminum producers with assistance from the Aluminum Association. The VAIP was created with the main goal of reducing PFC emissions and increasing the efficiency of aluminum production. In order to assure comparability of measurements over space and time, traceability to national or internal sources was desirable.

Approach: NIST developed a suite of eight primary gas standards for CF_4 and C_2F_6 . The suite of primary standards were then used to value assign transfer standards supplied to the aluminum industry. Three concentration levels were value assigned for the transfer standards: 1) CF_4 at $1378 \pm 15 \mu\text{mol/mol}$; C_2F_6 at $151.2 \pm 1.1 \mu\text{mol/mol}$, 2) CF_4 at $22.7 \pm 0.2 \mu\text{mol/mol}$; C_2F_6 at $2.54 \pm 0.04 \mu\text{mol/mol}$ and 3) CF_4 at $0.0902 \pm 0.0012 \mu\text{mol/mol}$; C_2F_6 at $0.0113 \pm 0.0006 \mu\text{mol/mol}$. Samples collected from an aluminum production line (pot-line) and emission stack were analyzed at NIST and value assigned. Participating aluminum companies analyzed these samples and submitted their data to NIST for analysis to determine the ability of individual facilities to analyze samples using a common source of standards.

Results and Future Plans: The results of this research illustrate that primary gravimetric standards for CF_4 and C_2F_6 in nitrogen can be accurately prepared in gas cylinders with combined uncertainties of $\pm 0.3 \%$. The primary standards can in turn be used to value assign working standards with expanded uncertainties of less than 2 % relative with the exception to the extreme low-level ($0.01 \mu\text{mol/mol}$) C_2F_6 , 5.3 %, at 2 sigma. Analysis of the data indicates that GC/FID is linear over at least a six-fold range. Even though the GC/FID is an extremely important and reliable analytical instrument at higher CF_4 and C_2F_6 concentrations, it does not

have the sensitivity necessary at the lower concentration levels. The limited dynamic range of GC/FID and GC/ECD requires one to employ the services of another analytical tool. FT-IR spectrometry was a more successful measurement tool for PFCs at the lower concentration levels. Results of the industry analysis of real pot-line and stack samples showed a maximum bias versus NIST of $\pm 15\%$.

The development of these transfer PFC gas standards has assisted the aluminum industry in establishing traceability and mutual confidence in data generated for PFC emissions. The improved accuracy of measurements with the aluminum industry facilitated by development of these standards allows better understanding and control of the “anode effects” that cause the production of PFCs. In 1999 the VAIP reported reductions in PFC emissions by 41 %, equivalent to a reduction of nearly 8 million metric tons of CO₂, from 1990 levels. These reductions were accomplished through reduced number of anode effects and the length of those effects.

Future standards development may require lower concentration levels to monitoring fugitive emissions from aluminum facilities. This would require the purchase and optimization of instrumentation to make the necessary measurements.

14. Wastewater Chlorination/Dechlorination Mechanisms

W.A. MacCrehan and M. Bedner, G. Helz (Univ. Md.)

Objective: To support national initiatives in developing environmentally-friendly, “green” chemical processing, NIST has partnered with the Water Resources Center of the University of Maryland to investigate new wastewater processing chemistries. To preserve human health, the majority of the 40 billion gallons per day of processed sewage wastewater entering the environment in the US is disinfected with chlorine. To limit the release of excess chlorine, wastewater is often treated with a reducing agent such as sulfur dioxide, prior to discharge. Although chlorination is highly effective and

inexpensive, chlorination by-products are thought to be muta-, estro- and androgenic. Furthermore, residual active chlorine from such processing has proven to be toxic to a number of aquatic species. Although wastewater treatment operations frequently use dechlorination with sulfur (IV) compounds such as sulfur dioxide, we have shown that N-chloramines of peptides and proteins exhibit slow (minutes to hours) dechlorination rates.

In this project we are seeking to understand and control chlorination residuals from two perspectives. The first is to develop a reliable measurement tool, liquid chromatography with post-column electrochemical detection, that can speciate the various forms of active residual chlorine. This has proven difficult as the highly reactive chlorination by-products, such as N-chloropeptides/proteins often decompose during chromatographic analysis. To evaluate these losses a custom separation system was designed that allows us to toggle between a flow injection analysis (FIA) (with no separation column) and the liquid chromatographic (LC) separation, see

Figure 1.

This has permitted the direct evaluation of the factors that influence on-column losses such as solvent composition, pH, and separation column material.

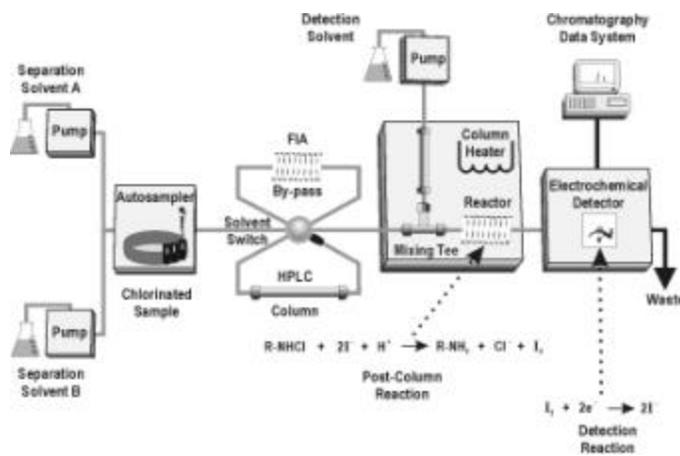
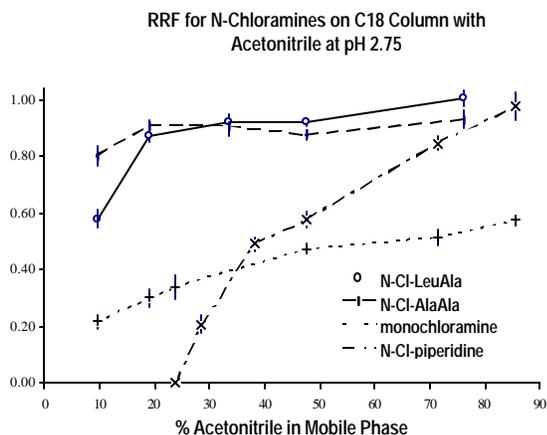


Figure 2 illustrates the effect of solvent composition on the LC recovery as indexed to the FIA measurement using the relative response factor (RRF). In addition, having these FIA measurements allows direct comparison of the separated chlorine species, as determined by LC, to the “total residual chlorine” (TRC) and allows

comparison of the NIST chlorine speciation measurements with the convention EPA-defined TRC. Using this new measurement system, we will evaluate the effectiveness of a series of new reducing agents to speed the slow dechlorination processes of N-chloropeptides. By understanding how the various chlorine species behave in their reaction with dechlorination agents, we hope to help sewage process engineers minimize the amount of wastewater chlorination by-products discharged into the environment.



15. Detoxification of Polychlorinated Compounds in Water, Oil, and Soil by Electron Beam Irradiation

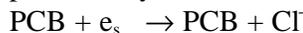
D.L. Poster, B. Huie and P. Neta (838), M. Chaychian, C. Jones, J. Silverman, and M. Al-Sheikhly (U. Md.)

Objective: To examine the radiolytic degradation of PCBs present in a variety of contaminated matrices. Specific objectives are: (1) to evaluate techniques which increase the efficiency of radiolytic dechlorination of PCBs through the use of nontoxic additives, surfactants, and sonication during the electron beam treatment, (2) to evaluate techniques which increase the efficiency of radiolytic dechlorination of PCBs in transformer oil and in organic solvents at various concentration levels through the use of additives (alcohol, alkali) and by applying an external high voltage electric field during electron beam irradiation, and (3) to evaluate cost and performance of radiolytic treatment methodologies for the remediation of the most

prevalent types of PCB contamination at polluted sites.

Problem: The widespread use of polychlorinated biphenyls (PCBs) in various industrial applications presents a major environmental issue due to the toxicity of these compounds and their long lifetime in ambient conditions. The method currently used to destroy most PCB containing materials is incineration. This method, however, suffers from two disadvantages: it incinerates the medium along with the PCB and it converts some of the PCB into more toxic materials, namely dioxins. Radiolytic degradation of PCBs is expected to overcome both of these disadvantages, but our previous studies have shown that radiation processing may also be inefficient due to the scavenging of solvated electrons by other constituents present in environmental matrices.

Approach: The radiolytic treatment of PCBs in organic solvents such as transformer oils leads to their reduction to benign inorganic chloride and practically nontoxic biphenyl, without formation of any dioxins. The approach is to reduce chlorinated compounds via solvated electrons produced by electron beam:



In principle, all of the chlorines can be removed from the PCBs. With the addition of safe, inexpensive additives the dechlorination yield may be enhanced.

Results and Future Plans: By means of electron irradiation and added triethylamine (TEA), we have successfully achieved 100% dechlorination of PCBs in BG&E transformer oil. The TEA not only increases the effectiveness of the irradiation, but also precipitates the inorganic chloride. Excess TEA remains dissolved in the oil, but TEA is very volatile and is easy to recover for reuse by distillation. The TEA that is precipitated with the chloride can also be recovered by distillation after addition of NaOH.

We also have significant findings on the effects of ionizing radiation on the concentrations of PCB congeners in a marine sediment (Standard Reference Material 1944 New York/New Jersey Waterway Sediment), both in the presence and absence of isopropanol. The latter is an effective hydroxyl radical scavenger; OH radicals can oxidize chloride ions to chlorine atoms, which can, in principle, rechlorinate organic products.

The same decrease in PCB concentrations with radiation dose was obtained whether this additive was present or absent. Its further use has been abandoned. We are measuring all PCB congener concentrations in SRM 1944, as a function of radiation dose, thereby determining the dose necessary to fully dechlorinate each component of the contaminated sediment.

Publications:

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16. Activities to Support Environmental Measurements

R.R. Greenberg, R. Demiralp, F.R. Guenther, W.R. Kelly, J. Kucklick, R.M. Lindstrom, S.E. Long, D.L. Poster, M.M. Schantz, W.J. Thorn III, S.A. Wise, and R. Zeisler

Objective: To provide support for environmental monitoring and research programs through a variety of quality assurance mechanisms including Standard Reference Materials (SRMs) with value-assigned concentrations of important toxic constituents. Such SRMs serve as quality assurance tools for environmental measurement and monitoring efforts worldwide.

Problem: There are a wide variety of environmental monitoring and research programs in the U.S. Inaccurate analytical results lead to inappropriate actions. Therefore, accurate

analytical measurements are of utmost importance for facilitating sound environmental decision making. Quality assurance programs to enhance the reliability of analytical data often depend upon the availability of a broad spectrum of different control and Certified Reference Materials with matrices similar to the actual environmental samples analyzed.

Approach: For nearly thirty years, NIST has developed measurement methods as well as provided a large number of natural-matrix SRMs to serve as accuracy benchmarks for environmental measurement and monitoring activities within the U.S. For many matrix and/or analyte combinations, NIST is the de facto source for such materials worldwide. Therefore, we are committed to developing new SRMs as needed, as well as recertifying high priority materials as their stocks are depleted and/or providing additional information for important new classes of analytes in both new and existing SRMs.

Results and Future Plans: A number of new measurement methods have recently been developed or improved, and the certification of a number of new and renewal environmentally-related SRMs has been completed. Several highlights in environmental measurement area are described below. In view of this important role for NIST in support of environmental programs, we plan to continue our work in developing the methods and standards needed to assure the quality of environmental measurements in the US.

Development of Mercury Methods to Support Emission Assessments from Electric Power Generation: The Environmental Protection Agency (EPA) has stated that mercury is the top priority elemental pollutant in the environment due to its ability to bio-accumulate in aquatic ecosystems in a highly toxic form. One of the first actions has been to require coal-fired electric power utilities over 25 megawatts, to measure mercury in their feedstock. However, in recent years there have been no available SRMs to provide the traceability for power industry measurements due to the extreme difficulty in making accurate and precise determinations of mercury in a coal matrix. The Electric Power Research Institute (EPRI) has been conducting a round-robin study to assess the status of the most commonly used industry methods for mercury in coal, with more than thirty external laboratories

participating. The lack of a reliably certified coal SRM has initially limited the assessment of data accuracy among these methods and laboratories.

A new primary method has been developed which utilizes a total destruction of the coal matrix by Carius tube pretreatment and quantification of the mercury by isotope dilution inductively coupled plasma-mass spectrometry (ID-ICP-MS). The digestion is achieved in a closed system, under high pressure, ensuring total equilibration of the added spike with no external loss of mercury. The mercury is measured by chemical reduction with tin chloride and generation of a "cold vapor." The elemental mercury vapor is separated from the matrix using a conventional gas-liquid separator and the mercury isotope ratio measured by quadrupole ICP-MS. There are considerable advantages to the new method, which include very-high sensitivity, accuracy and precision of the order of 1% or better, and complete freedom from both spectral and matrix interferences.

A radiochemical neutron activation analysis (RNAA) procedure to determine Hg was developed at NIST nearly 30 years ago. The procedure is based upon the distillation of radioactive Hg from the sample with non-radioactive Hg carrier during oxygen combustion and has been used in combination with other techniques to certify Hg in numerous, complex-matrix SRMs. This procedure, however, suffers from problems arising from the inability to determine chemical yield explicitly for each sample analyzed, and from poor detection limits caused by high baseline levels of radiation originating from the matrix. We have adapted the RNAA procedure to determine Hg via ^{203}Hg rather than the original ^{197}Hg for better sensitivity and with lower radiation exposure to personnel. In addition, mercury is now precipitated as the periodate. This greatly reduces ^{75}Se radioactivity (which would otherwise interfere), improves the counting geometry, and permits a gravimetric determination of the chemical yield for each sample.

Utilizing these improved methods, certification of mercury in SRM 1632c, a bituminous coal, has been completed. Results of the two methods are in good agreement: 94.3 ± 1.3 mg/kg via ID-ICP-MS and 93.4 ± 2.2 mg/kg via RNAA. Due to the difficulties of obtaining accurate

measurements using earlier methods, the original material had a reference value of 106 ± 23 mg/kg and therefore the new certified value of 93.8 ± 3.7 mg/kg, represents a significant advance in the quality of this reference material. The improved accuracy and precision should also permit reliable long-term stability testing of the material, which is an issue within the industry, as well as the certification of Hg several other coal SRMs. The sensitivity of the primary ICP-MS method should permit measurements at very low levels and it is planned to extend the method to other fossil fuel materials, such as crude oil and refined products such as diesel fuel. Knowledge of the mercury levels in such materials is extremely limited.

Chemical Composition of Respirable Particulate Matter: Newly issued National Ambient Air Quality Standards (NAAQS) for airborne particles require the review and assessment of major air pollutants including particulate matter less than 2.5 micrometers in diameter (PM_{2.5}). This new standard was issued primarily on the basis of evidence from epidemiological studies that consistently suggested associations between ambient concentrations of particulate matter and various adverse health effects including premature mortality. The chemical constituents associated with PM_{2.5} are the likely cause of the various adverse health effects.

As a result of the newly issued NAAQS, both the monitoring of ambient concentrations of PM_{2.5} and the determination of associated chemical constituents are necessary. Important chemical constituents include a suite of particle-associated inorganic toxic elements, organic compounds including polycyclic aromatic hydrocarbons (PAHs), alkanes and polychlorinated biphenyls (PCBs), as well as organic and inorganic carbon. Reliable measurements of trace constituents associated with particulate matter are critical for an accurate assessment of the detrimental effects on human health and on various ecosystems. PM_{2.5}-related SRMs are necessary to assure measurement quality assurance in support of such airborne toxic substance studies, surveillance, pollution monitoring/control strategy research, and health effects studies. These SRMs will also support industrial efforts in necessary remedial measures and are critical for cost effective operations.

The first of these new generation materials, SRM 2783, has been developed as a simulated PM_{2.5} urban aerosol that has been collected on filter media and is currently being certified for elemental concentrations. A unit of this SRM consists of two 42 mm diameter polycarbonate filter membranes that are loaded with 490 µg of the prepared aerosol, and two blank filter membranes. Concentration of about twenty elements including such toxic elements as Pb and V will be certified at levels down to tens of nanograms per filter.

The development of additional PM_{2.5} air quality related SRMs will be necessary in order to assist scientists in the determination of organic compounds and other elemental species associated with particles less than or equal to 2.5 micrometers. At least 200 g of PM_{2.5} from an urban location is required for the development of such an SRM. NIST has negotiated an interagency agreement with the US EPA to collect this quantity of contemporary PM_{2.5} using a specially designed ultra high volume sampling system. After the material has been collected, additional PM_{2.5} SRMs will be developed over a two-year work period. The primary focus will be to process and fully characterize the material for organic contaminants including the tier 1 and tier 2 prioritized pollutants, which include: PAHs, a suite of PCB congeners, a range of n-alkanes, organic carbon, and dioxins. The certification approach of such a material would be similar to that used previously for NIST atmospheric-particulate related SRMs.

Low-Level Nitric Oxides in Automobile and Stack Gas Emissions: American automobile manufacturers are building lower emission vehicles in response to stringent Federal Tier II and California LEV II clean air emission regulations. Since 1973, NIST has worked together with USEPA, Ann Arbor, MI, and the automobile industry to develop eighty gaseous Standard Reference Materials of mobile source pollutants such as propane (hydrocarbons: HCs), carbon monoxide (CO), carbon dioxide (CO₂), and oxides of nitrogen (NO_x). NIST has been committed to supporting the automobile industry by maintaining supplies of these accurate gas standards and providing new measurement expertise. As the trend of new vehicles having lower and lower emission levels continues, NIST

is developing infrastructural measurement capability and standards to allow new SRMs to be developed for use by the mobile source measurement community.

A parallel environmental support effort has NIST providing necessary SRMs to allow specialty gas companies to produce USEPA, Research Triangle Park, NC, protocol gas standards for calibration of continuous emission monitors on power plants and other stationary sources. New cogeneration technologies for power plants are requiring new SRMs with lower levels of sulfur dioxide and oxides of nitrogen.

In addition, the parties to the American Industry/Government Emissions Research (AIGER) Cooperative Research and Development Agreement have written to NIST requesting the development of two new NO SRMs at 0.5 µmol/mol and 1.0 µmol/mol in order to meet future stringent emission regulations. Similarly, gas fired turbine CEM users and specialty gas companies have identified new NO standards between 1.0 µmol/mol and 5.0 µmol/mol as highest priority for new NIST traceable gas standards.

The development and certification of new NO SRMs at 0.5 µmol/mol and 1.0 µmol/mol involves several challenges. Stability of these SRMs over a 2-3 year certification period is the major concern. Reactions can occur between NO and trace oxygen contamination in the nitrogen balance gas causing concentration decay. Low NO concentrations challenge our measurement detection limits. Such challenges to our methodology has limited our expanded uncertainty estimates to ± 2 percent relative below 10 µmol/mol; getting worse as the concentration goes below 1.0 µmol/mol. NIST started work on this problem in 1998. NIST and contractor Scott Specialty Gases worked together to gravimetrically dilute a SRM 2629 lot standard into fifteen gas standards. Three mixtures each at 0.5 µmol/mol, 0.75 µmol/mol, 0.95 µmol/mol, 1.05 µmol/mol, and 1.25 µmol/mol NO in nitrogen were prepared. The dilution nitrogen used was gettered to remove oxygen to less than 3 nmol/mol. These mixtures were analyzed against a dynamic NO₂ permeation standard (SRM1629) after three months and again after 1 year. All analyses suggest that these mixtures are

stable. A Third analysis at two years is planned for December 2000. A special treatment of the cylinder walls with NO₂ and the gettered nitrogen with less than 3 nmol/mol oxygen contamination are believed to be the keys to this unprecedented low NO stability. NIST will perform a two-year stability analysis on the fifteen infrastructural gas standards defining the instrument response curve from 0.5 μmol/mol to 1.25 μmol/mol NO.

NIST has also submitted an internal SRM development proposal to produce new SRMs at 0.5 μmol/mol and 1.0 μmol/mol. If the proposal is funded in FY 2001, these SRMs will be available in October 2003. In the meantime NIST will provide access to the infrastructure mixtures for NIST sponsored round robin testing or analysis of customer low NO standards.

Measurement Standards for Drinking and Wastewater Quality: A system has been established under which private sector companies and interested states are accredited by NIST to provide proficiency testing (PT) that meets the needs of EPA and states to those laboratories testing drinking water and wastewater for regulated chemicals, microbial, and radiochemical parameters. As part of NIST's oversight role in this system, NIST is to maintain SRMs to support the program. NIST is producing SRMs for those parameters not covered by existing SRMs. For the organic components, this includes 20 solution reference materials of a variety of organics in water soluble solvents and 6 solutions of different Aroclors in transformer oils. The list includes nine solutions of pesticides and herbicides, a solution of phthalates, and two solutions of organic disinfecting by-products.

The solutions are gravimetrically prepared and ampouled using an established standard operating procedure. The primary standards used for solution preparation are well characterized with purity determinations by multiple methods where possible, except for the analyte mixtures (Aroclors, chlordane, and toxaphene). These mixtures were obtained from the former EPA repository. The certified values are based on the gravimetric preparation of the solution, analytical verification of the gravimetry, and purities of the starting materials, except for the mixtures mentioned above.

Twenty-six semi-volatile solutions and 15 volatile organic solutions have been prepared so far. The analytical verification of the gravimetric concentrations is on going. The certificates are being completed as the analyses are finished. One important aspect of this project will be stability testing. A number of these analytes are potentially unstable in solution. Stability of the carbamate solution has already been shown to be a problem.

Work is continuing on preparing and certifying six gaseous organic compounds in methanol standards. The challenge is to develop a gravimetric preparation technique that will produce stable and accurate standards. This is quite a challenge in that the solutions produced lose the volatile compound rapidly under normal storage conditions in ampoules. The approach under test at NIST is to prepare the solutions under elevated pressures in a special cylinder. Quantitation of the contents of this cylinder would be accomplished using FTIR and the quantitative spectra in our Quantitative Infrared Database. This approach would allow NIST to verify solutions from PE producers; however, distribution of a traceable product would be difficult. A possible approach would be to prepare the solutions in a cylinder and send the cylinder to the PE producers for comparison to their standard solutions.

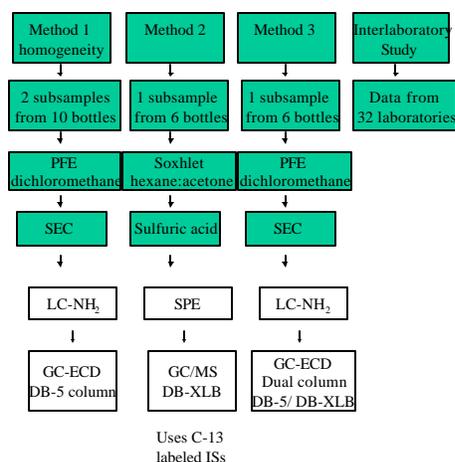
Contaminants in Edible Fish: An edible fish tissue SRM with concentration levels indicative of contaminated food web has been requested by both the environmental and food/nutrition measurement communities. To meet this need, NIST is developing a fish tissue SRM with certified and reference values for organic contaminants, a few inorganic contaminants, as well as nutritional content (see Methods and Standards to Support Nutritional Labeling of Food Products).

SRM 1946, Lake Superior Fish Tissue, is a frozen tissue homogenate prepared from adult lake trout (*Salvelinus namaycush*) fillet without the skin. The certification scheme for the ortho-polychlorinated biphenyl (PCB) congeners and chlorinated pesticides is shown below.

In addition to the ortho-PCB congeners and chlorinated pesticides, non-ortho-PCB congeners

and methylmercury have been determined in SRM 1946 using the selective fractionation and solid phase microextraction techniques, respectively, described elsewhere (see New Analytical Methodologies for Environmentally Significant Organic Species).

Based on the data from the different methods shown above, certified values will be available



for 20 to 30 ortho-PCB congeners and 10 to 15 chlorinated pesticides. In addition, total mercury (approximately 400 ng/g wet mass) and methylmercury (approximately 380 ng Hg/g wet mass) will be certified based on results from one method at NIST independent methods from two other laboratories. Reference values will be available for additional ortho-PCB congeners and chlorinated pesticides, three non-ortho-PCB congeners (PCB 77, PCB 126, and PCB 169), as well as several toxic elements.

A second fish tissue, SRM 1947 (Lake Michigan Fish Tissue) will be provided in the future with an emphasis on the trace element and nutritional value. In addition, based on EPA's recent draft reassessment suggesting that dioxin congeners may pose a significant health risk to some people who ingest them through foods in a normal diet, one of these SRMs will be evaluated for dioxin levels.

17. *Methods and Standards to Support Nutritional Labeling of Food Products*

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Objective: To develop food-matrix Standard Reference Materials (SRMs) to support nutrition labeling issues.

Problem: The Nutrition Labeling and Education Act (Public Law 96-359) requires that specific nutritional information be provided on all processed foods sold in the U.S. In a 1996 study by the U.S. Food and Drug Administration, the accuracy of label information ranged from 98% to 54% for nutrients for which labeling is required. As more food-matrix SRMs become available, label accuracy should improve when the food and nutrition communities employ these SRMs in their analyses. These SRMs will be used for validation of analytical methods as well as for quality assurance when assigning values to in-house control materials, and will facilitate compliance with nutritional labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of nutrition information that is provided to assist consumers in making sound dietary choices.

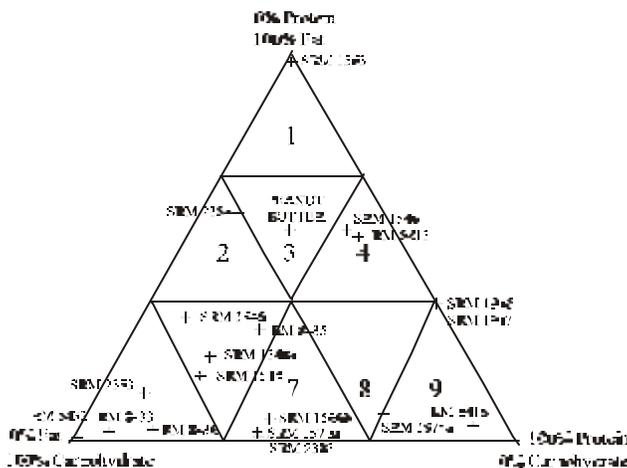
Approach: The composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed. AOAC (Association of Official Analytical Chemists) International has developed a nine-sectored triangle in which foods are positioned based on their fat, protein, and carbohydrate content. AOAC's belief is that one or two reference materials within each sector should be representative of other foods within that sector and could be used for quality assurance and method validation when analyzing those other foods. NIST has been working with other government agencies and the food industry over the past several years to provide an increased array of SRMs with values assigned for proximates (procedurally defined values for fat, protein, carbohydrate, etc.), fatty acids, cholesterol, vitamins, elements of nutritional interest, etc. Concentration values in the food-matrix SRMs are assigned based on a combination of data from NIST, from

interlaboratory comparison exercises involving approximately 20 member laboratories of the National Food Processors Association's (NFPA's) Food Industry Analytical Chemists Subcommittee (FIACS), and from additional collaborating laboratories.

Results and Future Plans: A workshop was held at NIST in 1997 to identify and prioritize the food industry's SRM needs. A material in sector 2 of the AOAC triangle was ranked as the highest priority, and in 2000, NIST began value assignment of SRM 2384 Baking Chocolate. Concentration values are expected to be assigned for proximates, individual fatty acids, total dietary fiber, several water-soluble vitamins, elements of nutritional interest, caffeine, theobromine, and catechins (polyphenolic compounds that may prevent cancer and heart disease). SRM 2384 will be the first reference material available from NIST with values assigned for caffeine, theobromine, and catechins.

The second and third highest priorities identified in the 1997 workshop were a material in sector 7 of the AOAC triangle, and a vegetable material with values assigned for persistent pesticides. (SRM 1570a Trace Elements in Spinach Leaves and SRM 1566b Oyster Tissue are located in sector 7, but these are freeze-dried finely ground powders rather than the "wet," more natural forms of the materials that are preferred by food laboratories.) In 2000, NIST acquired material for use as SRM 2385 Spinach. Normal levels of three pesticides were applied to the spinach by the grower, and the spinach was lightly rinsed prior to processing to remove soil but allow for possible retention of pesticide residues. Concentration values are expected to be assigned for proximates, individual fatty acids, total dietary fiber, several water-soluble vitamins including folate, elements of nutritional interest, carotenoids, and pesticides – including the degradation products of DDT, which remain in soil despite the ban on the use of DDT after 1972.

In 2000, NIST began value assignment of SRM 1946 Lake Superior Fish Tissue. SRM 1946 lies at the junction of sectors 4, 8, and 9 of the AOAC triangle. Concentration values are expected for proximates, individual fatty acids, and inorganic



analytes of nutritional interest. The material will also have values assigned for toxic trace metals, polychlorinated biphenyls, pesticides, and methylmercury; see Technical Activity 16.

In 1999, NIST held a workshop at the AOAC meeting in Houston, TX, to determine whether food-matrix reference materials needs had changed following the initial workshop held in 1997. High priority needs identified as a result of the follow-up workshop included SRMs for aflatoxins and allergens such as peanut protein. (Foods must be labeled if they contain – or may contain – unexpected allergens. Permissible levels of aflatoxins are also regulated.) A peanut butter SRM would address the needs for aflatoxin and allergenic peanut protein reference materials as well as providing a reference material in sector 3 of the AOAC triangle, which is not occupied by any other SRMs. Depending on the composition of the peanut butter, it could also serve as a reference material for *trans* fatty acids, for which labeling legislation is pending.

18. *Methods And Standards For Health Status Markers*

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Objective: To develop new methodologies and reference materials to support accuracy and traceability in health care measurements.

Problem: Inaccuracy in health-related measurements raises overall health care costs,

results in misdiagnoses, and leads to inaccurate conclusions in clinical studies. In addition, lack of scientifically sound reference methods and certified reference materials (CRMs) hampers traceability. With the EC IVD directive requiring traceability to recognized national standards, there is an increased need for reference systems for many analytes.

Approach, Results and Future Plans: NIST works closely with the American Association for Clinical Chemistry (AACC), the Centers for Disease Control and Prevention (CDC) and other organizations interested in health-related standards to prioritize our reference method and SRM development activities. Before SRM development can take place, NIST must have one or more reliable methods in house for certification. Consequently, NIST is undertaking development of methods for a list of analytes that have been identified as high priorities. Many of these analytes are large, complex biomolecules present at very low concentrations, requiring new approaches. The status of work on these analytes is described below.

Troponin-I Measurement of cardiac troponin I (cTnI) in serum provides a highly selective and sensitive means for diagnosing myocardial infarction. For clinical cTnI measurement, there are numerous cTnI assays that have been developed. Unfortunately, cTnI measurements using different methods on identical samples may differ by 100-fold, creating a serious problem for the clinical community. NIST, the American Association for Clinical Chemistry (AACC), and the International Federation of Clinical Chemistry (IFCC) have formed a subcommittee to address inter-method variability problems in clinical cTnI measurement, through development of a cTnI reference material to harmonize results. The subcommittee chose ten candidate reference materials to be evaluated through an interlaboratory comparison study. These ten materials include several different forms of troponin I, in frozen and freeze-dried states. The goal of the interlaboratory comparison study was to determine which form and preparation of cTnI material would best harmonize all cTnI clinical assays. The ten candidate reference materials were analyzed at NIST for purity and structural heterogeneity using liquid chromatography coupled with mass spectrometry (LC/MS). The relative cTnI concentration of each candidate

reference material was determined by LC and the materials were all diluted to a common cTnI concentration. The candidate reference materials were sent to all manufacturers of commercial cTnI assays used in the United States and Europe (13 manufacturers in total) for analysis using their cTnI assays. Results from these analyses were collected and subjected to statistical analysis that found that two candidate reference materials, both complexes of troponin I with other troponin isoforms, provided the most linear response for all thirteen cTnI assays. The next comparison study will involve these two materials and blood samples from patients with myocardial infarction. The candidate reference materials will be used to calibrate the commercial cTnI assays prior to measuring the patient samples. This study should determine which material that will best harmonize the commercial cTnI assays.

Folic Acid Folic acid is a water-soluble B vitamin that plays a significant role in the development of the central nervous system during the early weeks of gestation. A deficiency of folic acid during this time period prevents the neural tube from forming correctly that leads to spina bifida and other serious fetal malformations. Recently, the Food and Drug Administration (FDA) issued a regulation requiring that all enriched grain products be fortified with folic acid to reduce the risk of neural tube defects in newborns. Folic acid is directly involved in reducing the levels of the amino acid homocysteine in the body. High levels of homocysteine have been linked to the increased risk of cardiovascular disease and other diseases. The ability to determine folic acid status accurately is a significant challenge due to the existence of up to eight metabolites, many of which are unstable, and their low levels in plasma and serum (≤ 20 ng total folate/ml sample). A gradient elution liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) method for the separation and detection of the five most biologically relevant folates has been developed and applied to the quantitation of folates in human plasma/serum. The method involves the *in vitro* stabilization of plasma samples via a dual antioxidant system (*L*-ascorbic acid/*L*-cysteine), protein precipitation and sample concentration. The major circulating folate (5-methyl tetrahydrofolic acid) in human plasma/serum is detectable and quantifiable based on a standard

additions procedure using an endogenous plasma component as an internal standard. The other biologically relevant folates are not detectable in normal human plasma. Current efforts are focused on developing a folate quantitation procedure based on the use of external standard calibrants so that the LC/MS method might be more adaptable to routine clinical use. Additionally, our group is developing a second method based on the use of liquid chromatography with coulometric detection for the determination of folates in plasma.

Homocysteine Total homocysteine (tHcy) has emerged as an important independent risk factor for cardiovascular disease, as well as other serious health conditions. tHcy is measured clinically using a variety of methods including immunoassays and LC-based methods requiring extensive derivatization procedures and frequent analysis of quality control samples. Because clinical laboratories use a variety of different methods for its measurement, interlaboratory comparisons of tHcy measurements are poor. Work at NIST has focused on development of LC/MS and GC/MS methods. For the LC/MS method, chromatographic columns and elution conditions were investigated to achieve retention of homocysteine. This led to development of an elution system that allowed unambiguous detection of two homocysteine-specific ions and two isotopically labeled homocysteine-specific ions (internal standard) in human plasma. The quantitative capabilities of this approach will be tested in conjunction with the GC/MS method under development and in collaboration with scientists at the Mayo Clinic. Use of GC/MS requires additional separations and derivatization prior to analysis. An anion exchange method was found that provided the necessary analyte isolation. Thus, the GC/MS method should be ready for further testing in the near future. Once the methods have been validated, they will be applied to the determination of homocysteine in plasma-based reference materials.

Thyroxine Thyroxine is a hormone secreted by the thyroid gland that is a stimulator for a number of functions and is important in growth, development, and sexual maturation. The concentration of total thyroxine in serum is about 50-110 ng/mL, and is used as a measure of thyroid function.

A new method based on isotope dilution liquid chromatography/mass spectrometry using electrospray for ionization (LC/MS-ESI) has been developed for the determination of total thyroxine in serum. Thyroxine-d₅ is added as an internal standard and the serum treated with trichloroacetic acid for deproteinization to liberate thyroxine. Thyroxine is then extracted from the serum for LC/MS analyses. LC was performed on a C18 column with an isocratic mobile phase consisting of 0.1% acetic acid in acetonitrile/water (32:68) for positive ions, and 0.2% ammonium hydroxide in methanol/water (32:68) for negative ions. (M+H)⁺ ions at m/z 778 and 783 for thyroxine and its labeled internal standard were monitored for positive ions and (M-H)⁻ ions at m/z 776 and 781 for negative ions. Samples of CAP survey sera were prepared and measured on three separate sets. Excellent precision was obtained for all three levels of serum samples with within-set CVs ranging from 0.2-1.0%. Excellent linearity was obtained with the correlation coefficients of all linear regression lines ranging from 0.999-1.000. Positive and negative ion measurements agree within 0.8%. The detection limit at a signal to noise ratio of approximately 3 to 5 for thyroxine with this method is estimated to be 30 pg and 20 pg for positive and negative ion, respectively. The LC/MS-ESI method was tested against field methods (about 1900 laboratories from CAP surveys). The results of LC/MS-ESI method and field methods compared well with an average difference of 5% for all three levels. This method will be used to measure the thyroxine level in some existing serum-based SRMs. Future plans are to develop a new SRM with both low and elevated levels of thyroxine and cortisol. More than 99.9% of thyroxine in blood is bound to protein. There is considerable interest in measuring free thyroxine, but clinical methods give widely varying results. The isolation of the free thyroxine and its measurement by LC/MS will be investigated.

Thyroid-Stimulating Hormone Human thyroid-stimulating hormone (TSH) is a hormone secreted by the pituitary gland. Its role is in the stimulation of the thyroid gland to produce the thyroid hormones triiodothyroxine (T3) and thyroxine (T4). Serum TSH levels are used as a diagnostic tool for assessing thyroid function. Increased TSH levels can be indicative of hypothyroidism, a condition in which the thyroid

gland fails to recognize TSH and decreases its production of T3 and T4. TSH is structurally heterogeneous and present at very low levels in human serum, and therefore presents a significant measurement challenge. It is measured clinically using immunoassays, which are confounded by differences in antisera binding specificities, lack of internationally accepted standards, and the heterogeneity of TSH. These limitations often lead to significant variability in measurements of this analyte using different FDA-approved immunoassay kits. Work to date has focused on developing approaches for characterizing TSH preparations. Structural heterogeneity of untreated TSH preparations prevented accurate characterization using mass spectrometry. However, by treating TSH and other members of the glycoprotein hormone family with sialidase to remove sialic acid residues from the carbohydrate moieties on these proteins significantly improved their mass spectrometric characterization. Full characterization of the TSH preparations will require peptide mapping to verify identity and primary structure and characterization of carbohydrate heterogeneity. Future plans are to develop capabilities for measuring TSH levels in human serum using LC/MS or LC/MS/MS approaches and applying these methods to the certification of serum-based SRMs.

Iron-Containing Proteins Serum iron determinations are an important clinical measurement. Low serum iron levels are indicative of diseases such as anemia, rheumatoid arthritis, and certain infections. Hemochromatosis, the most common form of iron overload disease, is an inherited disorder that causes the body to absorb and store too much iron, often resulting in failure of the liver, heart, or pancreas. According to the Centers for Disease Control, hereditary hemochromatosis is the most common genetic disorder in the United States, with approximately one in one hundred people at risk for developing the disease. Typically, for the purpose of medical diagnosis, the total serum iron concentration is measured. However, in many iron-related diseases, the distribution of iron bound to serum proteins is altered. For improved medical diagnosis, it would be beneficial to determine the distribution of iron among serum proteins as well as the total serum iron concentration. Work has begun on development of methodologies that will lead to a reference material for speciated iron in serum, a material that could be used for the more routine

clinical measurements of total serum iron, but also used for the more advanced diagnosis of iron-related diseases. Initial work involved structural characterizations of iron-containing proteins found in human serum. The two important iron-containing proteins in serum are transferrin and ferritin. Transferrin is the most abundant iron-containing protein, and most clinical measurements involve transferrin determinations. The concentration of ferritin in serum is approximately one thousand times lower than that of transferrin. However, medical research is indicating that serum ferritin determination can often be a more selective diagnosis tool than determination using transferrin. Development of a quantitative method for serum transferrin using liquid chromatography coupled with mass spectrometry (LC/MS) and affinity chromatography is underway. The concentration of iron associated with the transferrin will be measured using high-resolution inductively coupled plasma mass spectrometry (ICP/MS). Together, the LC/MS and the ICP/MS measurements provide highly selective and high precision values for both serum transferrin and serum iron levels. The next goal to be accomplished is the development of quantitative methods for serum ferritin. This measurement will be more challenging due to the lower concentration of this protein and its structural heterogeneity. With quantitative methods to determine the concentrations of these proteins and their iron content, we will be able to produce a reference material that will meet the needs of the majority of clinical serum iron measurements performed.

Prostate-Specific Antigen Prostate-specific antigen (PSA) is a single chain glycoprotein (~ 7-8% carbohydrate) present in the prostate and seminal fluid. The presence of PSA in the serum is measured as a diagnostic indicator of prostatic cancer. Clinical laboratories currently measure PSA by immunoassays, which are confounded by lack of common standards, and the heterogeneity of the antisera and antigen being measured. Measurement of PSA using analytical methods is a significant challenge due to the structural heterogeneity of PSA in serum, its low concentration in this complex matrix (<10 ng/mL), and lack of knowledge regarding what form is actually being measured in clinical PSA tests. Current research efforts are focused on characterization of a variety of PSA standards at

the molecular level by LC/MS in order to understand further the heterogeneity of the analyte, arising predominately from differential glycosylation of the protein. Reproducible full scan (m/z 600-2500) positive charged mass spectra were obtained with a commercial source of PSA. Deconvolution of the mass spectrum showed a predominant molecular species at M_r 28,447; the predicted M_r for the glycosylated mature PSA is 28,430. Selected ion monitoring of specific positive charge states was utilized to detect PSA standards at levels as low as 50 ng. Further efforts will focus on: 1) production of a well-characterized SRM for improvement of between-method variations in calibration of immunoassay measurements, 2) determination of specific forms of PSA in patient samples to understand better the clinically relevant forms of this compound, 3) isolation of PSA from serum using non-immunological methodology including combinatorial production of aptamers with high binding affinities for PSA, 4) development of LC/MS and LC/MS/MS methods for the measurement of PSA in serum.

Glycated Hemoglobin Approximately 16 million people in the United States have diabetes, with nearly 2,200 new cases diagnosed each day. About 95% of the people with diabetes have type II disease (also called non-insulin-dependent diabetes mellitus). Those with type II produce insulin, but the cells in their bodies are "insulin resistant" -- they don't respond properly to the hormone, so glucose accumulates in their blood. Left untreated, diabetes can result in cardiovascular disease, kidney disease, eye diseases, and nervous-system maladies. In addition to the daily monitoring of blood glucose levels, the measurement of glycated hemoglobin (HbA_{1c}) plays an important role in the diagnosis and treatment of diabetes. When blood glucose levels rise above normal, the glucose can react with hemoglobin in the blood, forming HbA_{1c} . Since the lifetime of hemoglobin in the bloodstream is approximately 3 to 4 months, the measurement of HbA_{1c} provides a record of the levels of blood glucose over the course of 3 to 4 months. Glycated hemoglobin measurement provides doctors with information on the efficacy of diabetes treatment over a longer period than daily blood glucose measurements. While the medical utility of HbA_{1c} measurement has been clearly demonstrated, the clinical application of HbA_{1c} measurement has demonstrated

considerable problems with method-to-method variability. There is a strong need for HbA_{1c} measurement standardization. The International Federation for Clinical Chemistry (IFCC) has developed two reference methods for the determination of HbA_{1c} in blood hemolysates. One method uses liquid chromatography coupled with mass spectrometry (LC/MS) and the other used capillary electrophoresis (CE) for HbA_{1c} determination. Both methods have demonstrated high precision (average inter-laboratory coefficient of variance $\leq 2\%$) and excellent agreement with each other. These reference methods have been implemented in a network of laboratories worldwide. NIST has been a part of this network, participating in interlaboratory comparison studies of both IFCC reference methods and providing feedback to help improve these methods. Currently the IFCC reference methods are being used to develop an international HbA_{1c} reference material. Once this material is prepared, NIST will work with the IFCC and the Institute for Reference Materials and Methods (IRMM) in Belgium to validate the assigned value of glycated hemoglobin. The next stage in the development will be to assess how well this HbA_{1c} reference material harmonizes clinical HbA_{1c} measurements. This will be accomplished by an interlaboratory comparison study involving clinical laboratories throughout the United States and Europe.

Bilirubin Bilirubin, the orange-yellow bile pigment, is produced from protoporphyrin IX by microsomal heme oxygenase. Elevated levels of bilirubin in blood are indicative of impaired liver function. There are three principal isomers of bilirubin, which may be free or complexed with other blood constituents. Clinical laboratory measurements of bilirubin exhibit considerable variability because of method differences and calibration errors. Our goal is to develop an LC/MS reference method to measure bilirubin. Efforts have focused on first establishing the spectrophotometric reference method based upon a direct diazo reaction. Plans are to use this method as a tool for validation of the LC/MS method, and then use both methods for certification of bilirubin in serum-based SRMs.

Lead, Cadmium, Total Mercury, and Methyl Mercury in Whole Blood (SRM 966) Work has recently been completed for SRM 966, a whole blood reference material for evaluating the

accuracy of lead (Pb), cadmium (Cd), total mercury (Hg) and methyl mercury (MeHg) measurements in whole blood at natural and slightly elevated levels. Analysis of whole blood is one of the most common ways exposure to these toxic trace elements is monitored in the human population. New OSHA workplace monitoring standards now require measurements of blood cadmium and prior to this SRM, only proficiency samples with consensus cadmium values existed as “standards”. Also, a large number of prestigious organizations in the human health monitoring area (e.g. CDC, NIEHS, EPA, and WHO) have indicated their interest in biological SRMs (especially blood) certified for MeHg. A unit of this SRM consists of two vials from each level, containing approximately 2 mL of whole blood each. The bovine blood base material was prepared for NIST at a USDA licensed facility from cows bled after dosing with lead nitrate. The natural level pool was drawn without Pb dosing while the elevated level was taken after Pb dosing. The elevated pool was also spiked with MeHg (as iodide), inorganic Hg and inorganic Cd. The concentration levels of these toxic elements were adjusted to reflect realistic ranges observed for these analytes in affected human populations. For both levels, the endogenous Pb concentrations were certified by isotope dilution (ID) ICP-MS, a primary method for this analyte in clinical materials. Cd concentrations in the elevated level were certified by combining data from two independent methods, instrumental neutron activation analysis (INAA) and ID ICP-MS. Total Hg was certified in the elevated material by combining data from ID ICP-MS and cold vapor atomic absorption spectrometry (CVAAS). However, the extremely low concentrations in the natural level material posed significant measurement difficulties and resulted in informational values only for Cd and Total Hg concentrations in that material. MeHg was not measurable in the natural level material and its concentration was inferred in the elevated level blood by difference between measurements of the total Hg and the inorganic Hg at that level, in agreement with the spiked concentration. This material will support workplace and general public health monitoring for lead, cadmium, total mercury and MeHg in blood.

19A. *Methods and Standards to Facilitate Improved Industrial Processes*

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Objective: To promote U.S. economic growth by working with industry to develop and apply measurements and standards.

Problem: The development of new technology and products, coupled with the evolution and advancements of analytical instrumentation used by industry, results in an ever-expanding need for standards. The Division balances its broad program in compositional and chemical standards among continuously produced standards (e.g. primary materials), renewals (previously certified materials), and new standards. The Division works with broad-based industrial groups to identify and produce new standards—which typically will require the development of new analytical methodology or refinement of existing methodology—research in analytical methods.

Approach: Each project has its own unique characteristics. Yet, most projects also have common stages: an indication of need by some industry advocacy group; the development and definition of NIST capabilities; and, then, the application of these NIST capabilities in value assignment of a material available for broad distribution as an SRM.

Results and Future Plans: Examples of several on-going projects illustrate the range of industrial activities the Division supports. Note that several of the examples illustrate collaborations across the Divisions of CSTL.

SRM 2035/2035a Near Infrared Wavelength: NIR spectroscopy is a technique widely used in process control for a variety of industries. Through interactions with two committees of ASTM the need for optical filter wavelength standards in this wavelength region was established. In addition, technical workshops and meetings were held with users and regulators to refine these needs. Several prototype materials were evaluated; a round robin to potential end users in the chemical, polymer, pharmaceutical, and analytical instrumentation industries was carried out. Technical issues such as

homogeneity, temperature and environmental stability, polarization and bandwidth effects all had to be investigated. The first material has now been certified and is available. Work on adapting SRM 2035 for diffuse reflectance applications is under way.

Standards for Raman Spectroscopy: Raman spectroscopy is a technique that shares many of the advantages of NIR spectroscopy, but with enhanced information content. Unfortunately, the spectra acquired from this emission technique are highly instrument dependent. As a result no standard library of Raman spectra exists and the technique remains largely in the hands of specialists. The lack of performance validation standards also inhibits the technique's use in regulated industries, i.e. pharmaceutical, petroleum and health care.

The Analytical Chemistry, Process Measurements, and Surface and Microanalysis Science Divisions through interactions with the E13.08 ASTM committee, instrument vendors and specialists, have started a program for the development of intensity correction standards for Raman instrumentation. The first standard developed is a chromium-doped glass for use with spectrometers operating at 785 nm excitation, the most common industrially used excitation source. This reference material will be tested in FY2001 in a joint ASTM/NIST evaluation study. Additional standards are under study for the calibration of Raman systems operating with 532 nm, 1064 nm and 830 nm excitation sources. Development of these standards is anticipated to lead to the creation of an instrument-independent, Raman spectral library of organic compounds and the greater acceptance of the technique in regulated analytical applications.

SRM 1932 Fluorescein Concentration: The sensitivity and selectivity of fluorescence-based measurements have fueled a dramatic growth in biotechnology and drug discovery in recent years. Fluorescence detection is being used to map the human genome, to single out promising drug "leads" in combinatorial chemistry libraries of compounds, and to count diseased and drug-responsive cells in clinical diagnostics using flow cytometry. Standards are in growing demand in these areas, especially as assays become increasingly more quantitative.

Four NIST workshops were held with the fluorescence community over the past three years to establish the needs for standards in related industries. This fluorescein concentration standard is the first to arise from the planning effort and is scheduled to be released in 2001. Other fluorescence standards, including spectral emission, intensity and wavelength standards, are anticipated to follow.

SRMs 2850, 2851, and 2852 Zeolites: Zeolite catalysts are widely used in industry. Variations in silica-to-alumina ratios, pore sizes, surface area, cations, and incorporated metals produce a wide range of adsorbent and catalytic properties that are useful in many application areas. For instance, zeolites are extensively used as catalysts in the refining of crude oil into finished petroleum products. Zeolites are also environmentally friendly: non-hazardous, regenerable, and non-corrosive. They mitigate pollution by minimizing the production of pollutants and by treatment of effluents.

The need for these reference materials was identified at a National Science Foundation sponsored workshop with participants from the research community, users, and suppliers. The lack of common materials for comparative measurements was identified as an important barrier to the development of new materials. These SRMs (and uncertified reference materials that also will be made available) will provide a basis set of zeolite types to benchmark physical and chemical measurements.

Chemical composition measurements were made in the Analytical Chemistry Division using X-ray Fluorescence Spectrometry, and nuclear and classical methods. The physical parameters (particle size and lattice parameters) were measured in the Surface and Microanalysis Science Division, which championed this project, and the Materials Science and Engineering Laboratory.

SRM 2134 Ion-Implanted Arsenic in Silicon: Ion implantation is a key technology for the semiconductor industry, with spatial-concentration profiles characterized typically by secondary ion mass spectrometry (SIMS), a technique that requires standards for calibration. The variability among results for recent

interlaboratory comparisons have demonstrated a real need within the industry for these standards. The SEMATECH Analytical Managers' Working Group identified the development of this SRM as one of their top priorities for implants in silicon. An accuracy-based standard would permit the transfer of technology from one manufacturing site to another, and would allow a more useful comparison of experimental data with theoretical process models.

The Surface and Microanalysis Science and Analytical Chemistry Divisions have produced this SRM—a silicon wafer that has been certified for total dose of implanted ⁷⁵As per unit area—to meet the needs of the semiconductor industry. Additional information about the concentration of As atoms as a function of depth below the surface was provided by SIMS. The arsenic content was certified using instrumental neutron activation analysis (INAA) as a primary method. Since variability of SIMS measurements can be kept to within a few percent when the measurement process is carefully controlled, the relative (expanded) uncertainty for the arsenic certification in the SRM was targeted at 1 % on a relative basis. To achieve this level of accuracy, and to document the actual expanded measurement uncertainty, each individual uncertainty source was explicitly evaluated. A total of 29 individual uncertainty components were evaluated, and the final relative expanded uncertainty achieved was 0.38% for a total arsenic content of 90 ng/cm². The largest individual source of measurement uncertainty was observed to be that of the amount of arsenic in the calibration solutions.

SRM 1848 Lubricating Oil Additive Package: This SRM is the first certified reference material of its type and will benchmark accurate measurements of a very important industrial commodity (the value of the U.S. finished lubricants market was \$2.85 billion in 1999). Six industry laboratories collaborated in the certification and physical property characterization of the material. A total of 7 elements were certified: B, Mg, P, S, Cl, Ca, and Zn. Nuclear methods, atomic spectroscopy, XRF, and mass spectrometry were applied at NIST. The median uncertainty was 3 %, meeting the industry's need. In recognition of the successful completion of this project, the ASTM Committee on Elemental Analysis of Petroleum

Products awarded a Certificate of Appreciation to John Sieber for his technical leadership at NIST.

19B. *Methods and Standards to Facilitate Improved Industrial Processes: Moisture in Crude Oil*

S.A. Margolis, L.C. Sander, and S.A. Wise

Objective: To develop for the petroleum industry a method and Reference Materials to accurately measure the water in crude oil and other oils that contain significant amounts of substances that interfere with the Karl Fischer method for measuring water.

Problem: A variety of compounds (including mercaptans and other sulfur containing compounds) reduce iodine to iodide, and are present in crude oils and refined oils. These substances can bias the measurement of water in oil products, as determined with the primary assay technique, the Karl Fischer method. This is of significant economic importance in the crude oil industry because crude oils contain appreciable amounts of both sulfur compounds and water. Refineries do not wish to pay for water and the crude oil producers do not want the water content over estimated. There are no methods currently available for measuring these interfering substances in oils except for the method that we have developed.

Approach: In recent years research activities in the Organic Analytical Methods Group have been directed toward improving the accuracy of the measurement of water in all types of organic materials. This has led to SRMs for the calibration and standardization of instruments and refined oil reference materials for the measurement of water. In a recent collaborative study on the measurement of water in crude oil candidate SRMs 2721 and 2722 we observed a very large relative standard deviation that was method dependent. This indicated that the oils contained material that interfered with the measurement process and spuriously increased the amount of water detected. In order to evaluate this observation it was necessary to develop a new method for measuring these interfering substances coulometrically. The availability of a new instrument that allows the manipulation of measurement parameters has

facilitated our development of methodology to estimate non-water interferences. This approach uses methodology and reagents that are very similar to the Karl Fischer method; however, no reaction with water can occur.

Results and Future Plans: A reagent has been formulated which contains imidazole, trichloroacetic acid, potassium iodide, and sodium thiosulfate in methanol: xylene, 6:4 (v/v). The conditions for using this reagent with a Metrohm coulometer model 756 have been optimized. The standard used for this process was 0.1 mol/L sodium thiosulfate. Preliminary results with a representative group of refined and crude oils including SRMs and RMs indicate that refined oils may contain 5-10 mg/kg of interfering substances and that crude oils may contain from \approx 5 mg/kg (low sulfur crude) to \approx 800 mg/kg (high sulfur crude) of interfering substances. The total amount of material reacting with the Karl Fischer reagent in the high sulfur crude oil was 956 mg/kg indicating that 84% of the measured material was not water. In the case of RM 8506a the interfering substances were 7.6 mg/kg (40 % of the coulometrically measured water) and in RM 8507 the value was 0. These results demonstrate the utility of this method in the accurate measurement of small amounts of water in the presence of compounds that reduce iodine. It also has applications and economic significance in a great number of chemical industries. It has the potential to be used as a direct assay for compounds that reduce iodine.

Future plans: Plans include (1) the validation of this method, (2) its application to the measurement of the interfering materials in current RMs and the crude oil SRMs, (3) the measurement of water in oils such as motor and lubricating oils which also contain interfering substances, (4) the patenting of this method for which we already hold a preliminary patent and (5) the submission of this method to ASTM as a standard method to be used in conjunction with the Karl Fischer method for the measurement of water in a wide variety of oils.

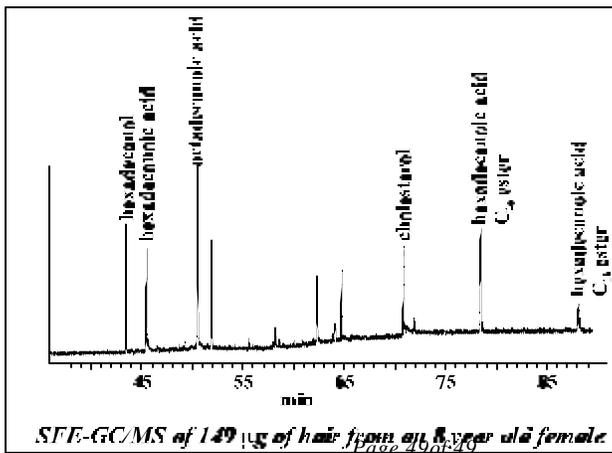
20. Providing Measurement Tools and Standards for Forensic Chemistry

G.W. Kramer, L.C. Sander, Bruce A. Benner, David L. Duewer, Margaret C. Kline (831), William A. MacCrehan, Karen Phinney, Janelle D. Secl, and Michael J. Welch

Objective: To provide a leadership role in developing and evaluating new measurement technology to aid forensic investigators in fighting crime. To support the validation of forensic measurement methods, we provide reference materials and conduct interlaboratory studies.

Problem: Crime laboratories are being called upon to investigate ever more sophisticated crime. Forensic investigators are heavily burdened with casework, providing little or no opportunity to develop new, more effective chemical measurement tools. Concurrently, the admission of scientific evidence is coming under increased scrutiny in the courtroom.

Approach: ACD is addressing the needs of the forensic chemical measurements community in two principal ways: (1) developing and evaluating new measurement technology that will have applicability in criminal investigations; and (2) providing reference materials and interlaboratory comparison studies to help forensic laboratories validate their methods and to provide compelling evidence of competent measurement performance. We are addressing forensic measurement needs in a number of key areas: human identification through the use of DNA and hydrocarbon signatures, accurate evaluation of drugs of abuse in hair, and the determination of handgun and pipe bomb use through organic gunpowder analysis.



Results and Future Plans:

Our division has a number of ongoing projects addressing specific measurement needs:

Human Identification Based on Hydrocarbon Analysis

Problem: Hair is routinely collected at crime scenes for subsequent forensic analysis. This typically involves inspection of the evidence by microscope techniques to determine the color, thickness, and shape of the hair. Recent advances in characterizing mitochondrial DNA (mtDNA) enable genetic profiling of small hair samples without root cells, but given that maternal relations yield indistinguishable mtDNA sequences, these techniques have their limitations. A method for determining chemical characteristics of small samples of hair could aid law enforcement officials in narrowing the pool of suspects of a crime.

Approach: For a number of years, workers in the Organic Analytical Methods Group have investigated methods for chemically characterizing small samples. One of the more promising techniques is on-line supercritical fluid extraction gas chromatography/mass spectrometry (SFE-GC/MS). The method offers a number of benefits including greater sensitivity compared with liquid extraction-based techniques because the entire extractable mass is transferred to the analytical system, compared with only a few percent from a conventional liquid extraction/injection. Another advantage of this technique is higher recoveries of volatile species, components that would be lost during multi-step liquid extraction and concentration procedures. Finally, the mass spectra of species detected by this method can confirm their identities. Application of SFE-GC/MS to small hair samples has yet to be discussed in the scientific literature, but might provide useful chemical information for forensic investigations, complementing morphological and genetic data. Toward this end, we have employed SFE-GC/MS to measure components associated with small samples (100 μ g to 1 mg) of human hair from 20 volunteers to investigate if the method yields consistent yet unique chemical profiles from peoples' hair.

Results and Future Plans: Components detected in hair after SFE-GC/MS include fatty acids, alcohols, fatty acid methyl esters, squalene, cholesterol, and a number of other species from secretions of the sebaceous and sweat glands in the scalp, shampoos and hair treatments, and from environmental exposure (Cf. figure). This preliminary study, while considering a modest number of head hair samples ($n = 20$), suggests that analysis of the surface components of small hair samples by SFE-GC/MS may help match hair samples taken at a crime scene with those of specific individuals. Further research is planned including utilizing a larger and more statistically developed data base of hair samples, investigating the effects of archiving on the chemical profiles of an individuals hair, and employing other analytical techniques in addition to GC/MS (e.g., liquid chromatography/mass spectrometry) for measurement of components in hair. A National Research Council post-doctoral fellow will be working in the Organic Analytical Methods Group for the next two years on refining both the analytical methods and data evaluation techniques for this project.

Validation of Multiplexed Short-Tandem Repeat (STR) DNA Typing Systems

NIST has conducted a series of interlaboratory studies of DNA typing methodologies used for human identification. These challenge studies evaluate forensic measurement, interpretation, and reporting practice using well-characterized samples of very different analytical difficulty. Most recently, Mixed Stain Study 1 (Apr-Nov 1997) and Mixed Stain Study 2 (Jan-May 1999) evaluated multiplexed short-tandem repeat (STR) DNA typing systems with samples containing DNA from more than one source. None of the relatively few errors reported in either exercise resulted in a false identification of a reference source; however, several errors in evaluating the unknown source in three-source samples would hinder profile matching in any archival database. None of the measurement anomalies reported is associated with any particular STR multiplex; all DNA amplification anomalies are associated with inefficient DNA extraction, inaccurate DNA quantitation, and/or analytical threshold policies. Mixed Stain Study 3, currently scheduled to begin sample distribution in Nov 2000, will more directly examine the linkages between DNA quantification and the intensity of the ultimate STR signals.

Drugs of Abuse In Human Hair

Objective: To develop analytical methodology and Standard Reference Materials for the determination of drugs of abuse in human hair.

Problem: The use of hair analysis to detect drug use is growing rapidly and is now used by many organizations in their hiring process as a tool for rejecting possible drug users. This type of drug testing is finding increased use in monitoring parolees for evidence of drug use and is even being used in custody cases to support or refute claims of drug use. While experienced laboratories can generally reliably detect most common drugs of abuse in hair following carefully tested and controlled procedures, there are still problems with this approach. One question that has never been satisfactorily resolved is whether drugs found in hair actually come from illegal drug use. Amphetamine and methamphetamine are commonly abused stimulants that can be prepared through a variety of synthetic pathways. Both compounds have been detected in hair samples of drug users. Because amphetamine and methamphetamine are chiral molecules, they can exist as pairs of enantiomers. The (l)-enantiomer of methamphetamine is found in legitimate pharmaceutical products, but clandestine synthesis often produces racemic compounds. Therefore, proof of illicit use of these stimulants requires an examination of enantiomeric composition of the compound of interest. In addition, the enantiomeric ratios can provide an indication of the synthetic route used to prepare the drug. Current analytical methods for the measurement of enantiomers of these stimulants typically involve derivatization with enantiomerically pure derivatizing agents to form diastereomers that are separated by gas chromatography (GC). However, most chiral derivatizing agents are not enantiomerically pure, and impure reagents may perturb the initial enantiomeric composition of the sample, leading to erroneous or misleading results.

For laboratories that are using hair testing to detect drug use, it is important that they validate their methods regularly. Reference materials are an important means that laboratories can use to assure that they are making accurate measurements.

Approach: Liquid chromatographic (LC) methods have not traditionally been used for the measurement of drugs of abuse in hair because they do not provide adequate sensitivity. However, the use of fluorescence detection can dramatically enhance the sensitivity of LC methods. We evaluated an achiral fluorescent tagging agent for the derivatization of amphetamine and methamphetamine enantiomers. The tagging agent serves the dual role of increasing sensitivity and facilitating enantiomeric separation of the compounds of interest.

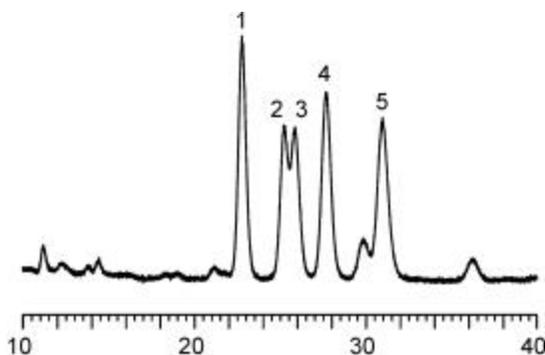
NIST has begun development of a new SRM consisting of two materials, both consisting of human haircut into short segments, and each containing four to five analytes. The compounds to be incorporated include: cocaine, benzoylecgonine, phencyclidine, amphetamine, methamphetamine, morphine, codeine, monoacetylmorphine, and tetrahydrocannabinol.

Present plans are to have the first five of these analytes in one material and the last four in the other. The material is prepared by collecting a large sample of drug-free hair, manually cutting it into short segments (1 – 5 mm), soaking it in dilute hydrochloric acid to neutralize basic residues, and then soaking the hair in solutions of dimethylsulfoxide containing the target analytes. The hair is soaked for several weeks to assure deep penetration of the analytes into the hair matrix. The hair is then rinsed in methanol to remove drugs from the surface and then is analyzed by GC/MS to determine the drug levels. If analyte levels are not within target ranges the hair is soaked further to either boost or reduce the levels. Once satisfactory levels are achieved, the hair is bottled and a statistical sampling is analyzed. Certification measurements will involve GC/MS and LC/MS or LC-fluorescence measurements at NIST and results from outside laboratories.

Results and Future Plans: We developed a liquid chromatographic method that incorporated a cyclodextrin-based chiral stationary phase for the separation of amphetamine and methamphetamine enantiomers. Although this chiral stationary phase provided the desired chiral resolution of the enantiomers, peak overlap prevented simultaneous determination of

amphetamine and methamphetamine enantiomers. A C₁₈ bonded phase was coupled in series with the chiral column to improve achiral separation of the compounds and to reduce interferences. The results shown illustrate the chromatographic separation on the coupled column system of amphetamine and methamphetamine enantiomers isolated from a spiked hair sample.

The fluorescent tagging reagent was easy to use, and the derivatives were stable for several days. We anticipate that this methodology will provide an alternative to GC methods for the separation of amphetamine and methamphetamine enantiomers from hair samples. Work is underway to evaluate this methodology for certification of amphetamine and methamphetamine in the new SRM for drugs of abuse in hair being developed. This material is being treated with the target compounds and will undergo testing to determine if appropriate levels have been achieved.



Separation of amphetamine and methamphetamine enantiomers extracted from a spiked hair sample. Peaks are (1) internal standard, (2) l-methamphetamine, (3) d-methamphetamine, (4) l-amphetamine, and (5) d-amphetamine.

Interlaboratory Comparison of Gunpowder Measurements

The accurate determination of the additive composition of gunpowder has impact in two important arenas: forensic investigation. Forensic labs determine the presence of propellant nitroglycerin and stabilizers such as diphenylamine and ethyl centralite as a means of individualizing gunpowder and its post-firing residues for investigation of handgun and pipe bomb crimes. Recently, the Analytical Chemistry Division (ACD) has been studying the use of accurate compositional measurements to associate handgun-fired and pipe-bomb residues

with source gunpowder. Using an ultrasonic solvent extraction/capillary electrophoresis technique, ACD researchers have evaluated the compositional characteristics of handgun residues in comparison to unfired gunpowder samples. Residues obtained by test firings of both known "reloaded" and commercial ammunition have allowed development of an approach for the association of residues with specific gunpowder samples based on calculating a numerical propellant/stabilizer ratio. Forensic investigators may someday use this compositional ratio to link residues recovered from a suspect's hands or pipe-bomb debris to unfired gunpowder samples.

Accurate gunpowder measurements are needed for forensic investigators to provide courtroom-defensible results and for military laboratories to provide reliable safety monitoring. To help these laboratories evaluate their measurement proficiency, NIST conducted an interlaboratory comparison study of gunpowder additive determination this last spring. Participating laboratories were quite enthusiastic about receiving the two test gunpowder samples, as there are currently no such test materials available for demonstrating their explosives/propellant measurement proficiency. The nineteen participants represented US national, state, and regional, international forensic agencies along with three military labs. Almost all of the additive component identifications were in agreement between laboratories. Quantitative compositional evaluations were in coarse agreement with NIST values, with the determination of the unstable nitroglycerin additive providing the most variability in the measurements. The interlaboratory study pointed to the need to develop a NIST Standard Reference Material, to evaluate and validate methods to assure the quality of forensic and military gunpowder measurements.

21. Formal Programs for Providing NIST Traceable Reference Materials from Commercial Sources

W.E. May, F. Guenther, W. Dorko, G.W. Kramer, J.C. Travis, G. Turk and J. Fassett and R. Greenberg

Objective: To establish formal programs to facilitate the commercial production and distribution of reference materials with a well-defined (and NIST recognized) traceability linkage to NIST.

Problem: 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 issued under NIST 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 provided to the user community for achieving chemical measurement quality assurance and traceability to national standards. Currently, NIST provides nearly 1400 different types of SRMs and in FY00 sold over 34,000 SRM units to approximately 6,550 unique customers; over 18,000 units of these represent about 840 different types that are certified for chemical composition. NIST does not have the resources necessary to provide — by itself — the increasing quantities and specific varieties of certified reference materials (exact sample types, unique compound combinations, concentrations, etc.) needed to meet increasing needs.

Approach: The NIST Traceable Reference Materials (NTRM) program has been created as part of the solution to this problem. 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. Initially the NTRM concept was implemented in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the “Emissions Trading” provisions of the Clean Air Act of 1990 (while facing the reality of constant human and financial resources at NIST). The program has been highly successful and since its inception, ten specialty gas companies

have worked with us to certify over 8500 NTRM cylinders of gas mixtures, which have been used to produce approximately 500,000 NIST-traceable gas standards for end-users. Production of an NTRM batch required 5 months on average, whereas, SRM production requires over 1 year. A formal study of the economic impact of the gas NTRM program has been initiated by NIST.

The NTRM model for the commercial production of reference materials is being extended to other mature and high volume areas so that more of our resources can be diverted to address new and/or more difficult high priority measurement problems. For the benefit and protection of both the users and providers of these materials, NIST has trademarked the term NIST Traceable Reference Material (NTRM) in order to restrict its use to only those materials that meet NIST-defined criteria and specifications. Brief descriptions of each of the four areas where NTRM programs are in place or are being explored follow.

Gas NTRM Program (*F. Guenther and W. Dorko*)

The Gas NTRM program was defined to achieve maximum customer confidence in NTRM gas mixture products from Specialty Gas Companies (SGCs). At the time the program was defined, it was thought that direct NIST involvement in the certification of the each NTRM batch was desirable and necessary. Through this approach, we have gained acceptance of the NTRMs by standards users, including the EPA and the stationary source monitoring community. We feel that the program can evolve to lessen the direct involvement of NIST in every NTRM batch. During the past year, NIST held a meeting with the SGCs and proposed an alternative certification approach available to SGCs that have demonstrated success through the production of NTRMs over a three-year period. In this new approach, a particular SGC facility could be certified such that any gas cylinder analyzed on its NIST certified analytical system would have the potential of being named an NTRM. Adequate controls would be imposed by NIST to assure quality control and traceability to NIST. It is hoped that this approach would enhance the availability of gas NTRMs such that they will replace gas SRMs as the primary traceability vehicle to U.S. Customers. Another eventual outcome of this new approach would be

the worldwide acceptance of gas NTRMs. At this time, the program is continuing in its original form while each SGC is evaluating how this new alternative approach would be implemented within its company. A Spring 2001 meeting with NIST and interested SGCs will address questions and/or modifications pertaining to the proposal and its implementation.

Reference:

NIST Special Publication 260-126: "The NIST Traceable Reference Material Program for Gas Standards" [see <http://www.cstl.nist.gov/nist839/839.03/index.html> and <http://www.cstl.nist.gov/nist839/839.03/ntrmdoc/default.htm>]

Optical Filters NTRM Program (*G.W. Kramer and J.C. Travis*)

NIST has produced Standard Reference Materials for calibrating the wavelength scale and verifying the absorbance accuracy of UV/visible chemical spectrophotometers for several decades. The NIST production capacity is rapidly becoming insufficient to meet the demand, and a recently developed program to leverage NIST measurement capability through the private sector is being adapted to these standards. These NIST Traceable Reference Materials are produced and marketed commercially, but with the active participation of NIST in the testing and value assignment of the standards. The first NTRMs for chemical spectrophotometry will be on the market within the coming year, and will be modeled on NIST SRM 930e and NIST SRM 1930, neutral density glasses (in a cuvette-simulation format) certified at five wavelengths in the visible spectral region and spanning absorbances between 0.3 and 2.0.

The expanded uncertainties for the certified values will be kept close to those of the corresponding NIST standards by reducing the recertification period from two years to one, which will be compatible with the renewal cycle of many quality control protocols and will provide more frequent cleaning of the filters. The certifying laboratories will be accredited through the NIST-based National Voluntary Laboratory Accreditation Program (NVLAP) and will maintain periodic intercomparison measurements with the National Reference Spectrophotometer in the Analytical Chemistry Division of NIST.

Other spectrophotometric NTRMs for wavelength calibration and UV absorbance verification are expected to follow.

References:

NIST Special Publication 260-140: "Technical Specifications for Certification of Spectrophotometric NTRMs"

NIST Handbook 150-21: NVLAP Chemical Calibration: Certifiers of Spectrophotometric NTRMs [see <http://ts.nist.gov/ts/htdocs/210/214/214.htm>]

Elemental Solutions NTRM Program (*G. Turk*)

At a 1999 workshop held at NIST for major commercial producers of elemental solution standards, a proposed NTRM program was presented. Key elements of this program include:
-*The introduction of a new series of SRMs. Elemental Primary Standards, or EPS SRMs.*
-*A calibration transfer method for comparing an NTRM against EPS SRMs.* These measurements would be performed or contracted by the NTRM provider.

-*Proficiency testing.* Assessing the ability of the NTRM provider to distinguish between slightly different amounts of analyte in different EPS containers has been proposed, and could be combined with the calibration function by keeping the EPS values unknown to the NTRM providers.

-*Software for value-assignment of the NTRM with the appropriate uncertainty based on the data of the calibration transfer method.* The software (which might be web-based) would "unlock" the assigned value only if the proficiency test is passed.

-*Periodic assessment of NTRM lots by NIST.*

Aspects of the described NTRM program are being developed as part of changes in the NIST SRM Single Element Solution Standards Certification Program such as the designation of selected materials as NIST Primary (NP) materials and the preparation and use of weighed aliquots of NP solutions (prototype EPS) for value assignment of SRMs in the current program using specially designed high precision ICPOES methods.

However, advances in the NIST SRM Single Element Solution Standards Certification Program has greatly increased the reliable availability of these SRMs from NIST. In addition, NIST is taking steps to facilitate self-asserted traceability for commercial standards to our SRMs in this program. A statement regarding traceability has been added to these SRM certificates and a publication is being developed that will provide additional information and guidance. Therefore, the need for an NTRM program in this area will be reevaluated during the coming year.

Metal Alloy NTRM Development (*J. Fassett and R. Greenberg*)

The steps in producing metal alloy NTRMs were outlined at a 1999 meeting with Analytical Reference Materials International, a secondary metal alloy standards producer that has agreed to work with NIST to pioneer the NTRM concept for metals. A protocol document has been prepared and discussed. However, this company recently changed ownership and, although the new management has stressed the desire to continue with the process, the company has been focusing on other aspects of their operations. Nonetheless, we hope to test the protocol this year with the production of the first three NTRM candidate materials as previously proposed:

- AISI Low Alloy Steel
- Chrome-Moly Steel F-11
- AISI 1030 Carbon Steel

22A The Micronutrients Measurement Quality Assurance Program

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

Objective: The Micronutrients Measurement Quality Assurance (QA) Program was organized to support measurement technology for selected fat- and water- vitamins and carotenoids in human serum and plasma.

Problem: Inaccurate results and data of unknown quality can have significant economic and health consequences. Also, for monitoring programs to compare data among laboratories, the performance of the participating laboratories needs to be evaluated on similar samples.

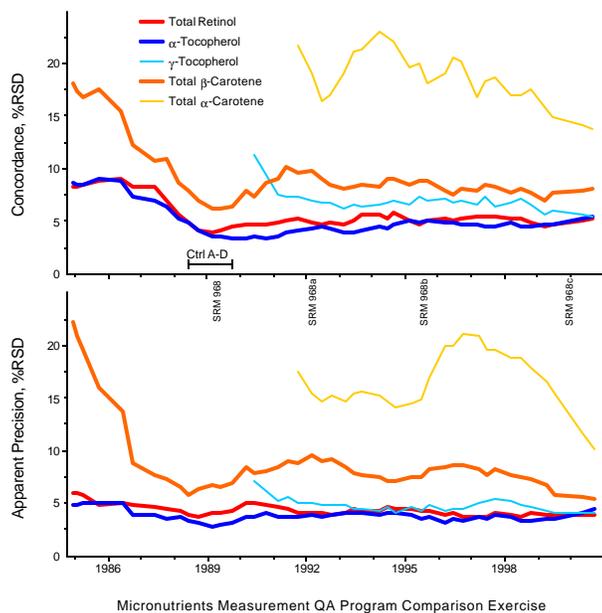
Approach: This program was initiated in 1984 as part of investigations supported by the National Cancer Institute (NCI) Division of Cancer Prevention and Control to study the possible role of these analytes in reducing the risk of developing certain types of cancers and diseases. The program initially supported a core of approximately 10-NCI grantee laboratories and has since expanded to include more than 60 laboratories worldwide. Today, the program is 100% fee-supported and is “the only QA program available for the fat-soluble vitamins and carotenoids.”

One of the services that the QA program provides to laboratories is measurement comparability assessment. NIST provides laboratories with the tools for comparability assessment through use of interlaboratory comparison studies, Standard Reference Materials (SRMs) and control materials, and methods development and validation. Serum-based samples with assigned values for the target analytes and performance-evaluation standards are distributed by NIST to laboratories for analysis. NIST staff provide the laboratories with technical feedback concerning their performance and suggestions for methods development and refinement. The results from the comparison studies are used to establish a laboratory performance database, which is used to help laboratories to improve their measurement comparability and to obtain reliable data needed to make accurate clinical and health-care decisions.

Results and Future Plans: As a result of the QA program, the accuracy of laboratory measurements resulting in increased interlaboratory comparability for retinol, α -tocopherol, and β -carotene has improved substantially over time (see figure below). The average estimated coefficient of variation for retinol and α -tocopherol has been approximately 5% for the past five years and about 10% for β -carotene for that same period of time.

Future plans for FY 2001 include the continuation of the round robin exercises for selected vitamins in serum, including the measurement of coenzyme Q10 and vitamin K1. As an effort to continue the program beyond FY 2001, a two-phase plan is currently underway to expand the program. Beginning November 2000,

an external QA web page will be available. Phase I will allow customers to enroll into the program via the internet. They will also be able to register on-line for the upcoming QA workshop, which will be held as a symposium at the Experimental Biology conference on April 4, 2001 in Orlando, Florida. Phase II (pending resources) will provide electronic data entry and feedback with security capabilities.



22B. Measurement Quality Assurance Programs: Contaminants in the Marine Environment

M.M. Schantz, R.M. Parris, and S.A. Wise

Objective: To assess the comparability of analytical measurements among the marine environmental measurement community.

Problem: Inaccurate results and data of unknown quality can have significant economic and health consequences. Also, for monitoring programs to compare data among laboratories, the performance of the participating laboratories needs to be evaluated on similar samples.

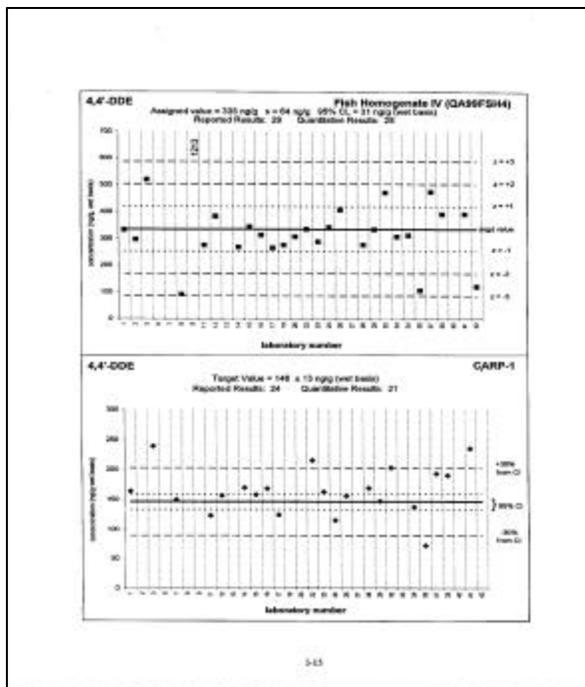
Approach: Since the beginning of the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Program in 1987, NIST has coordinated annual intercomparison exercises for the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl (PCB) congeners, and chlorinated pesticides in marine tissue and sediment samples. In the most recent exercise, over 40 federal and state government, private, and university laboratories reported results on 18 PCB congeners, and 24 chlorinated pesticides in a fresh frozen fish tissue homogenate and on 23 PAHs, 18 PCB congeners, and 24 chlorinated pesticides in a frozen marine sediment material. This is a performance-based program so each participating laboratory uses the methods currently being used by that laboratory for the analysis of similar materials. The laboratories are requested to submit data from three replicate determinations of the “unknown” samples along with results from concurrent analysis of appropriate Certified Reference Materials (CRM). Brief descriptions of extraction, cleanup, and analytical procedures are also requested.

The data received are combined to provide an assigned value for each “unknown” sample. In order to be used in the determination of the assigned values, a lab’s results for the associated CRM had to be within 30 % of the confidence interval for the analytes listed as certified on the Certificate of Analysis. Outliers are determined by statistical tests and knowledge of potential coelutions based on the laboratories methods.

Results and Future Plans : In the 1999 exercise, Fish Homogenate IV (candidate NIST SRM 1946) and Marine Sediment IX (candidate NIST SRM 1941b) were distributed. Results were received from 42 laboratories. An example of the results is shown below for 4,4’-DDE in the Fish Tissue.

Samples of Mussel Tissue X, candidate SRM 1974b, and Sediment X, the fine fraction from the preparation of SRM 1944, have been sent out for the current exercise. The target list of

analytes this year has been increased to 26 PAHs, 25 chlorinated pesticides, and 25 PCB congeners. This is the first year that all of the laboratories participating will reimburse NIST for the participation costs. Participants include federal, state/municipal, university/college, private sector, and international laboratories. Laboratories participate to demonstrate their capabilities when competing for grants and contracts. Participation in the program is written into a number of federal government solicitations, including at least one Mineral Management Service and eight U.S. Environmental Protection Agency solicitations in the last year.



22C. *First Year Implementation of Commercial Proficiency Testing Studies for EPA/States Water Programs by NIST-accredited Providers*

R.M. Parris, W.E. May, and C.D. Faison (NIST National Voluntary Laboratory Accreditation Program)

Objective: To provide a system under which private sector companies and interested states are accredited by NIST to provide proficiency testing (PT) that meets the needs of EPA and states to those laboratories testing drinking water and wastewater for regulated chemical, microbial, and radiochemical parameters.

Problem: Since the 1970's, EPA has conducted semiannual proficiency testing to assess the competence of over 6,000 public and private sector laboratories to conduct analyses required by the Clean Water and the Safe Drinking Water Acts. In 1998, the cost-free provision of these services was phased out to be replaced by a multiprovider system in which interested states and private companies provide these PT services on a fee-basis. NIST-provided mechanisms and tools are required for PT providers' use in measurement quality assurance and to enable appropriate government oversight of these programs.

Approach: In a government-private sector partnership, NIST worked with the EPA, States, and other public and commercial entities to establish appropriate oversight of this new effort to externalize and improve the nation's environmental laboratory PT programs. Under this arrangement:

EPA:

- provided NIST with support to develop a program for private sector/state provision of PT studies, and
- works with States and NIST to assure that the program developed for (1) preparing, value-assigning and distributing PT samples and (2) evaluating the quality of the Environmental Testing Laboratory data is sufficient to support national/state water program needs.

NIST:

- developed and manages program for third-party accreditation of private sector water PT study providers,
- establishes and maintains SRMs to support the program, and
- conducts blind sample audits of the commercially supplied PT samples on an ongoing basis as part of our QA responsibility for the program.

PT Study Providers:

- develop, manufacture, value-assign, and distribute PT samples,
- score results of Environmental Testing Laboratory analyses,
- report results to participants, EPA, NIST, States, and appropriate accrediting authorities, and
- maintain accreditation through NIST National Voluntary Laboratory Accreditation Program (NVLAP).

Results and Future Plans:

NIST Handbook 150-19, Chemical Calibration: Providers of Proficiency Testing handbook, developed to describing the technical requirements of this accreditation program, is used by NIST NVLAP in accrediting laboratories for their competence to characterize samples and to conduct proficiency test programs to support USEPA requirements for environmental laboratories as described in EPA's "National Standards for Water Proficiency Testing Studies: Criteria Document" (US EPA, December, 1998 Version).

The first class of accredited providers was announced by NIST/NVLAP in October 1999 and there are currently 12 accredited providers covering all 48 chemistry and microbiology PT program fields. In May 2000, NVLAP program designations for providers of radiochemistry PT studies were added to this program. A listing of the accredited providers and the specific program codes for which they are accredited are listed at the NVLAP website, <http://ts.nist.gov/nvlap>. The current June 1999 version of NIST Handbook 150-19 can also be obtained at this site.

NIST is producing primary benchmark materials such as SRMs for those parameters not covered by existing SRMs to provide the infrastructure needed by NIST to audit the commercially

supplied PT samples and to assist providers in value-assigning their PT materials. Twenty-six candidate semivolatile organic solution SRMs and 15 volatile organic solutions of semivolatile organics have been prepared and certification measurements have been completed on 29. Additional SRMs are planned. See Technical Activity Report 16 (Activities to Support Environmental Measurements) of this document for additional information.

Technical oversight of the program and the evaluation process is provided by the NIST/ACD with proficiency testing for the radiochemistry program designations to be conducted by NIST's Ionizing Radiation Division. The first year of commercial provision of these PT studies has proved to be quite successful - although many discussions have been required to provide information, to reach consensus about interpretation of various issues, etc. A series of working meetings with various stakeholder sectors are planned prior to the first Annual NIST/EPA PT Meeting that is scheduled for 16 August 2001 to provide a review and a forum for discussion of this program.

23. *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*

W.E. May, R.M. Parris, C.M. Beck II, J.D. Fassett, R.R. Greenberg, F.R. Guenther, G.W. Kramer, and S.A. Wise; and T. E. Gills, J.C. Colbert, R. Getting, and B. MacDonald (Div. 232)

Objective: To develop a document which provides definitions of terms and descriptions of current practices used at NIST for value-assigning Standard Reference Materials (SRMs) for chemical composition and related properties.

Problem: NIST SRMs are used worldwide for providing quality assurance for chemical measurements. Chemical measurements are becoming increasingly important in international trade decisions and addressing healthcare, environmental and safety-related issues. There has been a recent proliferation of commercial and government-based reference material producers

and the current ISO definitions for Reference Materials (RMs) and certified Reference Materials (CRMs). For example, a Reference Material is defined as *a material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials [ISO VIM: 1993, 6.13 [7]].* A Certified Reference Material is defined as *a Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence [ISO VIM: 1993, 6.14].* These definitions say nothing about the quality of the measurement science infrastructure that supports the value-assignment process for RMs and CRMs. This causes confusion for chemical measurement analysts worldwide as they attempt to establish increasingly required traceability for their chemical measurements.

Approach: The quality of assigned values for any CRM or RM is based on the existence and application of sound metrological principles and practices to the value-assignment process. It is with this basic premise that we have developed **NIST Special Publication 260-136**, “Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements”. This document provides a complete description of the seven modes used at NIST to acquire analytical data for the value assignment of our SRMs and RMs for chemical measurements and links these modes to three data

Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements	Certified Value Reference Value Information Value
1. Certification at NIST Using a Primary Method with Confirmation by Other Method(s)	✓
2. Certification at NIST Using Two Independent Critically-Evaluated Methods	✓ ✓
3. Certification/Value-Assignment Using One Method at NIST and Different Methods by Outside Collaborating Laboratories	✓ ✓
4. Value-Assignment Based On Measurements by Two or More Laboratories Using Different Methods in Collaboration with NIST	✓ ✓
5. Value-Assignment Based on a Method-Specific Protocol	✓ ✓
6. Value-Assignment Based on NIST Measurements Using a Single Method or Measurements by an Outside Collaborating Laboratory Using a Single Method	✓ ✓
7. Value-Assignment Based on Selected Data from Interlaboratory Studies	✓ ✓

quality descriptors: NIST Certified Values, NIST Reference Values and NIST Information Values.

Results and Future Plans: A NIST Standard Reference Material® (SRM®) is a CRM issued by NIST that also meets additional NIST-specified certification criteria. NIST SRMs are issued with Certificates of Analysis or Certificates that report the results of their characterizations and provide information regarding the appropriate use(s) of the material. A *NIST Certified Value* is a value reported on an SRM Certificate/Certificate of Analysis for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been fully investigated or accounted for by NIST. Values are generally referred to as certified when Modes 1, 2, or 3 have been used for value-assignment and all the criteria for that mode are fulfilled. These three modes all require NIST measurements and oversight of the experimental design for the value-assignment process. The uncertainty associated with a certified value generally specifies a range within which the true value is expected to lie at a level of confidence of approximately 95 % if the sample is homogeneous. If significant sample heterogeneity is included, the uncertainty generally represents a prediction interval within which the true values of 95 % of all samples are expected to lie at a stated level of confidence.

A *NIST Reference Value* (formerly called Noncertified Value) is a best estimate of the true value provided on a NIST Certificate/Certificate of Analysis/Report of Investigation where all known or suspected sources of bias may not have been fully investigated by NIST. Reference values are generally determined using the following modes:

- Mode 2 or 3 is used when there is lack of sufficient agreement among the multiple methods.
- Modes 4, 5, or 6 are used when the intended use of the value by the measurement community does not require that it be a certified value.
- Mode 7 can be used in special cases, e.g., when results are obtained from another national metrology laboratory with whom NIST has historical comparability data for the

method(s) used for the specific matrix/analyte combination.

The uncertainty associated with a NIST Reference Value may not include all sources of uncertainty and may represent only a measure of the precision of the measurement method(s).

A *NIST Information Value* is considered to be a value that will be of interest and use to the SRM/RM user, but insufficient information is available to assess the uncertainty associated with the value. Typically, the information value has no reported uncertainty listed on the certificate and has been derived from one of the following value-assignment modes:

- Results from modes 4, 5, 6, or 7 in which the intended use of the value by the measurement community does not require that it be a certified or reference value, e.g., information about the composition of the matrix such as the value of “total organic carbon” of a sediment material may be useful to the user in selecting an appropriate analytical method.
- The results from modes 4, 5, 6, or 7 lack sufficient information to assess the uncertainty.
- Results are provided from outside NIST as supplemental information on the SRM matrix and are not measurements typically made at NIST but may be of interest to the user.

NIST has met the chemical reference materials needs of U.S. industry and commerce for nearly 100 years. 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. The establishment of these seven modes for value assignment of NIST SRMs and RMs and communication of their linkage to the three quality descriptors (NIST Certified Values, NIST Reference Values and NIST Information Values) is a critical component of our strategy to produce an increasing number of SRMs and RMs through collaborative efforts with selected expert

laboratories and other National Metrology Institutes/Standards Laboratories.

Publication:

“Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements”, National Institute of Standards and Technology Special Publication 260-136, NIST Spec. Pub. 260-136 January 2000

24. *Inhaled Nitric Oxide Therapy*

W.J. Thorn III

Objective: NIST measurements, research, and gas standards are supporting the implementation of Inhaled Nitric Oxide (INO) Therapy that could help save the lives of 2,000 U.S. newborns annually. Nitric oxide, which helps babies breathe easier, was approved as a drug by the FDA in December 1999. Currently, NIST and the FDA are working with doctors and instrument manufacturers to develop written protocols so that new INO devices can gain FDA approval; before becoming commonplace in hospital neonatal care units.

Problem: Dilute concentrations (<100) of nitric oxide have shown dramatic results as a pulmonary vasodilator in some newborn patients when inhaled in oxygen via a ventilator. However once the nitric oxide is mixed with oxygen, the clock starts for the spontaneous formation of nitrogen dioxide. During INO clinical trials in 1995, the National Institute for Child Health and Development sought NIST assistance in developing a NIST Traceable Reference Material (NTRM) at 85 $\mu\text{mol/mol}$ NO in nitrogen.

Accurate measurements of nitrogen dioxide is important because nitrogen dioxide (>5 $\mu\text{mol/mol}$) is considered detrimental—possibly leading to pulmonary edema and other negative consequences;

Approach: NIST and NICHD researchers performed a collaborative kinetics study [1] to determine how fast nitrogen dioxide forms. This helped the pediatric researchers determine the proper conditions for delivering the right amount

of nitric oxide to the babies while minimizing the risks from inhaling nitrogen dioxide.

NIST's role now is to serve on ASTM subcommittee F29.1.11 working with FDA anesthesiologists, medical doctors, INO medical device manufacturers on a device acceptance "standard" which when followed will facilitate FDA device approval. NIST provides input to NO and NO₂ performance tests for gauging accuracy and the negative effects of interfering anesthesia gases.

Finally, NIST and an interested device manufacturer will perform all of the tests called for in the standard to demonstrate their viability and to document unforeseen problems. These comments are then documented to the subcommittee.

Results and Future Plans: ASTM Subcommittee F29.1.11 work on a standard for NO devices has been balloted to the full subcommittee. NIST and Pulmonox collaborated on performing the 17 accuracy tests required in June 2000. Similar testing in September 2000 evaluated sensor response changes to interfering anesthesia gases. In discussing the interference testing results with the subcommittee; a different procedure was recommend which will be tested at NIST in late November 2000. The subcommittee is now drafting a similar standard for NO₂ devices.

Publications:

Sokol, G.M., Van M., Krisa P., Wright, Linda L., Thorn, W.J. III, Chu, P.M., and Sams, R.L. "Nitrogen Dioxide Formation During Inhaled Nitric Oxide," *Clinical Chemistry* 45:3 382-387 1999

ASTM "Standardization News," Cover, September 2000

25. Key Comparison on the Determination of Cholesterol in Serum (CCQM – K6)

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INTRODUCTION

At the CCQM Organic Working Group meetings in 1999, a Key Comparison on the determination of total serum cholesterol in human serum was approved and outlined, based upon a successful 1999 Pilot Study (CCQM-7) in this area. In January 2000, the designated pilot laboratory, NIST, sent serum samples to participating laboratories for determination of total serum cholesterol. The six NMI laboratories that submitted results in the pilot study were included in this study. In addition, one laboratory (NARL) that did not participate in CCQM-7 elected to participate in the Key Comparison. By prior agreement of the CCQM, only results from those laboratories that participated in the pilot study would be eligible to be included in calculation of the Key Comparison Reference Values.

The participants were:

- National Institute of Standards and Technology (NIST) (USA) *Pilot Laboratory*
- LGC, Inc. (UK)
- National Institute of Materials and Chemical Research (NIMC) (Japan)
- Institute for Metrology and Technology (NMI) (Netherlands)
- National Research Center for Certified Reference Materials (NRCCRM) (China)
- Physikalisch-Technische Bundesstalt (PTB) (Germany)
- National Analytical Reference Laboratory (NARL) (Australia)

PILOT STUDY

A pilot study for the determination of total serum cholesterol was organized by NIST in 1999. Seven institutes agreed to participate and were sent samples from two frozen human serum pools. These pools were unknown to the participants, except for the pilot laboratory, and were NIST SRM 965 certified for glucose, but not cholesterol (Material A_p), and SRM 1951a, Level 2, (Material B_p) certified for cholesterol, using the published isotope dilution/gas chromatography /mass spectrometry (ID/GC/MS) "definitive" method for serum cholesterol¹ described briefly below. Each participant also

received a sample of SRM 911b to use for calibration. The participants were free to use whatever method they chose. Results were received from six participants, all of whom used ID/GC/MS-based methods. The results are shown in Table 1. The scatter in the data was acceptable for such an exercise, but was greater than would be expected for experienced laboratories, probably because most of the participants had no previous experience with cholesterol measurements. The two most experienced laboratories (NIST and Deutsche Gesellschaft für Klinische Chemie E.V. (DGKC)) reported the highest values, suggesting that the other laboratories were likely experiencing a low bias, perhaps due to incomplete hydrolysis of esters of cholesterol. DGKC did not participate in the Key Comparison study since they are not an NMI or an official representative of an NMI at this time. Participants in pilot study met at the 1999 meeting of the Organic Working Group of the CCQM to discuss the results and possible sources of bias and how such biases might be overcome. With the expectation that further exercises would show improvement as laboratories became more familiar with the methods they used for the determination of cholesterol, the CCQM directed the Organic Working Group to proceed with a Key Comparison.

KEY COMPARISON

The Key Comparison study involved sending samples from two pools of human serum with significantly different concentrations of cholesterol for the participants to analyze using a method of their choice based upon isotope dilution/gas chromatography/mass spectrometry. Two unfortified frozen serum materials, with concentrations unknown except by the Pilot Laboratory, were sent on dry ice. For each material there were four vials included, one as a test sample to determine how much internal standard was needed and three vials for measurement, two aliquots per vial. Material A was a material prepared at NIST by thoroughly mixing sera from two volunteers known to have high blood cholesterol levels. Material B was SRM 965 Glucose in Frozen Human Serum. This SRM is not value assigned for cholesterol, but has a cholesterol level in the normal range for humans.

As all of the laboratories used isotope dilution/gas chromatography/mass spectrometry-based methods for the pilot study, only this technique was permitted for the Key Comparison. All of the laboratories followed the published ID/MS "definitive" method for serum cholesterol.¹ This method involves adding a known mass of a cholesterol material with a stable isotope label to a known mass of serum. After an equilibration period, the serum is treated with a strong base to hydrolyze all cholesteryl esters to free cholesterol. After neutralization of the solution, the cholesterol is extracted with a non-polar solvent. The cholesterol is converted to a trimethylsilyl derivative to improve its gas chromatographic and mass spectrometric properties. The derivatized material is injected into a gas chromatograph/mass spectrometer system to carefully measure the ratio between the native cholesterol and the isotope labeled material that was added. By comparing this ratio with that of known mixtures of the same labeled cholesterol and unlabeled cholesterol of known purity (calibration material), it is possible to accurately and precisely determine the cholesterol content of the serum.

In contrast to the pilot study, no calibration material was supplied by the organizers. Thus each participant was responsible for selecting a calibration material that had a stated purity and uncertainty, which were to be incorporated in calculations of concentration and uncertainty.

The laboratories were instructed to prepare samples for measurement on three separate days, with one vial of each level sampled in a day. The measurements were also to be done on separate days. The laboratories were further instructed to provide a detailed description of their uncertainty budget and were encouraged to attend the April 2000 meeting of the CCQM to discuss their uncertainties.

RESULTS

Results were received from all of the participants in the Key Comparison. The composite results including means and expanded uncertainties for all of the participants are shown in the Table 2. Because NARL did not participate in the Pilot Study, their results are not considered to be part of the calculation of the Key Comparison Reference Values and thus were not used to calculate the means and uncertainties in the table.

If their results were included the interlaboratory mean values would increase to 2.198 and 1.733 mg/g for Materials A and B, respectively. The results for Materials A and B are shown in Figures 1a and 1b, respectively. The shaded areas represent the ranges of the Key Comparison Reference Values, which are listed and described in Appendix 1. The Table of Equivalence, which enumerates the relationship among the results of the participants in the Key Comparison, is shown in Appendix 2.

DISCUSSION

Material A was prepared only for use in the Key Comparison and thus had not been previously measured. Results from five of the six participants were in good agreement, but the other participant provided a result that was low relative to the others. This result is statistically an outlier by Dixon's Test². When this result is excluded from the calculation, the mean increases by 0.5% and the expanded uncertainty decreases by 42%.

Although unknown to the participants, Material B was one of the materials that had been used in the pilot study (Material A_p), approximately one year prior to the Key Comparison. Consequently, it is possible to compare the performance of the laboratories on the same material over time. This comparison is shown in Figure 2. It is clear from this figure that interlaboratory precision was much better for the Key Comparison than it was for the pilot study on this material. For the pilot study, most of the participants had little experience with cholesterol measurements. From discussions after the pilot study, it appeared that these laboratories may not have used conditions that assured complete hydrolysis of cholesteryl esters, resulting in a low bias. For the Key Comparison, these laboratories had more experience with the cholesterol method and therefore, used more rigorous hydrolysis conditions, resulting in much better interlaboratory precision for the Key Comparison.

Uncertainty calculations involved both Type A and Type B components for all of the participants. All of the laboratories used the repeatability of their measurements as the type A component. Repeatability of measurements was the largest source of uncertainty for most of the laboratories and contributed significantly to the

overall uncertainty for the others. The type B component consisted of uncertainty in the purity of the reference compounds that they used plus other factors that could introduce a systematic bias. The complete uncertainty budget used for the NIST measurements is shown in Table 3, using material A from this study as an example. From the NIST perspective, based upon many years experience with using an ID/MS-based method for determining cholesterol, two of the most significant contributors to the type B uncertainty are the completeness of the hydrolysis of the cholesteryl esters and the stability of free cholesterol in the basic solution used for the hydrolysis. Incomplete hydrolysis results in a low bias, but it is not possible to prove complete hydrolysis. Model systems can show that the most common esters are completely hydrolyzed, but it is possible that small quantities of some less abundant species may resist hydrolysis. Cholesterol is known to be unstable in basic solution over long periods. If some degradation occurs before equilibration is achieved, a bias will occur. Recovery studies have shown that very little cholesterol is lost in the entire sample preparation procedure¹, but some losses in the hydrolysis step cannot be ruled out. Therefore, degradation of cholesterol may contribute significantly to the uncertainty for methods with high precision measurements such as those used in this study. None of the other laboratories in this study explicitly used these factors. However, one laboratory included a component for unknown systematic effects that would encompass effects such as these. Several laboratories included components related to the uncertainties in weighing. Uncertainties from weighings should be very small and, in most cases, will contribute to the type A measurement uncertainty. However, non-linearity in balance responses would be a systematic effect that could make a small contribution to the type B uncertainty. For all of the laboratories that used this approach, the uncertainties attributable to weighing steps contributed minimally to the overall uncertainty.

CONCLUSIONS

This Key Comparison study demonstrated that the participating NMIs could successfully measure serum cholesterol for normal and elevated levels, using ID/MS-based methods, with interlaboratory expanded uncertainties of less than 1%. Comparison of results from the

Key Comparison and the earlier pilot study demonstrated that laboratories previously inexperienced in the determination of serum cholesterol showed dramatic improvement as they gained experience and better understood potential sources of bias, such as incomplete hydrolysis. However, every serum analyte of interest as a health marker provides a unique set of challenges. To provide a more comprehensive measure of the capabilities of NMIs for measuring well-defined serum analytes, the CCQM is conducting pilot studies for the determinations of serum glucose and creatinine, that if successful, will be followed by Key Comparisons. These two analytes were chosen because they present very different challenges than does cholesterol, thus providing a more complete picture of the capabilities of participating NMIs. 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. The combination of these three Key Comparisons may provide a basis for the evaluation of measurement capabilities of participating NMIs for other well-defined organic analytes in serum, without having to actually conduct a Key Comparison for all such analytes.

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