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Automotive and Aerospace

Title: New Gas Standards for Calibrating Instrumentation used for Measuring Emissions from Next Generation Low Emission Vehicles

Authors: W.J. Thorn III and W.D. Dorko

Abstract: Stakeholders in the American Industry / Government Emissions Research (AIGER) group are working together to facilitate the automobile industry meeting more stringent 2003 Federal Tier II and California LEV II emission regulations. AIGER members include the U.S.EPA, California Air Resources Board (CARB), General Motors, Ford, and Daimler-Chrysler. In 1998, NIST worked with a Specialty Gas contractor to blend two cylinders each of low NO standards at concentrations of 0.5 µmolmol, 0.75 µmol/mol, 0.95 µmol/mol, 1.05 µmol/mol and 1.25 µmol/mol. The ten new standards were prepared employing a newly developed cylinder pretreatment, a NIST SRM 2629a as the parent NO source and a diluent balance gas of specially gettered nitrogen (O_2 < 2 nmol/mol). The new standards have been analyzed periodically against NIST dynamic permeation standards and have exhibited excellent NO concentration stability for more than 4 years. In 2001, AIGER provided direct funding to NIST's contractor to expedite the development of two new NO SRMs at the lower concentrations of 0.5 µmol/mol and 1.0 µmol/mol. NIST and its contractor used the same pretreatment etcetera to blend forty cylinders of 0.5 µmol/mol and forty cylinders of 1.0 µmol/mol NO in nitrogen candidate SRM mixtures. The ownership of 27 candidate cylinders of each new SRM (54 total) was transferred from AIGER to NIST; who is certifying twenty-five (25) stable cylinders of NIST SRM 2737 (0.5 µmol/mol) and twenty-five (25) stable cylinders of NIST SRM 2738 (1.0 µmol/mol), to be completed by January, 2004. Additionally, thirteen cylinders of each concentration were recently analyzed by NIST and returned to AIGER members for their interim use − until the new SRMs become available in March, 2004.

Purpose: NIST and the U.S. Motor Vehicles Manufacturers have worked together since 1975 to develop sixty (60) gaseous Standard Reference Materials, which are the Nation's benchmarks against which all U.S. EPA mandated fuel economy and mobile source emission measurements must be traceable; by federal law. NIST currently supports AIGER members by maintaining a reasonable inventories of required SRMs which consist of dilute mixtures of key pollutants such as hydrocarbons, carbon monoxide and nitric oxide. Newer vehicles produce lower levels of these pollutants because their engines employ fuel injection whose air to fuel ratio is optimized by an on-board computer, and their emissions are further reduced by more efficient catalytic converters. During testing the exhaust levels are diluted by the use of constant volume sampling bags or by new mini-diluter technology. AIGER stakeholders have identified the need for significantly lower NIST gas standards containing carbon monoxide, hydrocarbons and nitric oxide; with the completion of SRM 2737 (0.5 µmol/mol) and SRM 2738 (1.0 µmol/mol) nitric oxide in nitrogen being their highest priority.

Major Accomplishment: NIST assigned NO and NO_X values to the ten NIST low NO standards by two different methods. Two calibration curves (NO and NO_X) where plotted the assigned NO or NO_X concentration (*x-value)* for each of the ten vs. their measured ratios to LS 2738- AL-22 (*y-value*). All eighty candidate SRM mixtures at 0.5 ppm and 1.0 ppm were

analyzed in December, 2002 sixteen months after blending. All cylinders are stable and all contain0.2 µmol/mol nitrogen dioxide. The candidate SRM cylinders remain at NIST awaiting a final analysis in December, 2003. The remaining AIGER mixtures were returned to their owners for interim use until the certified SRMs are available. NIST completed some additional Special analyses for CARB in June, 2003.

Impact: The project has technically resolved NIST's low NO SRM stability problems, provided AIGER members with interim standards to work with and will soon provide much needed lower concentration NIST certified NO SRMs in the near future; and thus help facilitate vehicle manufacturer's in meeting U.S.EPA and CARB's current and future lower emission regulations.

Future Plans: NIST will complete certification of 25 cylinders each of SRM 2737 and SRM 2738 by January, 2004 and available for sale soon after March 2004. AIGER and NIST are discussing technical issues to upgrade the NTRM protocol to meet mobile source accuracy and stability criteria for emission measurements. The NTRM "prime" (NTRM') addendum to NIST SP 260-126 has been submitted to AIGER for comment. A Rollout of the new NTRM' gas standards are scheduled at a meeting between AIGER, specialty gas companies and NIST to be held in parallel with the March 2004 Pittsburgh Conference meeting in Chicago.

Automotive and Aerospace

Title: Fourier-Transform Microwave Spectroscopy Critically Evaluated for Real-time Measurements of Automotive Exhaust Emissions

Authors: R.R. Bousquet and P.M. Chu

Abstract: Fourier-transform microwave (FTMW) spectroscopy is being investigated as a means for quantitative real-time measurements. Briefly, FTMW spectroscopy combines the high spectral resolution of microwave spectroscopy and the high sensitivity of molecular beam and cavity-enhanced methods creating a technique that can provide unambiguous identification of vapor phase analytes that possess permanent electric dipole moments. The principal goal of the present program is to critically evaluate the technique's potential as a sensitive, reliable, and robust tool for quantitative measurements of trace gases. Current work has focused on improving the sensitivity of the detection system and characterizing the effects of species such as water and carbon dioxide present in the sample gas.

Purpose: The purpose of this research is to develop and demonstrate FTMW spectroscopy as an analytical tool for accurate measurements of single species in complex gaseous mixtures for real-time analysis of automobile exhaust emissions, environmental monitoring, process control, and homeland security applications.

Major Accomplishments: Continued efforts to improve the signal stability and reproducibility pushed the stability to approximately 1% over 24 h. Additional effort has been focused on characterizing the factors which determine the overall sensitivity of the detection system and upgrading the software and hardware to enhance the signal to noise. Further development continues in a number of areas including: use of low noise preamplifiers in the initial signal processing, implementation of a dual phase modulation/detection system, improvements of the cavity mode quality, and addition of adjustable antenna for optimization of the microwave radiation transmission at specific analyte transition frequencies. Finally, detection limits ranging from 0.1 μmol/mol to 10 μmol/mol have been realized for several analytes in pure nitrogen matrices. Furthermore, minimal changes in the detection limits were observed for the addition of ambient levels of oxygen, carbon dioxide and water to the sample matrix suggesting that this is a viable technique for real-time ambient monitoring.

Impact: Accurate quantitative measurements of trace species in bulk gases are becoming more vital to both science and industry with detection requirements to the low part per billion regions and beyond. Analytical methods that exhibit high chemical selectivity and sensitivity are particularly advantageous for the analysis of complex matrices. Through this research we hope to demonstrate the potential of FTMW spectroscopy to help meet these measurement needs.

Future Plans: The remaining targeted areas of improvement for the FTMW spectrometer will be implemented in FY2004. These improvements should push the detection limits of the FTMW spectrometer to the nmol/mol to pmol/mol region. Additionally, to facilitate the deployment of the FTMW spectrometer in field and laboratory applications, higher pressure measurements will be investigated.

Energy Systems

Title: The Measurement of Ultra Low Sulfur in Fuel Products for Next Generation Internal Combustion Systems

Authors: R.D. Vocke and W.R. Kelly

Abstract: Fuel sulfur is the principal poison affecting the sophisticated catalytic converters and on-board diagnostic systems that are central to reducing pollution from advanced internal combustion technologies. Through a staged process, petroleum producers are seeking to produce gasoline and diesel fuels that are functionally "sulfur-free" by the end of this decade. In support of these efforts, we have produced two new low sulfur fuel SRMs and one near-zero sulfur fuel SRM that will help producers meet current and evolving regulatory limits. Capitalizing on recently improved analytical capabilities, we are also completing work on another near-zero sulfur liquid fuel whose relative total uncertainty has been reduced by more than an order of magnitude compared with uncertainties for previously certified low sulfur fuel materials. We are also the pilot institution for a CCQM Key comparison and pilot study aimed at assessing participating NMI's ability to provide accurate measurements of low and very low sulfur diesel fuels. These exercises will be completed during FY04.

Purpose: Nitrogen oxides and non-methane organic gases are the primary emissions from internal combustion engines that impact ozone and particulate matter pollutants. As the requirements for reducing these emissions become more stringent, the need for extremely efficient and long-lived after-treatment systems are being nationally and internationally mandated by regulatory actions. Sulfur is the most deleterious "poison" to these systems and impedes the implementation of all major air pollution reduction strategies in the transportation sector. Consequently, there is a strong movement towards "sulfur-free" fuels. Depending on the applicable national emission standard, near-zero or sulfur-free means a maximum S concentration between 5 and 10 µg/g. Such near-zero levels of S pose significant analytical challenges, which NIST is meeting with new SRMs.

Major Accomplishments: We have completed the accurate characterization of the sulfur content of two low S fuels and one near-zero S fuel. Certificates have been issued for SRM 2299, Sulfur in Gasoline (Reformulated) and SRM 2723a, Sulfur in Diesel Fuel Oil, which have sulfur mass fractions of 13.6 µg/g ± 1.5 µg/g and 11.0 mg/kg ± 1.1 mg/kg, respectively. SRM 2298, Sulfur in Gasoline (High Octane) was certified for a sulfur mass fraction of 4.7 µg/g ± 1.3 µg/g. Capitalizing on recently improved analytical capabilities stemming from lower sulfur blanks and improved blank control, we are currently completing work on another near-zero sulfur fuel (SRM 16XX, Sulfur in Kerosene) whose relative total uncertainty has been reduced by more than an order of magnitude from that reported for SRM 2298.

Furthermore, we anticipate that, at the conclusion of the CCQM Key comparison and Pilot Study, we will have another diesel fuel SRM with sulfur mass fraction near 40 µg/g.

Impact: The United States has called for a limit of 15 μg g^{-1} S in diesel fuel by 2006 and a limit of 30 μg g^{-1} S in gasoline by 2007. The aforementioned SRMs are in place and should fully meet the sulfur regulatory concerns.

Future Plans: We plan to continue development of an analytical approach to sulfur analyses on our high resolution ICP-MS. This approach should simplify the process of making high accuracy sulfur measurements by eliminating the necessity of a chemical separation of sulfur from its matrix.

Energy Systems

Title: Determination of Hg in Crude Oils in the Department of Energy Strategic Petroleum Reserve by ID-CV-ICPMS

Authors: W.R. Kelly, S.E. Long, and J.L. Mann

Abstract: The Hg concentration has been determined in almost 100 different crude oils from the U.S. Department of Energy Strategic Petroleum Reserve (SPR) by isotope dilution cold vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS). The Hg concentration ranged from 0.02 to 10 ng/g with about 80 % of the samples falling below 2 ng/g. The high accuracy of this technique is superior to existing techniques because of three unique capabilities: 1) very low and controllable blanks which average 3 ± 2 pg (± 1s), 2) high sensitivity of the ICPMS, and 3) a digestion method using only high purity nitric acid in closed system (Carius tube) which prevents loss of the highly volatile Hg and prevents contamination of the sample. This is the first and only high accuracy database on Hg in crude oils and indicates that the Hg concentration is about an order of magnitude lower than currently believed.

Purpose: The Clean Air Act (CAA) as amended in 1990 required the U.S. Environmental Protection Agency (EPA) to submit a report to Congress on Hg emissions to the atmosphere. The EPA responded with the Mercury Study Report to Congress (1) that identified a number of sources of Hg emissions to the atmosphere and quantified those emissions with the best available estimates, but no estimates were given for crude oil consumption because of the lack of reliable data. It is well established that coal is a major source of Hg emissions to the atmosphere. The Hg content of coal combusted in the US is about 100 ng/g. Given the fact that in the US the magnitude of crude oil consumed is equal to that of coal, many scientists have speculated that crude oil could be a major unknown component to the global Hg cycle. The EPA, concerned with the well-known toxic behavior of Hg, now requires reporting of Hg emissions from companies that process more than 10 pounds of Hg per year and this reporting requirement may be prelude to a regulation on Hg emissions. If the mean content of Hg in crude is 10 ng/g, as commonly believed, then all refineries would be over the limit.

Major Accomplishments: We have developed a high accuracy method for the determination of Hg in crude oil. This method was used to certify Hg in two NIST crude oil SRMs at levels never before achieved: SRM 2721 – 41.7 ± 5.7 pg/g and SRM 2722 – 129 ±13 pg/g. The new technique and these data were published this year (2).

Our high accuracy data base is now almost 100 different crude oil samples most of which have come from the SPR. But we have also measured the Hg content in several Canadian and Russian crude oils. This increasing data base will be the most extensive and the most accurate currently available.

Impact: We have developed and described (2) a high accuracy technique for Hg in crude oil. Heretofore, this element in this matrix has been a major analytical challenge. Any laboratory that has an ICP-MS could use this technique. This high accuracy data base will fill a void in the

current efforts to establish an accurate account of Hg cycling on a global scale. The low Hg concentrations from this study indicate that Hg in crude oil is not as high as formerly believed.

Future Plans: This project has been funded by Department of Energy for two years. We have just received funding for Phase 3, which will finish and complete the survey of crude oils consumed in the US. We also will measure Hg in water and brines from the SPR caverns to complete a mass balance of Hg in the SPR. At the conclusion of this next phase, we anticipate the data base of about 150 different crude oil samples and these will probably represent 80 to 90 % of the crude processed in the US. This will be the largest and most accurate database on Hg in crude oil.

1. Mercury Study Report to Congress, 8 Volumes, EPA-452R/R-97-003 to EPA-452R/R-97-0010 (1997).

2. Kelly, W.R., Long, S.E., and Mann, J.L., ***"Determination of Mercury in SRM Crude Oils and Refined Products by Isotope Dilution Cold Vapor ICP-MS using Closed-System Combustion,"*** Anal. Bioanal. Chem., 376, 753-758 (2003).

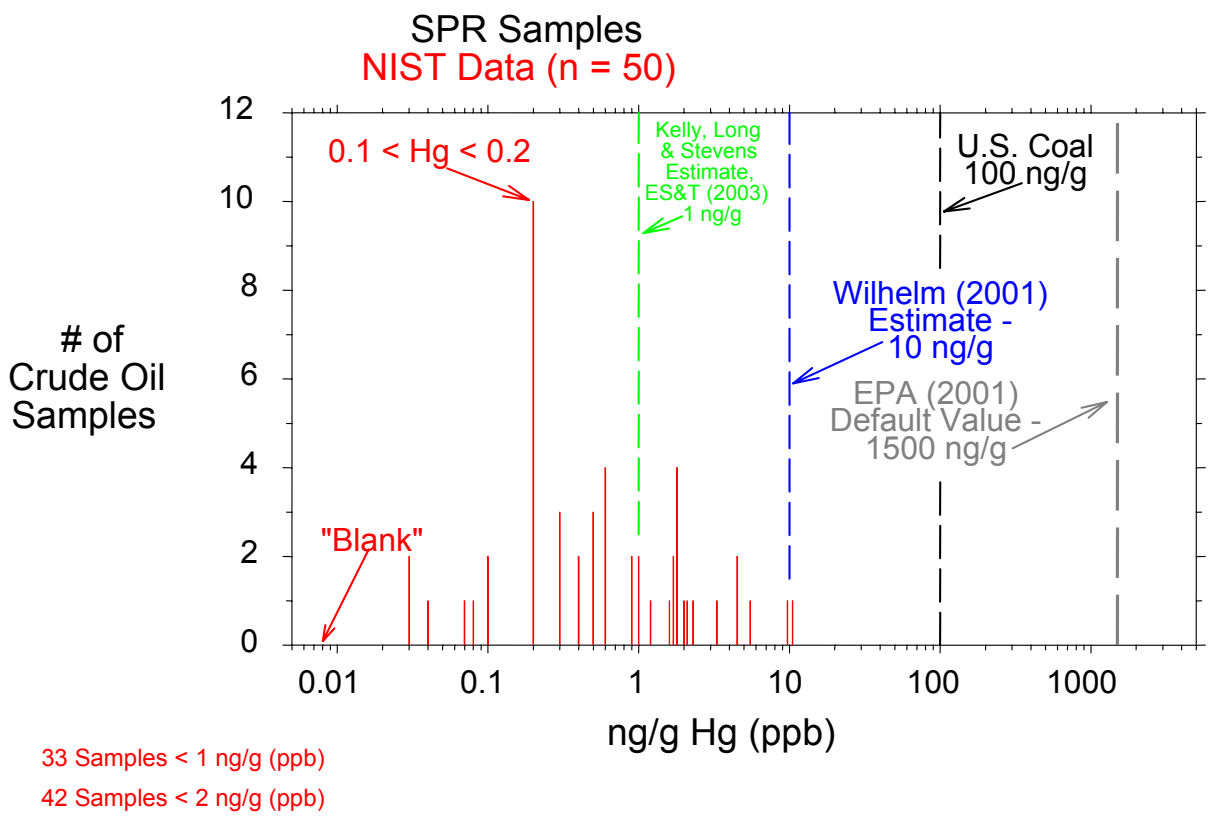


Figure 1. A subset of recent NIST high accuracy determinations of Hg in crude oil. Note that all samples fall below the current estimate of the mean concentration of Hg in crude oil of 10 ng/g. Our data indicate the value may be an order of magnitude lower

Environmental Technologies

Title: SRMs for Contaminants on Air Particulate Matter less than 2.5 µm ($PM_{2.5}$)

Authors: R. Zeisler, J.R. Kucklick, B.J. Porter, D.L. Poster, M.M Schantz, R.O. Spatz, and S.A. Wise

Abstract: The Environmental Protection Agency (EPA) issued new standards in 1997 for air particulate matter (PM) under the national ambient air quality standards. These standards added new regulations for PM less than 2.5 µm ($PM_{2.5}$), which is the respirable PM fraction, in addition to the existing PM10 standards. Research recommendations have already been made by the National Research Council at the request of Congress and EPA to focus on evaluating what types of particles cause detrimental health effects. To support compositional analyses and other investigations on the fine PM, quality assurance materials are necessary; however, few appropriate materials are currently available to support this research. NIST has entered an agreement with the US-EPA to develop, certify, and issue a suite of SRMs that will be used for the evaluation of analytical methods to provide national measurement traceability and to improve inter-laboratory comparability of measurements of $PM_{2.5}$.

Purpose: NIST, in cooperation with EPA, will establish critical benchmark SRMs for air particulate matter monitoring covering elemental, organic, and other species that will be used for the evaluation of analytical methods, for helping to provide national measurement traceability, and for improving inter-laboratory comparability of measurements of today's PM.

Major Accomplishments: Newly released SRM 2783 air particulate on filter media, provides 0.5 mg of fine air particulate matter on a polycarbonate filter membrane with certified mass loadings for 24 toxic and environmentally important elements. This SRM is intended for elemental analysis using X-ray fluorescence, neutron activation analysis, and other analytical techniques. For organic compounds on $PM_{2.5}$, we are in the process of collecting a 200-g bulk sample of ultra-fine particulate matter at a site in Baltimore, MD with a high-volume sampler. Because the collection, preparation, and certification of this $PM_{2.5}$ SRM will require 2 to 3 years, an interim reference material was prepared from a 20-g batch of $PM_{2.5}$ collected earlier at the Baltimore site during different sampling periods in 1998-99 and 2001. This interim reference material has been characterized at NIST for polycyclic aromatic hydrocarbons (PAHs) and nitro-substituted PAHs. The interim reference material has been distributed to 18 laboratories as part of a NIST/EPA interlaboratory comparison exercise for the determination of organic compounds in fine particulate matter. The results of this interlaboratory study will be used in conjunction with NIST measurements to assign concentration values for the compounds of interest; this interim reference material will then be made available to laboratories involved in EPA $PM_{2.5}$ research programs for use as a control or reference material.

Impact: The certification of SRM 2783 provides to testing laboratories world-wide the first chemical composition SRM to validate chemical measurements on PM that has been collected from ambient air. The development of a SRM for organic species will expand the quality assurance capabilities to important source markers and species critical to human health.

Future Plans: After the collection of the 200 g $PM_{2.5}$ sample from the Baltimore site, the candidate SRM will be prepared and analyzed to provide concentration values for the organic constituents of interest to EPA as both pollutants and combustion source markers. Research on new forms of SRMs for inorganic and organic species includes preparations of size-fractionated samples and evaluation of the chemical mobility of selected species to address new and more advanced monitoring requirements.

Environmental Technologies

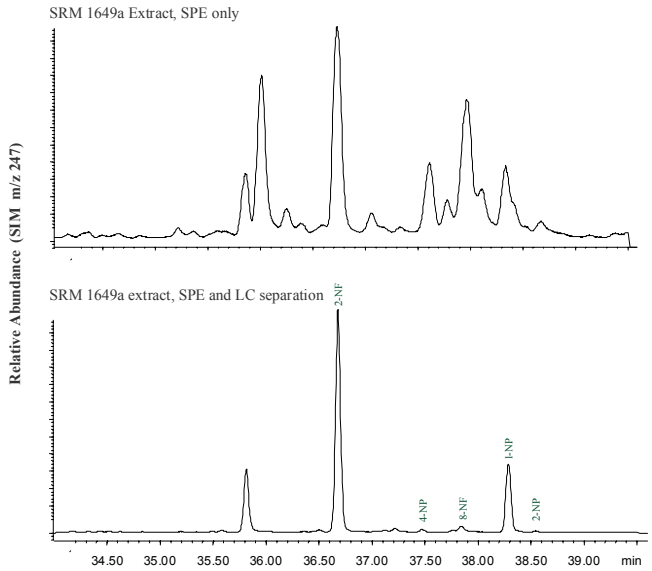
Title: Development and Evaluation of Analytical Method for Nitro-substituted Polycyclic Aromatic Hydrocarbons in Atmospheric Particulate Matter

Authors: H.A. Bamford, M.M. Schantz, and S.A. Wise

Abstract: NIST currently has two air particulate SRMs available, SRM 1648 Urban Particulate Matter and SRM 1649a Urban Dust. These SRMs which were collected in the late 1970s are certified for trace elements and organics, primarily polycyclic aromatic hydrocarbons (PAHs), respectively. In the late 1990s, a fine air particulate matter was collected as part of the NIST/EPA effort to develop a fine particulate matter reference material (particle size <2.5 µm). Nitrated PAHs (nitro-PAHs) are often present on ambient atmospheric particles from either direct sources, such as diesel and gasoline exhaust or gas-phase reactions of PAHs with nitrogen oxides. The nitro-PAHs are persistent in the environment and have been shown to be more mutagenic and carcinogenic in some cases compared to the parent PAHs. An analytical method has been developed using pressurized fluid extraction followed by isolation of the nitro-PAH fraction from the parent PAH fraction using normal-phase liquid chromatography (LC) with final analysis using gas chromatography/mass spectrometry in the negative chemical ionization mode (NICI). Using this method, 28 mononitro- and dinitro-PAHs were quantified in the air particulate SRMs (1648 and 1649a) and the fine particulate matter interim reference material.

Purpose: Nitro-PAHs are present in air particulate samples at concentrations in the low ng/g range. These compounds, however, may be a significant fraction of the direct-acting mutagenic compounds present in air particles. Using a selective fractionation and analytical method, nitro-PAH concentrations have been determined in SRM 1648 and SRM 1649a, as well as a contemporary fine particulate matter. These air particulate materials will aid in assuring the accuracy of results for monitoring studies of nitro-PAHs in air particulate.

Major Accomplishments: The normal-phase LC fractionation step was critical for the correct determination of nitro-PAH concentrations in air particulate samples as shown below.



This figure compares the GC/MS chromatogram of the nitrofluoranthene and nitropyrene molecular ion (m/z of 247) before and after extracts of SRM 1649a were fractionated using the normal-phase LC method. Note that before the normal-phase LC fractionation (top chromatogram), there are a large number of interfering peaks; whereas, after the LC fractionation (bottom chromatogram), quantitative analysis of the nitrofluoranthenes and nitropyrenes is possible. In addition, the use of a moderately polar GC column resulted in the separation (and thus quantification) of some isomers that coelute on the typically used non-polar GC columns. For example, the 2-nitrofluoranthene (the most prominent peak in the chromatogram above) coelutes with 3-nitrofluoranthene when using a non-polar GC column. The 2-nitrofluoranthene is more prevalent in air samples as opposed to the 3-nitrofluoranthene which is more prevalent in diesel samples.

Impact: Value assigning the concentrations of nitro-PAHs in the existing air particulate reference materials aids in assuring the accuracy of results from monitoring studies. The fine particulate material is of primary interest to EPA due to its health impact. By assigning values in the fine particulate matter, the EPA studies will have an interim reference material for their use.

Future Plans: Sufficient fine particulate matter will be collected to prepare an SRM. Determination of the nitro-PAH concentrations in this material will be an important addition to the value assignment process for this new air particulate SRM.

Environmental Technologies

Title: Developing Methods for the Determination of Past-Use and Current-Use Persistent Organic Pollutants in Standard Reference Materials

Authors: J.R. Kucklick, M.M. Schantz, S.A. VanderPol, K.J. Tuerk, and S.A. Wise

Abstract: Both past-use compounds and those of emerging environmental concern are often challenging to measure. Many banned pollutants continue to be of concern due to their persistence in the environment, accumulation in people and wildlife, and toxic effects. These so called "legacy pollutants" and those of recent environmental concern often do not have established analytical methods which are needed to assess human and wildlife exposure. NIST has developed methods for many of these pollutants, including polychlorinated biphenyls (PCBs) and chlorinated pesticides, to assign certified and reference concentrations in natural environmental matrix SRMs. The compounds under current study include toxaphene, a banned pesticide; polychlorinated naphthalenes (PCNs), mainly used in the electrical industry; and polybrominated diphenyl ethers (PBDEs), which are current use flame-retardants. Recent methods development and measurements have provided reference values for SRM 1946 Lake Superior Fish Tissue (toxaphene), SRM 1588a Organics in Cod Liver Oil (toxaphene and PBDE congeners), SRM 1945 Organics in Whale Blubber (toxaphene and PBDE congeners), SRM 1649a Urban Dust (PCN congeners), SRM 1944 New York/New Jersey Waterway Sediment, and SRM 1941b Organics in Marine Sediment (PCN congeners). Future work will focus on providing additional concentration values for toxaphene and PBDE congeners in these and additional natural environmental matrix SRMs. PBDEs are of particular interest because they are increasing exponentially in concentration in humans.

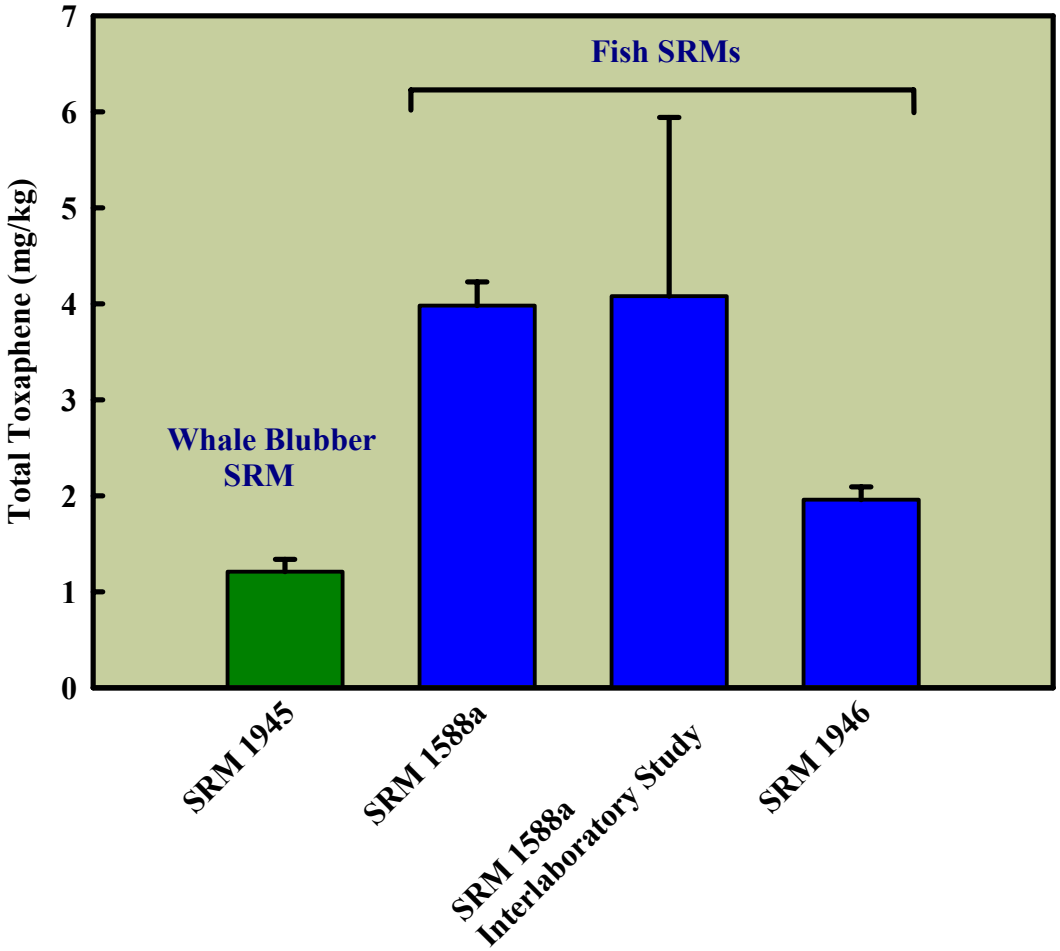
Purpose: Many persistent organic pollutants (POPs), such as polychlorinated biphenyl (PCB) congeners and chlorinated pesticides, have well-established measurement methods and reference materials available in several different matrices. However, some pollutants, such as toxaphene, polychlorinated naphthalenes (PCNs), and polybrominated diphenyl ethers (PBDEs), do not have established analytical methods or natural matrix certified reference materials available because these compounds are either more challenging to measure or they have only recently emerged as compounds of environmental concern. Toxaphene is listed in the Stockholm Convention on POPs for elimination, and the PCNs are candidates for elimination by this treaty. PBDEs are a class of fire retardant compounds that are increasing exponentially in concentration in humans and are being extensively monitored and investigated by environmental scientists. To meet the need for reference materials for these compound classes, we have developed analytical methods and provided reference values for these compounds in selected Standard Reference Materials (SRMs) that can be used by the environmental measurement community to help improve their data quality.

Major Accomplishments: Toxaphene and selected toxaphene congeners were measured in SRMs 1945 Organics in Whale Blubber, SRM 1946 Lake Superior Fish Tissue, and SRM 1588a Organics in Cod Liver Oil using gas chromatography/mass spectrometry with negative chemical ionization (GC/MS-NCI). Average total toxaphene values determined in SRM 1588a were in excellent agreement with a prior interlaboratory study using this material, and average values in SRM 1946 were at the Food and Drug Administration's Consumption Advisory Limit (2 mg/kg).

Toxaphene levels in SRM 1945 were about 50% of this value. The five major PBDE congeners present in most biological samples were also measured in SRMs 1588a and 1945 using GC/MS with electron impact ionization. The levels are in a useful range for routine environmental analysis and agreed well with measurements made on these two materials by other laboratories. PCN congeners were measured in selected abiotic SRMs including SRM 1944 New York/New Jersey Waterway Sediment, SRM 1649a Urban Dust, and SRM 1941b Organic in Marine Sediment. PCN Levels were determined in the SRMs using GC/MS-NCI following isolation using a liquid chromatography technique that isolates planar compounds. As a result of this work, SRM 1944 and SRM 1649a were chosen as interlaboratory comparison materials for the Second International Intercomparison Exercise for the PCNs, which is administered both by NIST and Environment Canada. Results from this comparison will be used in conjunction with measurements made at NIST to assign reference concentration values for individual PCN congeners in these materials. Work continues on measuring toxaphene, PCNs, and PBDEs in environmental matrix SRMs.

Impact: Reference values for the PCNs, toxaphene, and the PBDEs on relevant environmental matrix SRMs will provide laboratories a tool to help assess and their data quality. By producing quality data, laboratories will be better suited to address research and regulatory questions.

Future Plans: Future efforts will be directed at providing values for individual toxaphene components and additional PBDE congeners not determined in this initial research. For the PBDEs emphasis will be placed on PBDE 209 (decabrominated diphenyl ether), which occurs in sediments and is an analytically challenging compound to measure. Much of the current use of PBDEs as flame-retardants by industry is of the so-called deca formulation consisting mainly of PBDE 209.


Fish SRMs
Whale Blubber SRM
Total Toxaphene (mg/kg)
0
1
2
3
4
5
6
7
SRM 1945
SRM 1588a
SRM 1588a Interlaboratory Study
SRM 1946

Environmental Technologies

Title: Expansion of NIST's Environmental Specimen Banking Activities

Authors: P.R. Becker, R.S. Pugh, S.S. Vander Pol, J.R. Kucklick, R.D. Day, S.J. Christopher, B.J. Porter, M.B. Ellisor, and S.A. Wise

Abstract: NIST's capability in environmental specimen banking has expanded with the completion of its new cryogenic banking facility at the Hollings Marine Laboratory in Charleston, SC. This facility is being devoted to the expansion of specimen banking to support monitoring and research on marine environmental health issues. The banking of marine mammal tissues (blubber, kidney and liver) is a major part of this effort; however, additional programs have been added including the banking of bird eggs and feathers and marine mammal blood. These programs have required that NIST develop and establish collection and banking protocols for these new matrices. All of these programs have been established at the request of other agency sponsors, i.e., National Oceanic and Atmospheric Administration (NOAA), U.S. Geological Survey (USGS), and U.S. Fish and Wildlife Service (USFWS). Realizing the importance of environmental specimen banking to environmental research and monitoring programs, the banking of other kinds of specimens is being considered by these and other potential sponsors.

Purpose: The Analytical Chemistry Division maintains the National Biomonitoring Specimen Bank (NBSB) at the Neutron Research Facility on the NIST Gaithersburg campus and in Charleston, South Carolina, at the recently completed Hollings Marine Laboratory (HML). The NBSB, established in 1979, is a cryogenic environmental specimen bank, that stores specimens (e.g., human livers, marine sediments, fish tissues, mussels, oysters, human diet samples, marine mammal tissues, and bird eggs and feathers) collected as part of several monitoring and research programs supported by the Environmental Protection Agency (EPA), Food and Drug Administration (FDA), National Oceanic and Atmospheric Administration (NOAA), Minerals Management Service (MMS), US Geological Survey (USGS), and US Fish and Wildlife Service (USFWS). The NBSB is specifically designed to store environmental specimens over long periods of time (50-100 years). A systematic well-designed specimen bank program, such as the NBSB, is not only a valuable component of real-time monitoring and basic research, but it also enables future investigators to extend their research into the past (hind casting) and provides for future verification of analytical results (quality assurance).

Major Accomplishments: Through the NIST facility at the HML, environmental specimen banking is being expanded for marine environmental health monitoring and research. Current projects include the NMFS and USGS sponsored National Marine Mammal Tissue Bank (NMMTB), which archives marine mammal tissues and has been an active ongoing part of the NBSB since 1987 (the National Marine Mammal Tissue Bank was established by federal legislation in 1992). In 1999, NIST established egg collection and banking protocols and began to archive the contents of seabird eggs for the Seabird Tissue and Archival Project (STAMP). This project is a collaborative effort among NIST, USGS, and USFWS to monitor contaminants in eggs of seabirds of the Alaska Maritime National Wildlife Refuge (AMNWR). This refuge, which encompasses 4.5 million acres of discontinuous islands, reefs, and headlands, extends from above the

Arctic Circle to the end of the Aleutian Islands and eastward to the Alaska-British Columbia border. Eggs are particularly useful for temporal and spatial monitoring of persistent organic pollutants and mercury. In 2002, NIST began the development of a blood banking program for NOAA's Marine Mammal Health and Stranding Response Program (MMHSRP). NIST staff developed and established collection and storage protocols for whole blood, plasma, and serum as part of health assessment studies being conducted on populations of bottlenose dolphins in the Gulf of Mexico and on the US Atlantic coast. Marine mammal blood is now being banked at the HML facility. The latest addition to banking has been an effort begun in 2003 to bank peregrine falcon eggs and feathers for the USFWS.

Marine mammals have the largest number of specimens in the NBSB. The Gaithersburg and Charleston facilities together contain 2,182 tissue specimens from 784 individual animals representing 35 species from the Atlantic and Pacific oceans, Gulf of Mexico, and Alaska. The egg collection by STAMP has resulted in the banking of contents from 321 eggs representing three species of seabirds (common and thick-billed murres, and black-legged kittiwakes) from seabird colonies in Alaska. NIST has recently completed analysis of banked tissues from 47 white-sided dolphins from the Atlantic coast and 15 rough-toothed dolphins from the Gulf of Mexico for persistent organic contaminants and heavy metals [1,2]. The contents of 67 murre eggs from colonies in Alaska have been analyzed for persistent organic contaminants and mercury [3-5]. The geographic and species-specific patterns found in the seabird eggs are being verified through additional analyses that are presently underway.

Impact: The success of NIST's environmental specimen bank has convinced several agencies as to the value of such a concept to their own research programs. The banking program developed for STAMP has resulted directly in the incorporation of specimen banking into a newly emerging peregrine falcon monitoring program by the USFWS. The protocols developed by NIST for STAMP have also been taken by The Alaska Sea Otter and Steller Sea Lion Commission and applied to their own subsistence monitoring program for sea gulls. Data on banked specimens generated by NIST in collaboration with NOAA's Northwest Fisheries Science Laboratory represents the major portion of the US contribution to the international Arctic Monitoring and Assessment Programme on contaminant levels in marine mammals and seabirds in the US Arctic.

Future Plans: The value of environmental specimen banking is being recognized by scientists and various agencies and organizations. Future expansion being considered by NIST and its sponsors and collaborators include the banking of: sea turtle tissues, genetic material for marine biota, marine mammal biopsies from live-captures, and expansion of the seabird and peregrine falcon specimen bank to include other species (marine and terrestrial) and other kinds of tissues.

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Environmental Technologies

Title: SRMs for Contaminants in Marine Sediment and Tissue

Authors: S.A. Wise, S.J. Christopher, R. Demiralp Oflaz, R.R. Greenberg, J.R. Kucklick, S.E. Long, E.A. Mackey, B.J. Porter, D.L. Poster, M.M. Schantz, and R. Zeisler

Abstract: Recent marine sediment and tissue matrix SRMs have been developed to address the need for natural matrix SRMs for quality assurance in environmental measurements including marine sediment (SRM 1941b, SRM 2702, SRM 2703), mussel (SRM 1974b) and fish tissue (SRM 1946 and SRM 1947). SRM 1941b Organics in Marine Sediment and SRM 1974b Organics in Mussel Tissue are renewals of these popular marine matrices for determination of organic contaminants with certified values for over 60 constituents. SRM 2702 Inorganics in Marine Sediment, and SRM 2703 Marine Sediment for Microsampling are intended for the determination of elemental composition. SRM 1946 Lake Superior Fish Tissue is the first frozen fish tissue SRM and is intended primarily for the determination of contaminants such as polychlorinated biphenyls (PCBs), chlorinated pesticides, total mercury and methylmercury, as well as food matrix components such as proximates and fatty acids. SRM 1947 Lake Michigan Fish Tissue, which is currently in progress, is a second frozen fish tissue with certified concentrations for both inorganic and organic contaminants.

Purpose: Validation of the complete analytical procedure requires the use of natural matrix SRMs similar to those matrices typically encountered in the analysis of environmental samples. NIST natural matrix SRMs for organic and inorganic contaminants in marine sediment and tissue are necessary for the development and validation of analytical procedures and to provide quality assurance of chemical measurements in the marine environmental community.

Major Accomplishments: The certification of several sediment and tissue matrix SRMs has been completed in the past year including two renewals of previous SRMs and two new materials. SRM 1941b Organics in Marine Sediment and SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*) are the third issue of these popular marine matrix SRMs for organic contaminants, which have found widespread use in a number of marine monitoring programs supported by the Environmental Protection Agency (EPA) and the National Oceanic and Atmospheric Administration (NOAA). Both SRM 1941b and SRM 1974b have certified concentrations for 60 polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides compared to only 11 and 9 certified constituents in the original sediment and mussel tissue SRMs issued in 1989 and 1990, respectively. Two new marine matrix SRMs were issued in the past year including SRM 1946 Lake Superior Fish Tissue and SRM 2702 Inorganics in Marine Sediment. SRM 1946 is the first NIST fish tissue SRM and is intended for both the environmental measurement community with certified values assigned for 30 PCBs, 15 chlorinated pesticides, total mercury, and methylmercury, and the food and nutrition measurement community with certified values assigned for proximates and 13 fatty acids. Both SRM 1946 and SRM 1974b are issued as frozen tissue homogenates rather than freeze-dried tissues to provide a matrix similar to that typically analyzed in analytical laboratories. A second fish tissue (SRM 1947 Lake Michigan Fish Tissue) has been prepared and measurements are in progress to provide a fish tissue SRM with emphasis on the trace element content to complement SRM 1946, which focuses primarily on the organochlorine contaminants.

SRM 2702, which replaces the popular SRM 2704 Buffalo River Sediment, has certified values for 25 elements, reference values for 8 elements, and information value for 11 elements covering all of the priority pollutant elements. In addition, the stock material used for SRM 2702 is also being used to develop the first reference material (SRM 2703) for solid sampling direct analysis techniques that commonly use small analytical test portions.

Impact: SRMs 1946, 1941b, 1974b, and 2702 were developed to support the numerous laboratories involved in marine environmental monitoring.

Future Plans: As new classes of compounds become environmental concerns, e.g., polybrominated diphenyl ethers (PBDEs), toxaphene, polychlorinated naphthalenes (PCNs), and organometal speciation, these marine sediment and tissue SRMs will be evaluated for potential value assignment for these new environmental contaminants.

Environmental Technologies

Title: Detoxification of PCB-contaminated Materials using Electron Beam Technology and Ultra-violet Radiation

Authors: D.L. Poster, P. Neta and R.E. Huie (Div. 838), O. Kantoglu, J. Silverman, and M. Al-Sheikhly (University of Maryland)

Purpose: The widespread use of chlorinated organic compounds in a wide range of applications presents environmental issues due to not only the toxicity of these compounds but also their proven persistence in the environment. Many of these compounds, such as polychlorinated biphenyls (PCBs), were used previously in industry though a large portion consists of current-use compounds that are intentionally released to the environment, such as pesticides. Unfortunately, these compounds are typically very hydrophobic and eventually accumulate in fat- or organic-rich environments such as the fatty tissues of organisms in the food chain or in freshwater (i.e., aquifers, lakes, rivers) or marine (i.e., estuary or ocean) sediments. Reduction or removal of chlorinated organic contaminants from environmental systems is a complex task. Most materials that contain chlorinated species are incinerated. This method, however, suffers from two disadvantages: the medium is incinerated along with the contaminants and the process may convert some of the contaminants into other toxic materials such as dioxins. Degradation of chlorinated species by radiolytic or photolytic methods possibly overcomes these disadvantages, however when applied to organic-rich compartments, such as sediment, the process can be inefficient. Other constituents in sediment compete or react with secondary electrons and organic radicals produced during radiolysis. We have investigated procedures to suppress these competing processes while at the same time enhancing the dechlorination yield.

Major Accomplishments: For several years, the Analytical Chemistry Division has collaborated with the Physical and Chemical Properties Division and the University of Maryland on studies of the radiolytic and photolytic dechlorination of polychlorinated biphenyls (PCBs). In earlier work, PCBs in water and transformer oil were successfully dechlorinated[1-3] using electron beam and gamma irradiation technology (Figure 1), but more recently, we have moved to the more complex matrix of marine sediment. Studies involving the use of aqueous-based additives (alcohol solutions, triethylamine) to enhance the dechlorination processes have demonstrated the effective degradation of PCBs and a metabolite of the chlorinated pesticide DDT (DDE) in marine sediment (SRM 1944) with electron beam and ultra-violet technologies. In the electron beam irradiated samples, the concentrations of 29 PCB congeners were found to decrease with dose (83 % at the highest dose). Photolysis led to little dechlorination but photolysis with added base led to dechlorination (about 60 %)[4]. Current investigations are focused on the dechlorination of contaminants in the presence of food-grade (i.e., edible) surfactants. Initial results suggest that these additives enhance the electron beam and ultra-violet induced destruction of PCBs in marine sediment.

Impact: The feasibility of electron beam treatment for PCB-contaminated systems has been demonstrated and initial design parameters for possible scale-up have been established. Photochemical destruction of PCBs in transformer oil and sediment has also been shown to be effective. Other compounds present in these systems, such as pesticides, can also be reduced or

likely removed with optimum conditions (greater irradiation dose or exposure time). However, the specific methods are likely limited in their practical applications with regard to sediments. A combination of dredging and subsequent treatment by either radiolytic or photolytic methods with relatively expensive additives would be considered only in very special cases. Regardless, minor revisions to the approach, such as the use of benign, environmentally-friendly additives or lime for pH adjustment, could make these technologies very competitive for the clean-up or remediation of sediments.

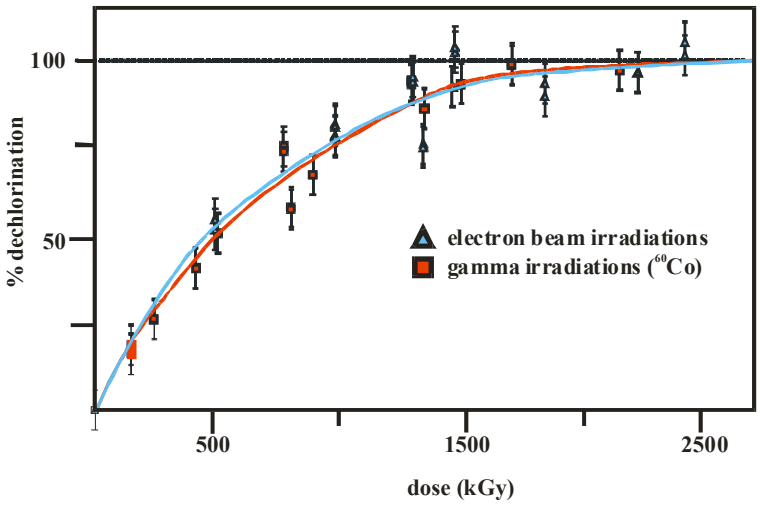

Figure 1. Dechlorination of PCBs in transformer oil using electron beam and gamma irradiation technologies. Both technologies are comparable although a longer exposure time is required with gamma irradiations due to the lower energy of the gamma source (^{60}Co) relative to the electron beam. Complete dechlorination is achieved with both types of radiation[3]. Current studies are focused on the use of the electron beam.

Future Plans: This activity is on-going. Experiments are underway to identify optimal conditions for PCB and pesticide degradation in marine sediment using different food-grade surfactants and electron beam and ultra-violet technologies. Biosurfactants produced by microorganisms may also be examined. The rates of reaction between chlorinated species and the solvated (secondary) electrons as well as electron scavenging by the surfactants will be studied using pulse radiolysis. Both of these parameters are necessary for fabricating environments that are conducive to the efficient and quick degradation of chlorinated compounds in marine sediment.

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Environmental Technologies

Title: Global Climate Change Measurement Needs Development of Gas Mixture Standards to Support

Authors: R.C. Myers and G.C. Rhoderick

Abstract: Several species of gases found in the atmosphere that can contribute change to the radiative environment of the earth have been developed as gaseous primary standards (PSMs). These gases are considered greenhouse gases and have been monitored throughout the atmospheric environment community worldwide for many years. The gases have also gained increased importance since the Kyoto protocol was designed and implemented to promote reduced emissions of greenhouse gases. PSMs were developed for methane (CH_4), carbon dioxide (CO_2), tetrafluoromethane (CF_4), nitrous oxide (N_2O), and sulfur hexafluoride (SF_6).
Development of NIST primary standards (PSMs) regarding global climate change measurements has served three purposes this past year. Firstly, there was a need to replenish the NIST in-house inventory of methane and carbon dioxide gas primary standards to continue support of several NIST SRMs containing CH_4 and CO_2 that are frequently used by the atmospheric research community. Secondly, our laboratory is currently participating in three comparison studies for PSMs. One study, CCQM-P41 Greenhouse Gases, involves several national metrology institutes (NMIs) and laboratories from the World Meteorological Organization (WMO) in an effort to evaluate and improve the preparation and measurement capability of gravimetrically prepared methane and carbon dioxide mixtures. A second separate study in collaboration with NOAA/CMDL for comparing methane PSMs is also currently ongoing. The third is also an international comparison, CCQM-K15, for CF_4 and SF_6.

Purpose: PSMs were developed to support the in-house standards base for SRM certification, international intercomparison programs between national NMIs to determine equivalence, and the NIST FTIR spectral database project.

Major Accomplishments: A total of 22 gravimetrically prepared CH_4 in air primary standards, many of which were prepared this past year, now exist and are used to define the NIST primary calibration methane scale for laboratory measurements and traceability. The entire suite of primary standards range in concentrations from 0.8 µmol/mol to 10 µmol/mol. Two PSMs were submitted by NIST for evaluation in the CCQM-P41 Greenhouse Gas comparison study. One PSM contains methane in air and the second mixture contains carbon dioxide in air. Three PSMs containing methane in air were also compared to methane in air PSMs prepared at the Climate and Diagnostics Laboratory at NOAA. A suite of three standards containing CF_4 and SF_6 were prepared to value assign the CCQM-K15 sample. Three standards each of CF_4 and N_2O were prepared for band strength intensities for the FTIR spectral database. These standards are currently being analyzed by FTIR to characterize band strength intensities, which in turn are archived into the NIST FTIR database. This database can then be used as a standard to determine amount-of-substance fractions (concentrations) of chemical species in unknown gas samples and real time measurements.

Impact: Development of these PSMs will underpin the SRM program and serve to establish equivalency between national NMIs.

Future Plans: Additions to the FTIR database will continue in the future with characterization of chemical species that contribute to changes in the atmospheric composition and global warming. Also, it is anticipated that PSMs will be developed for many species that contribute to global warming. These PSMs will lay the foundation for which atmospheric measurements can be underpinned and possible development of new SRMs to support national ambient monitoring measurements of those species.

Environmental Technologies

Title: International Infrastructure for Traceability in Atmospheric Ozone Measurements

Authors: P.M. Chu and J.E. Norris

Abstract: The concentration of ozone (O_3) in the atmosphere remains a significant issue from both scientific and political perspectives. At tropospheric levels, O_3 is a health concern and contributes to global climate change as a greenhouse gas, while stratospheric O_3 protects earth from harmful UV radiation. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to ten US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest has prompted sixteen foreign laboratories to acquire SRPs. Current efforts focus on further anchoring the SRP through international comparisons, comparisons with NIST's primary nitric oxide (NO) gravimetric standards and a nitrogen dioxide (NO_2) permeation tube system, and the development of an advanced primary reference standard. Additionally, NIST and BIPM have been collaborating to transfer the responsibility of international O_3 measurement traceability to the BIPM.

Purpose: To support the growing national and international interest in the comparability and SI-traceability of global O_3 measurements, NIST is pursuing several efforts. NIST is partnering with BIPM to develop advanced primary ozone reference standards and to share the dissemination of secondary or transfer standards. The basic project plan includes 1) Transfer the current ozone "national reference photometer" technology to BIPM. 2) Assess the performance of the current SRP with respect to current and future ozone measurement requirements. 3) Develop a primary reference standard with capabilities of SI-traceability verification.

Major Accomplishments: Over the past two years, NIST has been working extensively with BIPM to transfer the responsibility of international O_3 measurement traceability to BIPM. This effort has included the construction of five new SRPs, including extensive training of BIPM staff during the construction process. This work completes a major portion of NIST's collaboration with BIPM to transfer the current SRP capabilities to BIPM. As an initial effort to evaluate the current level of international comparability of O_3 standards, NIST is participating, along with 19 other National Metrology Institutes in the CCQM-P28 pilot study, led by BIPM. During FY 2003, a gas phase titration experiment was performed to check the consistency of the current SRP ozone measurement capability with NIST NO gravimetric standards and an NO_2 permeation calibration system. The results indicate the O_3, NO and NO_2 standards agree to within 1 %. Under NIST's Small Business Innovative Research (SBIR) Phase II funding, Aerodyne Research Inc. is developing a quantum cascade (QC) laser system to serve as NIST's authoritative O_3 reference standard. Aerodyne's successful Phase I results have demonstrated that this new technology will significantly extend NIST's measurement capabilities for O_3, NO and NO_2.

Impact: It is anticipated that the advanced primary ozone reference standard will improve the SI-traceability of O_3 measurements. Furthermore, successful completion of this work may also lead to a superior O_3 reference instrument that is produced by a commercial supplier.

Future Plans: After completion of the SBIR Phase II prototype, NIST will fully evaluate the feasibility of the QC laser system to serve as NIST's primary reference standard for ozone.

Environmental Technologies

Title: Research for Development and Measurement of Heavy Hydrocarbon (C_6-C_{16}) Gas Standards

Authors: G.C. Rhoderick

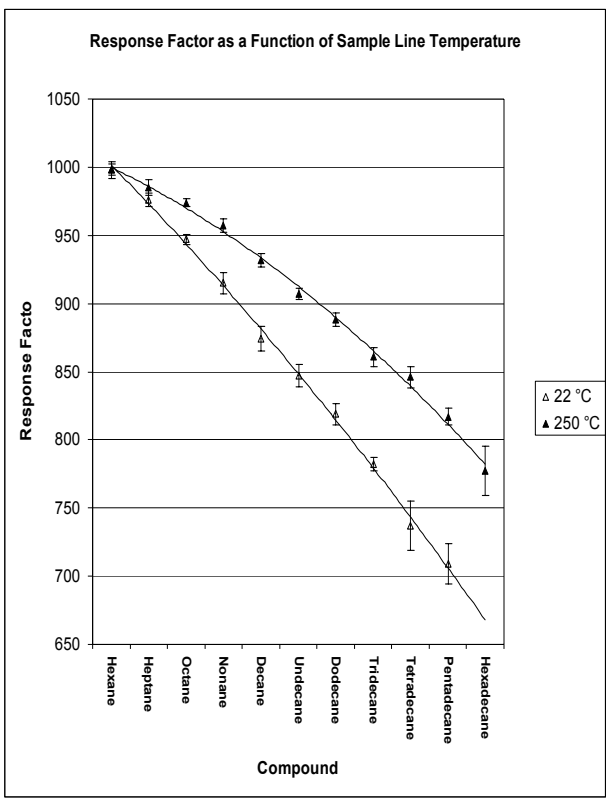
Abstract: In recent years many health risk statements have been issued about long-term exposure to diesel exhaust. Diesel exhaust contains soluble organic compounds including the alkane class. Laboratory results indicate that these diesel exhaust products are toxic, mutagenic and carcinogenic. The wide use of diesel engines, including larger trucks, buses, fire engines and equipment, railroad locomotives, farm tractors, construction equipment, ships, mining equipment, and stationary equipment such as electricity generators, puts a large percentage of people at risk to exposure of these compounds from exhaust. In some areas where diesel engines and equipment are used, efforts are being made to reduce worker exposure. State and federal agencies are beginning to monitor ambient air for compounds related to diesel exhaust. The National Institute of Standards and Technology (NIST) was asked to research and develop standards containing heavy (C_{10} – C_{16}) alkanes which could then be used in air monitoring and exhaust studies. Several primary gravimetric standards were developed and analyzed by gas chromatography (GC) with a flame-ionization detector (FID). The results of this research indicate that accurate gas standards containing these hydrocarbons can be prepared. However, the analytical results show that the temperature of the transfer system from the gas cylinder to the GC column (including the gas sample valve) must be heated in order to prevent adsorption of these compounds within the analytical system. The results indicate that even at elevated temperatures these compounds are being absorbed within the system. Quantitative results cannot rely on the use of one compound, such as hexane, as an internal standard but can be obtained if the compounds of interest are determined from standards containing the relevant compounds.

Purpose: Discussions have begun on the future implementation of diesel exhaust test much the same as the automobile testing that many states require. This will also lead to ambient air monitoring of these species as NIST has already supplied the state of California Air Resources Board with gas standards containing C_{10} – C_{13} alkanes. Appropriate gas standards will be needed to measure the components of diesel exhaust as well as an understanding of the measurement system.

Major Accomplishment: Previous standards development of C_6-C_{10} compounds had been highly successful. However, the high boiling points of C_{11}-C_{16} (having no prior experience) prompted a question as to the ability to accurately transfer a quantity of these compounds into an aluminum gas mixture and then retrieve them for analysis. Several standards containing the C_6-C_{10} alkanes were gravimetrically prepared. The standards were intercompared using gas chromatography (GC) with flame ionization detection (FID). System temperatures were elevated to the maximum levels taking into account safety issues including the FID at 350 °C and the sample transfer system from the gas cylinder to the GC column at 250 °C. The concentration determined by analysis agreed very well with the gravimetric value for all of the hydrocarbons with all showing a difference of /nor more than ± 0.6%. All the gravimetric concentrations fall within the uncertainty bounds for the analyzed concentrations. These results show that accurate

standards of heavy hydrocarbons up to C_{16} can be prepared. However, analyses using varying system temperatures showed major differences in results. Theoretically, the response factors should be the same for each hydrocarbon since the FID is proportional to the number of carbons/unit time, reported as FID relative sensitivities. However, figure 1 shows that the response factors at 250 °C are larger than at 22 °C, except for hexane. These results suggest that the heavier hydrocarbons absorb on the regulator and sample transfer line from the cylinder to the GC. This may or may not present a problem when trying to determine the concentration of a heavy hydrocarbon in a gas mixture. The results do show that a consistent response for an individual hydrocarbon can be obtained. Therefore, if the efficiency of sample transfer from the cylinder to the GC remains constant, one should be able to use a standard to determine an unknown. The one restriction would be that each hydrocarbon would have to be determined using the respective hydrocarbon in a standard. For example, dodecane would have to be determined using dodecane and not another compound such as hexane or decane.

Figure 1. Comparison of response factors at 22 °C and 250 °C.



Impact: The results of this research indicate that gas standards, containing heavy, straight chain hydrocarbons found in diesel fuel (C_{10} – C_{16}), can be gravimetrically prepared. Consistent measurements of these compounds by GC-FID can be obtained using elevated system temperatures.

Future Plans: More research into elevated temperatures is needed to more fully understand the effects this might have on the analytical measurements. Research is planned to determine the transfer efficiency of the hydrocarbon into the gas cylinder during the preparation procedure.

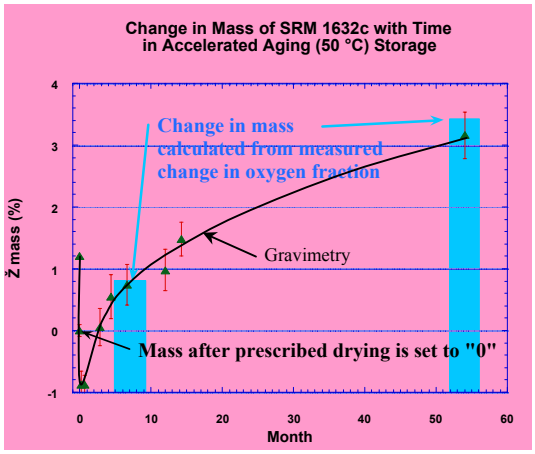
Environmental Technologies

Title: Influence of Oxygen on the Shelf Life of Coal SRMs

Authors: R. Zeisler and W.D. James (TAMU)

Abstract: In 1997 users of the previously (1985) issued SRM® 1632b Trace Elements in Coal reported a change in the measured values of the carbon mass fraction, the new values being about 1.6% lower than previously measured. Additional evidence of change was provided by trace element data in this material, which indicated approximately the same change in mass fraction values for a number of elements determined by INAA, although the new measurements were still within the bounds of uncertainty given in the certificate. While a change in carbon content through loss of CO_2 may have been a plausible explanation for lower than previously measured carbon values, the measured decrease of a larger number of elemental content values may be explained by a gain of matrix mass, such as by irreversible oxidation and therefore increased matrix mass. To directly measure mass fractions of oxygen in coal samples, a fast (14-MeV) neutron activation analysis (NAA) procedure was employed. The procedure was applied to test samples of SRM 1632c Trace Elements in Coal (Bituminous). The samples had been stored at three conditions: in liquid nitrogen vapor and at room temperature in the original containers packaged under argon, and for accelerated aging at 50 °C open to air. Samples were found stable during the more than 4-year observation, except for the ones stored open at higher temperature.

Purpose: Validation of the long-term stability of a coal SRMs under proper storage.



Major Accomplishments: Twenty-six samples each were selected and compared for three storage conditions. The 14-MeV NAA is an effective procedure for the direct determination of oxygen. It demonstrated sufficient precision and accuracy to determine a relative change of about 5 % or more, in the oxygen mass fraction of about 11 %, which is equivalent to about 0.5 % of the total mass. To evaluate the sample stability, test points were set at 6 and 54 months for the oxygen determination, more frequent gravimetric tests were carried out for the samples in the open 50 °C storage. The gain in mass and in oxygen for the latter samples is illustrated in the figure. All gain in mass can be explained by the gain in oxygen content. No change in oxygen content was measured in the other storage conditions.

Impact: The measurements give assurance that properly packaged coal SRMs, protected from oxygen and heat, are stable during their shelf life.

Future Plans: Gravimetric monitoring of coal SRMs during shelf storage should be a cost-effective quality measure.

Food Safety and Nutrition

Title: Development of Analytical Methods and SRMs for Chemical Characterization of Botanical Dietary Supplements

Authors: S.A. Wise, T.A. Butler, T. Ihara (National Metrology Institute of Japan, AIST), S.E. Long, M.C. Mildner, E.A. Mackey, K.E. Murphy, K.W. Phinney, B.J. Porter, L.C. Sander, M.B. Satterfield, K.E. Sharpless, L.J. Wood, and L.L. Yu

Abstract: More than 50% of the U.S. population uses dietary supplements, accounting for roughly $10 billion in sales every year. A variety of botanical-containing products are marketed as dietary supplements; common examples include St. John's wort, ephedra, ginkgo, and saw palmetto. Taxonomically authentic botanical reference materials with assigned values for active and/or marker compounds are needed for quality assurance of analytical measurements associated with the manufacturing process and for the verification of manufacturers' label claims for the dietary supplement industry. Assigned values for contaminants and adulterants are also necessary to address public health and safety concerns. In 2001 NIST, the Food and Drug Administration (FDA), and the National Institutes of Health's Office of Dietary Supplements (NIH-ODS) initiated a multi-year program to develop Standard Reference Materials (SRMs) and analytical methods for a number of botanical and botanical-containing dietary supplements. The goal of this collaborative program is to provide SRMs for eight to ten different botanical dietary supplements over a six-year period. Ephedra-containing materials were identified as the highest priority by FDA and NIH-ODS, and we are currently producing a suite of five ephedra-related SRMs to address this need.

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

Major Accomplishments: A suite of ephedra-based materials has been prepared consisting of authentic ephedra plant material, SRM 3240 *Ephedra sinica* Stapf (Aerial Parts); two dried extracts of the plant material (natural and fortified to contain 8% total ephedrine alkaloids), SRM 3241 *Ephedra sinica* Stapf (Native Extract) and SRM 3242 *Ephedra sinica* Stapf (Commercial Extract); and two finished products, SRM 3243 Ephedra-Containing Powdered Solid Oral Dosage Form and SRM 3244 Ephedra-Containing Protein Powder. These materials have been ground and sieved to 80 mesh, as necessary, blended, packaged, and radiation sterilized for distribution as SRMs. Qualitative and quantitative chemical characterization of these five materials is nearing completion. The six ephedrine alkaloids, the primary active markers in ephedra, have been measured at NIST using several analytical methods (i.e., liquid chromatography with UV absorbance detection (LC-UV), LC/mass spectrometry (MS), LC/tandem MS (MS/MS), and capillary electrophoresis (CE-UV) to assign concentration values.

Measurements for contaminants such as toxic metals (As, Cd, Hg, and Pb) using inductively coupled plasma MS (ICP-MS) and instrumental neutron activation analysis (INAA) are also in progress. Additional analyses of the ephedra SRM suite are being provided by collaborating laboratories including FDA, National Research Council Canada, and ChromaDex. An LC and thin-layer chromatography (TLC) profile or "fingerprint" of the plant material will also be provided for use as a reference for comparison to other similar materials.

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

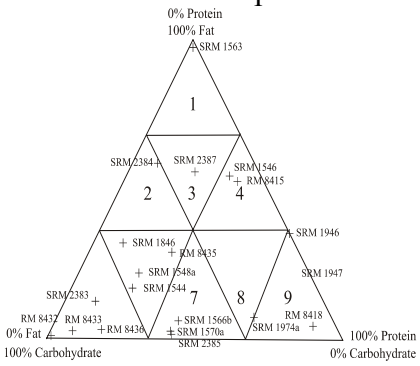
Future Plans: This program is a six-year effort to develop SRMs for eight to ten different botanical dietary supplements. After ephedra the next botanical materials for development of SRMs are: g*inkgo biloba*, St. John's wort, saw palmetto, and green tea. The ginkgo materials (plant, extract, and solid oral dosage form) have been obtained and the preparation (grinding, blending, and bottling) of this SRM suite is in progress. Acquisition of the St. John's wort, saw palmetto, and green tea are in progress and will be completed in early 2004. The next priorities for SRM development include: β-Carotene (e.g., a mixture of carotenoids in an oil matrix and a mixture of β-carotene isomers), vitamin E (e.g., d-α-tocopheryl acetate/d,l-α-tocopheryl acetate and a mixture of the tocopherol and tocotrienol isomers), and *Citrus aurantium*. NIH-ODS also plans to expand the scope of this project to include multivitamin and multielement supplements. The development of a multivitamin and multielement dietary supplement SRM, with values assigned for the 35 vitamins and elements listed on the supplement label, will be a major effort in FY04 and FY05.

Food Safety and Nutrition

Title: Completion of a Complementary Suite of Food-Matrix SRMs to Support Industry Compliance with Nutritional Labeling Requirements

Authors: K.E. Sharpless, J. Brown Thomas, C.S. Phinney, B.J. Porter, and L.J. Wood

Abstract: The Analytical Chemistry Division (ACD) is working within the broad category of "food and nutrition" to facilitate compliance with nutritional labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices. The composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed; a fat-protein-carbohydrate food composition model developed by AOAC International is a useful way to do that. NIST has been working with other federal agencies and the food industry over the past several years to provide a suite of SRMs within this model to address nutrition labeling issues. Value-assignment of two food-matrix SRMs was completed this year: SRMs 2385 Slurried Spinach and 2387 Peanut Butter. Their positions in the AOAC triangle, along with those of the other food-matrix reference materials available from NIST, are shown.



Purpose: To develop reference materials and analytical methods to underpin measurements made for compliance with nutritional labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices

Major Accomplishments: Value-assignment of two food-matrix SRMs was completed this year: SRM 2385 Slurried Spinach and SRM 2387 Peanut Butter.

Impact: Food-matrix reference materials with values assigned for nutrient concentrations are now available within all sectors, or on the borders between two sectors. Ideally, these materials will improve the accuracy of nutrition information provided on product labels.

Future Plans: Now that materials are available within or on the borders of all sectors, this particular aspect of NIST's food and nutrition program can be expected to decrease. Focus will then shift to more specialized analytes (e.g., individual amino acids, allergens, mycotoxins) in foods, as well as to dietary supplements.

Food Safety and Nutrition

Title: Certification of SRM 1946 Lake Superior Fish Tissue – Frozen Fish Tissue for Environmental Contaminants and Food Constituent Measurements

Authors: S.A. Wise, S.J. Christopher, J.R. Kucklick, S.E. Long, E.A. Mackey, C.S. Phinney, B.J. Porter, R.S. Pugh, D.L. Poster, M. Rearick, KE. Sharpless, and M.M. Schantz

Abstract: SRM 1946 Lake Superior Fish Tissue, which is the first fish tissue SRM, is intended to address the needs of both the environmental contaminants and food measurement communities. SRM 1946 is a fresh frozen tissue homogenate with certified and reference concentration values for over 100 constituents. SRM 1946 was developed primarily for use in the determination of organic contaminants with certified and reference concentrations for 42 polychlorinated biphenyls (PCBs) and 17 chlorinated pesticides, which is the largest number of organochlorine compounds with values assigned in any natural matrix SRM or in any certified reference material (CRM) from other sources. SRM 1946 has values assigned for 13 PCB congeners that have not been previously measured in natural matrix SRMs. This is the first natural matrix SRM with certified values for the three non-*ortho*-substituted PCB congeners, which are present at 10 to 100 times lower concentrations than the other PCB congeners measured, but they are considered to be the most toxic congeners with respect to mammalian cells and biological systems. The increased number of PCB congeners with assigned values in this SRM was a result of improvements in the analytical approach used for these measurements.

Purpose: Validation of the complete analytical procedure requires the use of natural matrix SRMs similar to those matrices typically encountered in the analysis of environmental samples. NIST natural matrix SRMs for organic and inorganic contaminants in marine tissue are necessary for the development and validation of analytical procedures and to provide quality assurance of chemical measurements in the marine environmental and food measurement communities.

Major Accomplishments: SRM 1946 Lake Superior Fish is a fresh frozen tissue homogenate prepared from trout collected in Lake Superior and was developed primarily for use in the determination of organic contaminants with certified and reference concentrations for 42 polychlorinated biphenyls (PCBs) and 17 chlorinated pesticides, which is the largest number of organochlorine compounds with values assigned in any natural matrix SRM or in any certified reference material (CRM) from other national or international sources. SRM 1946 has values assigned for 13 PCB congeners that have not been previously measured in any natural matrix SRMs. This is the first natural matrix SRM with certified values for the three non-*ortho*-substituted PCB congeners, which are present at 10 to 100 times lower concentrations than the other PCB congeners measured, but they are considered to be the most toxic congeners with respect to mammalian cells and biological systems. The increased number of PCB congeners with assigned values in this SRM was a result of improvements in the analytical methodology used for these measurements [1], particularly for the non-ortho-substituted PCB congeners [2] and the methylmercury [3].

Food Safety and Nutrition

Title: SRM® 1727 and Its Use in Keeping Tin Cans Lead-Free

Authors: J.R. Sieber, K.E. Murphy, and S.E. Long

Abstract: SRM 1727 Anode Tin was developed in cooperation with ASTM International to provide a tool for improving the accuracy of Pb determinations at trace levels in tin, tinplate products, and plating bath solutions. SRM 1727 was certified on the basis of determinations using isotope-dilution mass spectrometry. It is anticipated that tinplate producers and container manufacturers will use SRM 1727 to help control the levels of Pb in coatings applied to food and beverage containers.

Purpose: When steel mills manufacture tin-plated sheet steel for food cans, they must test the tin and the electroplating bath for lead content. If lead is allowed to accumulate in the tin or the bath, it will become part of the tin coating and may leach into food stored in cans. In recent years, the allowable Pb content of tin plate has been reduced in recognition of potential health risks in products used for foods. Steel mills typically purchase Sn anodes according to ASTM B 339 Standard Specification for Pig Tin, with a restriction of 50 ppm Pb maximum. Residual amounts of anode tin are typically remelted on-site to form new anodes. Incoming and remelted tin anodes are analyzed by atomic emission spectrometry to meet material acceptance and process control requirements.

Major Accomplishments: Developed in cooperation with ASTM International, Standard Reference Material® 1727 Anode Tin is intended primarily for use in evaluating chemical and instrumental methods of analysis of refined pig tin for Pb content. SRM® 1727 is certified for Pb content on the basis of analyses by isotope-dilution inductively-coupled plasma mass spectrometry. Information values are provided for ten additional elements. SRM® 1727 is sold in solid form (a block 30 mm on each side) for spectrometric analysis and may be chipped for use with chemical methods of analysis.

Impact: Tinplate is a high volume commodity made by many US and foreign steel companies. About 3 million tons of tinplate are manufactured and shipped from US steel producers annually, at a typical selling value of $800 per ton during 1999. Container manufacturers purchase much of the tinplate to make products such as food and beverage cans, automotive filter cases, and paint cans. SRM 1727 will provide a means for validation of the accuracy of analytical determinations of Pb at trace levels enabling these industries to control their tinplate lines more economically and provide safer products.

Future Plans: Currently there are no future plans for work in this area. However, ASTM Specification B 339 lists a number of additional elements that may require monitoring. If the steel and container industries require better analytical performance for additional elements, it may be possible to certify additional elements in SRM 1727.

Because SRM 1946 is comprised of edible fish tissue, the food chemistry community can also use this material for measurements that are necessary to support nutritional labeling requirements. SRM 1946 is characterized for nutrient concentrations including proximates (fat, protein, carbohydrates, ash, and solids), 25 individual fatty acids (the most fatty acids measured in any SRM), and 12 nutritive minerals and trace elements. The individual fatty acids that are certified in SRM 1946 include the nutritionally important omega-3 fatty acids, which have not been certified previously in a natural matrix SRM.

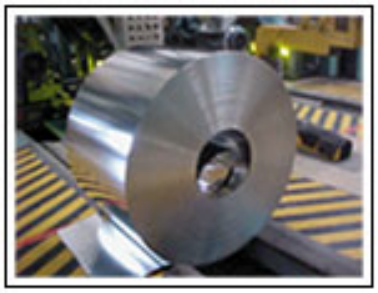
Of particular importance in both the environmental contaminants and food measurement communities is the determination of total mercury and the more toxic mercury species, methylmercury. Both of these environmentally relevant species are characterized in SRM 1946. This is the first certification of these compounds in a fresh fish tissue homogenate CRM. The certified concentration of methylmercury in SRM 1946 is approximately at the level proposed by the U.S. EPA for freshwater and estuarine water quality criterion (0.3 mg/kg fish). Thus this SRM will be valuable in assessing methods used to monitor methylmercury levels in fish to determine allowable fish consumption advisories.

Impact: The development of SRM 1946, with certified and reference values for over 100 chemical constituents, including 3 coplanar PCB congeners and the omega-3 fatty acids, represented a significant technical challenge. This SRM is the only CRM of its kind in the world and will serve as an invaluable quality assurance tool for quality assurance of contaminant measurement programs related to the marine environment on local (i.e., Chesapeake Bay fish monitoring), regional, (i.e., the Great Lakes) and continental scales (i.e., fish pollution monitoring within 200 miles of U.S. shores for healthy consumable fish trade).

Future plans: A companion fish tissue material, SRM 1947 Lake Michigan Fish Tissue has been prepared and measurements are in progress to provide a fish tissue SRM with emphasis on the trace element content to complement SRM 1946, which focuses primarily on the organochlorine contaminants. SRM 1947 will be completed in FY04.

References:

1. Poster, D.L., Kucklick, J.R., Schantz, M.M., Porter, B.J., Leigh, S.D., and Wise, S.A. **"*Determination of Polychlorinated Biphenyl Congeners and Chlorinated Pesticides in a Fish Tissue Standard Reference Material,*"** Anal. Bioanal. Chem., 375, 223-241 (2003).
2. Brubaker, Jr. W.W., Schantz, M.M., and Wise, S.A. **"*Determination of Non-ortho Polychlorinated Biphenyls in Environmental Standard Reference Materials,*"** Fresenius'J. Anal. Chem., 367, 401-406 (2000).
3. Tutschku, S., Schantz, M.M., and Wise, S.A. **"*Determination of Methylmercury and Butyltin Compounds in Marine Biota and Sediments using Microwave-Assisted Acid Extraction/Digestion, Solid-Phase Microextraction, and Gas Chromatography with Atomic Emission Spectrometric Detection,*"** Anal. Chem., 74, 4694-4701 (2002).



Forensics and Homeland Security

Title: NIST Support of the CDC Chemical Counter-Terrorism Laboratory Network

Authors: K.E. Murphy, M.M. Schantz, B.A. Benner, Jr., T.A. Butler

Abstract: NIST has developed an improved method for the determination of cyanide concentrations in whole blood samples and is collaborating with the Emergency Response and Air Toxicants Branch of the Centers for Disease Control and Prevention (CDC) to help provide quality assurance and measurement proficiency standards for the "Chemical Counter-Terrorism Laboratory Network" (CTLN). This laboratory network has been established by CDC to provide rapid measurement support in the event of a terrorist chemical attack. Cyanide is considered a likely candidate for use in chemical terrorism because it is easy to obtain and has been used in previous attacks. Cyanide is analytically challenging and because it is unstable, blood-based standards are not commercially available. NIST developed an improved method for the determination of cyanide in whole blood based on headspace gas chromatography with mass spectrometric detection (GC/MS), employing isotopically labeled cyanide ($K^{13}C^{15}N$) as the internal standard. Use of the isotopically labeled internal standard coupled with GC/MS resulted in decreased analysis times and improved accuracy and precision. The improved method has been adapted by CDC for transfer to state laboratories within the CTLN and is being used to provide value assignment and stability testing data for a pool of cyanide-spiked blood samples produced by CDC for the CTLN.

Purpose: To develop a method for the precise and accurate quantitation of cyanide in human whole blood and implement the new procedure to provide measurement data to support the CDC CTLN.

Major Accomplishments: At the start of this project, NIST did not have a method in- house for the determination of cyanide. A method based on headspace analysis with GC/MS detection was implemented and improved by the use of an isotopically labeled internal standard. An interagency agreement with CDC has been established to evaluate the content and stability of cyanide in spiked, frozen human whole blood. Proficiency standards containing different levels of cyanide have been prepared by a commercial supplier and approximately 700 vials per level have been packaged. NIST has started a series of periodic analyses which will proceed over the upcoming year to provide value assignment and monitor the stability of the cyanide concentrations in the proficiency standards. Preliminary results indicate that the cyanide concentrations in all levels of the frozen proficiency standards have remained stable for the past two months.

Impact: It is imperative that measurements made in response to a chemical terrorism event be both rapid and accurate. There are currently no standards to underpin the accuracy of blood cyanide measurements. NIST's value assignment and stability monitoring of these blood based cyanide proficiency standards will enhance CDC and state public health preparedness in the event of a chemical terrorism incident involving cyanide.

Future Plans: NIST will monitor the stability of cyanide in the frozen human whole blood proficiency standards over the upcoming year and will continue to work closely with CDC to meet the needs of the CTLN which has grown from the original five members to include laboratories in 46 states across the U.S.

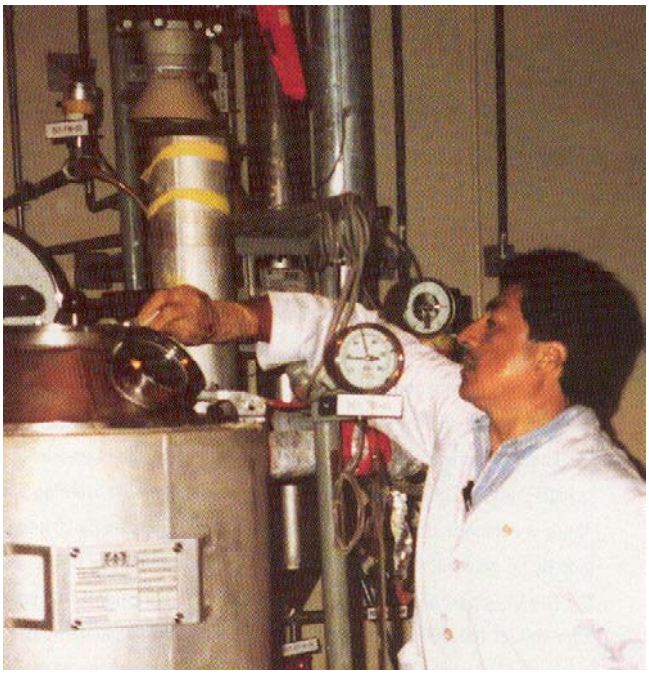
Forensics and Homeland Security

Title: Determination of Traces of Fissionable Materials using Delayed Neutron Activation Analysis

Authors: R.M. Lindstrom, E.A. Mackey, and G.P. Lamaze

Abstract: Detection and measurement of small traces of fissionable uranium and plutonium can be done by delayed neutron activation analysis. The method is intrinsically specific to nuclear fission, the sensitivity is excellent, and the procedure is simple, rapid, and readily automated for high throughput.

Purpose: As shown in the figure, an important tool in nuclear forensics is the collection and analysis of "swipe" samples at sites where materials of interest may be, or may have been at one time. Tiny traces of fissionable uranium or plutonium can be left behind whenever these materials are handled or transported. Using neutrons from the NIST research reactor, the delayed neutrons from fission in these traces can be used to detect and quantitate U and Pu in swipe samples with excellent speed, sensitivity, and specificity.



IAEA inspector collecting a swipe sample from process equipment in a nuclear fuel fabrication facility in 1994 (*Anal. Chem.* **74** (2002), 28A)

Major accomplishments: Delayed Neutron Activation Analysis is being established at NIST for the measurement of small quantities of fissionable nuclides such as ^{235}U and ^{239}Pu. After a brief neutron irradiation, the sample is placed quickly into a neutron detector array and the neutron emission rate measured and compared with that of a standard. The method is well-tested, rapid, specific, matrix independent, nondestructive, and sensitive. The system being built at NIST is calculated to have a detection limit for either of these species about 10 picograms, based on a straightforward extrapolation from published practice. The analysis time is less than 2 minutes per sample.

Preliminary tests have been performed using uranium standards prepared by depositing solutions containing known amounts of uranium onto filter papers, irradiating, and counting on a detection system with one ^{3}He detector surrounded by hydrogenous moderator. Two shielding configurations were tested, one using water as the moderator and the other using polyethylene. Based on these results and a review of the literature, a final detection system was designed. This consists of ten neutron detectors in a 30 x 30 cm cylindrical moderator of polyethylene, lined with 2 cm of lead to absorb gamma radiation. The design of the manual system incorporates the existing pneumatic rabbit assembly for irradiation control. Incorporated in its design is the ability

to unload a sample from the receiver after irradiation with compressed air. This feature will be used to move the sample rapidly to the neutron detector through a polyethylene flight tube.

Impact: On completion and verification, the Nation will have a readily accessible, rapid means of measuring traces of fissionable U and Pu in samples of forensic interest. In addition, the specificity and sensitivity of this method of analysis will be put to use in certifying trace uranium in Standard Reference Materials.

Future Plans: To improve sample throughput, the transfer system can be readily automated with computer control in the coming year. It has been demonstrated elsewhere that ^{233}U, ^{235}U, and ^{239}Pu can be distinguished by the relative yields of delayed neutron precursors with different half-lives, and also of several fission products. We plan to add a gamma-ray detector into the neutron moderator to exploit this signature.

Forensics and Homeland Security

Title: Certification of NIST Reference Material Supporting Improvised Explosive Device Measurements

Authors: W.A. MacCrehan and M. Bedner

Abstract: As part of their investigations of explosives crime, national, state and local forensic laboratories measure the additives in smokeless powder (gunpowder). US military laboratories also measure the chemical composition of such propellant powders to determine the stability of munitions. CSTL is providing a reference material to support the quality of these forensic and military smokeless powder measurements, RM 8107 Additives in Smokeless Powder.

Purpose: Improvised explosives devices (IEDs), such as pipe bombs, most commonly have smokeless powder as the active filler ingredient. When investigating an explosives incident, forensic investigators collect residues of the incompletely burned powder from the crime scene. As part of the evidence investigation process in the forensic laboratory, the physical appearance and chemical composition of the powder are carefully measured. This permits a categorization of the residue powder for comparison to samples of known powders. The resulting information can often be used to link residues to powders recently purchased by or collected in evidence from the perpetrator.

To help assure the quality of forensic measurements of evidence such as explosives residues, the forensic community has a certification program for laboratory measurements, called ASCLD/LAB (American Society of Crime Lab Directors/Laboratory Accreditation Board). As part of a five-year certification process, laboratory methods and operator proficiency are tested with challenge samples of known properties. Currently, there are no test or reference materials to support explosives measurements. To address the need for forensic explosives reference materials, the NIST Office of Law Enforcement Standards (OLES, in conjunction with the National Institute of Justice) and the NIST SRMP have sponsored the development of RM 8107 Additives in Smokeless Powder. In addition to the need of forensic laboratories, precise measurement of the chemical composition of smokeless propellants is critical to the US military community as a means of assuring the safety of munitions. Certification of NIST RM 8107 will promote accurate forensic and military smokeless powder measurements.

Major Accomplishments: Smokeless powder is a blend of nitrocellulose and a series of additives including the propellant nitroglycerin (NG) and stabilizers such as diphenylamine (DPA) and ethyl centralite (EC, N, N'-diethyl, N, N'-diphenylamine) along with. N-nitrosodiphenylamine (NnDPA) that forms during aging of DPA-stabilized nitrocellulose. These four key analytes are used in forensic and military smokeless powder measurements and are the targets for the NIST RM certification measurements.

RM 8107 consists of a rifle-type ball powder. A major powder manufacturer, Primex Technologies, provided 4 kg of rifle powder from a single, carefully blended manufacturing

batch. For the certification measurements of the four additives, an ultrasonic solvent extraction method was developed to recover the analytes. Two separation techniques were used to determine the NG, DPA, NnDPA, and EC content: micellar capillary electrophoresis (CE) and liquid chromatography (LC). The mean values obtained by the two techniques were in good agreement. Since the LC technique provided lower uncertainty in the measurements, it is being used for the certification measurements. We hope to release RM 8107 for sale in 2004.

Impact: With the development of this smokeless powder RM, forensic laboratories will have a reliable material for the development and validation of measurement methods for improvised explosives devices. The RM may also be used as a proficiency challenge sample to test operator and laboratory performance in explosives measurements as part of laboratory accreditation activities. In addition, US military laboratories can use the additive certified reference values in the NIST RM to assure accurate monitoring of munitions stability.

Future Plans: The smokeless powder RM 8107 was developed to help assure the quality of 'low explosives' type measurements. However, 'high explosives' such as TNT and RDX (ingredients of military and plastic explosives) are often used in terrorist explosive devices. In conjunction with OLES and the Office of Domestic Preparedness, we plan to develop a reference material for these high explosives. A solid matrix material will be coated with commonly used military explosives. The goal is to develop a particulate explosives material that can be used to test explosives detection equipment used for airport security screening, evaluation of suspicious packages by first responders, and forensic laboratory post-blast investigations.

Forensics and Homeland Security

Title: Cell-based Sensors for Screening Toxins

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

Abstract: This project is a continuation from last year and involves a collaboration between the NIST Microanalytical Laboratory competence project (now in its final year of funding) and Virginia Polytechnic and State University. The goal of this work is to develop a cell-based microfluidic biosensor that can serve as an early warning system for the detection of chemicals in the wastewater that may cause bioreactor failure. In particular, we have focused our joint effort on the detection of electrophilic chemicals in water since for many years Love's group has studied the negative biological response of bacterial cells to this class of toxins. In the presence of electrophilic toxins, *E. coli* cells rapidly efflux a large amount of potassium into the surrounding media, a response that contributes to bacterial deflocculation and ultimately bioreactor failure. Prototype bioreactor devices are designed as microfluidic chips containing immobilized bacteria with integrated optodes to detect potassium efflux upon exposure to electrophiles. We are targeting the device for the detection of toxins in wastewater streams but also hope to extend this work to screen for toxins in drinking water.

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

Major Accomplishments: Contamination of the bioreactor, the heart of the wastewater treatment plant, from chemical dumping can have a devastating impact on wastewater processing. In fact, system shut-down caused by bioreactor failure often results in the diversion of large amounts of raw untreated sewage to rivers and streams with an obviously detrimental effect. Several different classes of chemicals, including electrophilic toxins, feeding into water treatment plants have been known to cause bioreactor degradation leading to system processing failures. Recent results from Love's group have led to the hypothesis that sludge deflocculation or biofilm detachment, a mechanism by which the bioreactor can fail, occurs through the activation of the glutathione-gated K^+ efflux (GGKE) system stimulated by electrophilic toxins. Therefore, prior to biofilm detachment, bacterial cells expel large amounts of potassium from inside of the cell to the outside. In this collaborative effort, we have designed a cell-based microfluidic biosensor to detect the presence of electrophilic toxins in water streams entering wastewater treatment plants in an effort to prevent failure of the bioreactor. The approach is to immobilize bacterial cells in a microfluidic system and monitor cell response, in particular potassium efflux, as the water flows through a small bed of immobilized cells. In essence, we have created a micro-bioreactor. The behavior of our micro-bioreactor is then used to predict the behavior of the process bioreactor when exposed to the same water supply. A negative response in the microsystem can be used to decide when to divert part of the incoming stream to prevent process failure. The microbioreactor has several key components including an immobilized bed

of live bacterial cells (*Escherichia coli* K-12); an optode film that is responsive to potassium ions; an optical detection unit; and microfluidic channels to move cell media and the water samples through the cells and past the optode film. This year, we have focused our attention on two aspects of this project: (1) cell immobilization in polymer microchannels and (2) development of a miniaturized optical detection component.

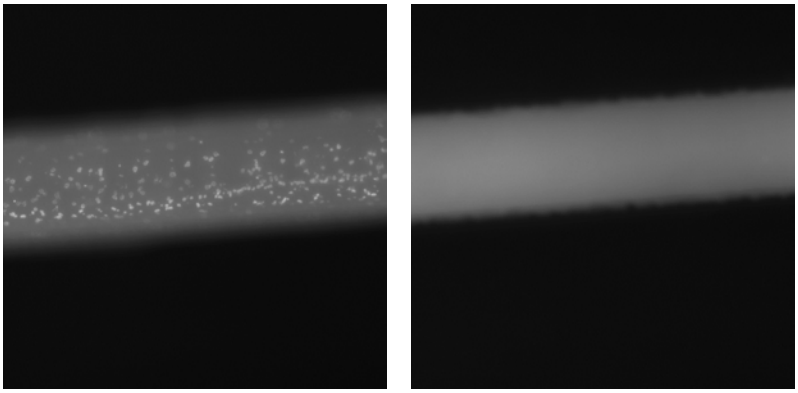


Figure 0. Fluorescence image of PETG microchannels after exposure to *E. coli* cells. Fluorescent dots indicate the presence of live cells. A) Uncoated PETG channel, and B) channel coated with polystyrene sulfonate.

Love's group hypothesized that cell adhesion to an abiotic polymeric surface would be influenced by several factors including the chemical characteristics of the bacterial membranes; the physiological state of the cells at the time of adhesion; and the characteristics of the polymer material used to fabricate microfluidic channels. Earlier work[1] was performed to determine the characteristics of the bacterial membrane, specifically the relative hydrophobicity of *E. coli* K-12 cells in different growth media, that could affect cell adhesion. Experiments showed that cells grown in an N-limited medium (20:1 C:N) exhibited greater cell surface hydrophobicity than a balanced C:N media supporting previous findings[2]. Based on these results, polymers were chosen that were expected to enhance cell adhesion and these were tested in cell binding experiments. *E. coli* cells were also immobilized onto polymer surfaces coated with polyelectrolyte multilayers. It was determined that the cells adhered better to uncoated polyethylene terephlalate (PETG) surfaces than to PETG surfaces coated with polyelectrolyte (shown in Figure 1).

The detection system was also miniaturized this year to make the device more suitable as a prototype field device. A microfluidic device was mounted on a small holder and a miniaturized light source was coupled to the channel using fiber optics. Light was collected through fiber optics into a hand-held spectrometer (Ocean Optics) coupled to a laptop computer for data processing. This device is now being evaluated to measure the optode film response in the presence of varying concentrations of potassium.

Impact: The goal of this collaborative effort is to develop a hand-held device for in-situ monitoring of environmental toxins that could seriously impact the efficacy of bioreactors used in wastewater treatment processes. This problem is not often addressed until after reactor failure occurs resulting in the dumping of unprocessed sewage into streams and rivers. A reliable early

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[1] van Loosdrecht, Mark, C.M, Lyklema, Johannes, Norde, Willem, Schraa, Gosse and Zehnder, Alexander J.B *Applied and Environmental Microbiology*, **53** (8) 1898-1901, **1987**.

[2] Cowell, B.A., Willcox, M.D.P., Herbert, B., and Schneider, R.P. *Journal of Applied Microbiology*. **86** 944-954, **1999**.

warning system can have a profound environmental and financial impact by detecting the problem before failure occurs.

Future Plans: We are currently seeking funding to continue this work and to apply it to the development of a microfluidic biosensor for detection of chemical and biological toxins in drinking water.

Technologies for Future Measurements and Standards

Title: Microfluidics and the Microanalytical Laboratory

Authors: L. Locascio, D. Ross (836), A. Henry, W. Vreeland, M. Gaitan (812)

Abstract: The Microanalytical Laboratory project was established as a competence project in 1998 and has just completed its last year of funding. The work performed in this project is collaborative between the Analytical Chemistry and Process Measurements Divisions in CSTL and the Semiconductor Electronics Division in EEEL, and involves the development of new techniques to improve measurement capabilities in microsystems based on microfluidic technology.

Purpose: The goals of this competence project are to advance the knowledge and understanding of Lab-on-a-Chip devices based on microfluidics technology in order to promote higher performance and improved measurements in these miniaturized analytical systems. To accomplish this goal, our objectives of this proposal are three-fold:

1) Establish a fundamental understanding of the fluid/wall interactions that affect microflow;
2) Devise novel approaches for achieving chemical selectivity for analyte separation applications;
3) Promote new integrated detection concepts.

Major Accomplishments: To date, most of the separations performed using microfluidic systems utilize electroosmotic pumping for fluid flow and separate analytes on the basis of their electrophoretic mobilities. While this mode of separation is a useful one for many bioseparations, scenarios arise in which it is necessary to separate neutral analytes. For these separations, it is necessary to incorporate a stationary phase in the microchannel; the analytes can be flowed through the microchannel using electroosmotic pumping but the basis of the separation is the interaction of the analytes with the stationary phase. This mode of separation science is known as capillary electrochromatography (CEC) and is the hybridization of capillary electrophoresis and high-performance liquid chromatography. With CEC, different species interact with the stationary phase for different periods of time; thus, a separation of the neutral species occurs. This year, we have focused some of our effort on the development of microchip-based CEC (μCEC) devices fabricated using polymer laser ablation.

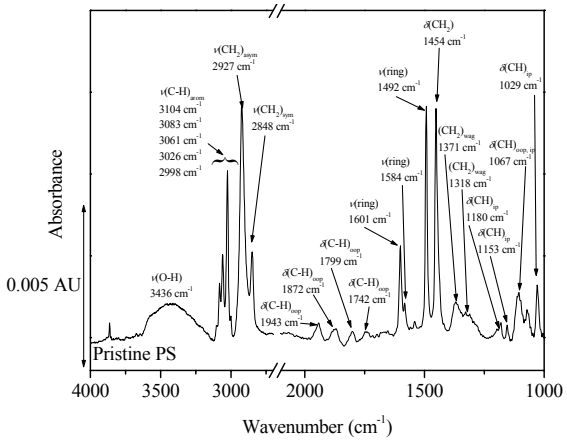


Figure 1a: Transmission infrared spectrum at Brewster's angle of poly(styrene) spin-

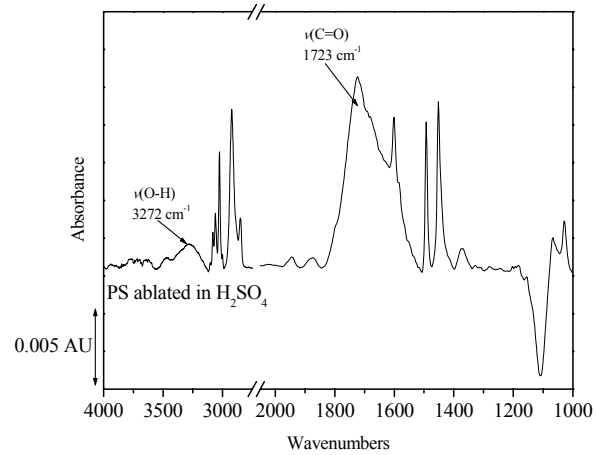


Figure 0b. Transmission infrared spectrum at Brewster's angle of poly(styrene) spin-coated in Si amnd ablated under H_2SO_4 vapor.

The laser ablation of polymeric materials results in a microchannel surface that is significantly rougher than embossed microchannels. Additionally, the chemical species present on ablated surfaces are much different than those present on a pristine or embossed polymer substrate, and can be modified by changing the atmosphere under which the channel is ablated. Figures 1a, 1b show the transmission infrared spectra at Brewster's angle of poly(styrene) (PS) spin-coated on Si, and PS ablated in an atmosphere of H_2SO_4 vapor, respectively. The two spectra are quite different with the H_2SO_4-ablated PS spectrum showing bands indicative of carboxylic acid species suggesting that this channel would have a higher density of negative charges at pH values > ~4. Because electroosmotic flow (EOF) rates increase with increasing surface charge density, we expect a higher EOF in carboxylate-terminated PS surfaces than in their pristine counterparts. Figure 2 shows the dependence of EOF on pH for microchannels ablated under a variety of atmospheres. The microchannel ablated in H_2SO_4 supports the highest EOF, indicating higher density of charged moieties on this microchannel surface.

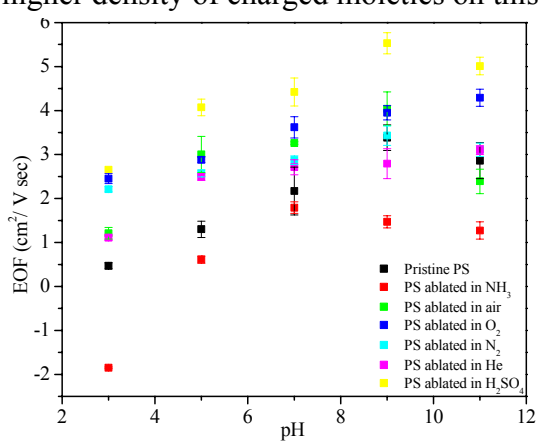


Figure 2. Measurement of EOF using current monitoring method in PS microchannels fabricated by laser ablation

While the presence of charged species on microchannel surfaces is essential to drive EOF, it is also important for the covalent addition of stationary phases to the microchannel surfaces for μCEC separations. We have modified H_2SO_4 ablated microchannels to link various stationary phases suitable for μCEC, and experiments are now underway to use those stationary phases in the first PS-based device.

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

work with CFD Research Corporation (initiated last year) on the development of microflow models for polymer microchannels that will be ultimately applied to predict and optimize results of separations performed in polymer-based systems.

We are also continuing the development of temperature gradient focusing methods for microanalytical separation and pre-concentration. This year, the method was extended to the separation and concentration of neutral and chiral analytes *(see technical activity report, Div. 836*). We continue to develop methods to enhance mixing in microfluidic formats. This year, we introduced methods for mixing based on selective release of reagents from liposomes (*see technical activity report, Div. 839, Rapid mixing in Microfluidic Channels*).

Finally, our work continues to involve several active collaborations both internally (EEEL, PL, CSTL, and MSEL) and externally (Virginia Tech, Cornell, Stanford, Clemson, CFD Research Corporation, University of North Carolina, University of Maryland). One of these collaborations will also be highlighted *(see technical activity report, Div. 839, Cell-based Sensors for Screening Toxins*).

Impact: In the past year, we have developed essential collaborations for the propagation of fundamental data and measurement methods. We have also developed several key collaborations for the development of systems that have application to NIST strategic focus areas including Homeland Defense, Nanotechnology and Health Care. We have continued to be very productive in information dissemination. In the past 5 years of this project, we have produced approximately 50 publications with 8 patents/invention disclosures.

Future Plans: In the next year, we plan to become more involved in the newly formed standards activities groups to support microtechnologies. We also will initiate a new effort with the National Institute of Justice for the development of microsystems based for DNA forensic analysis. Our research efforts in the development of novel analytical methods for microchemical separation and detection will continue to be a major part of our program.

Technologies for Future Measurements and Standards

Title: Nanobiotechnology: Nanofluidics and Nanoscale Chemical Reactions

Authors: L.E. Locascio, W. Vreeland, K. Brazhnik (800), R. Kishore (842), S. Kulin (842), K. Helmerson (842)

Abstract: This project focuses on the development of nanoscale structures to facilitate the performance of ultra-small volume chemical reactions and separations. The work is associated with the Single Molecule Manipulation and Measurement competence program whose purpose is to study the behavior of biomolecules one molecule at a time to elucidate the differences that make them uniquely beneficial or detrimental. The nanoscale structures that we are designing are composed most often of phospholipid molecules and are self-assembled spherical or tubular structures with diameters ranging from tens to hundreds of nanometers. We have demonstrated the use of these nanometer structures for controlled chemical reactions using picoliters of reagents.

Purpose: The purpose of this work is to develop nanometer-sized structures that can ultimately be incorporated into microsystems (microfluidics and MEMS) for use in studying the behavior of very small numbers of biological molecules with fine control.

Major Accomplishments: There were two major accomplishments associated with the project during the last fiscal year:

- *Use of liposomes for the performance of picoliter chemical reactions*
- *Development of methods for fabricating rigid and stable lipid nanotubes*

Due to their amphiphilic nature, when phospholipid molecules are dispersed in water they self-assemble into bilayer membranes to form structures called liposomes that are often spherical and encapsulate an aqueous internal volume. Liposomes range in size from 50 nm to 10's of micrometers encapsulating volumes that are measured in attoliters to picoliters. Water-soluble molecules, including DNA, RNA, proteins and small ionic molecules, can be readily incorporated into the liposomes upon formation. In this work, we use liposomes to sequester very small amounts of reagents in discrete packages to control their reaction. The liposomes are trapped and manipulated in a microenvironment using optical tweezers. Liposomes containing two different reagents that are brought into contact using the optical tweezers do not bind or fuse, therefore their contents cannot react. However, if the liposome

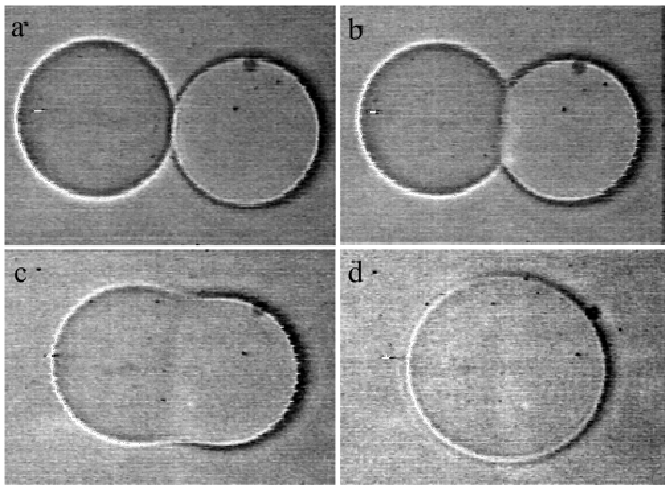


Figure 0. A. Two liposomes brought into contact using optical tweezers. B. A UV laser pulse at the interface initiates fusion of the two membranes. C, D. Fusion is completed in the next 2 successive video frames.

membrane is exposed to a short UV laser pulse while in contact with another liposome, the membrane destabilizes and the two liposomes fuse together to form a single liposome structure as shown in Figure 1. During fusion, the contents of the two liposomes react immediately. The time scale of liposome fusion events varies in length from milliseconds to seconds and is most likely dependent on the lamellarity of the liposome membrane.

Phospholipids can be forced to form non-spherical structures under certain conditions. We recently reported the formation of phospholipid tubes in a microfluidic device under conditions of high shear. Above the phase transition temperature of the phospholipid, lipid hydration in the microchannels resulted in self-assembly of lipids into tubes with diameters of several hundred nanometers to a few micrometers and lengths of up to several centimeters as shown in Figure 2. In general, tubes aligned parallel to the lateral dimension of the microchannel. Elasticity of tubes was assessed via secondary manipulation with optical tweezers and with an optical scalpel. It was determined that lipid tubes formed and stored in a microfluidic setup became mechanically rigid after a few days of storage at room temperature or after a month of storage at 4 ^{o}C.

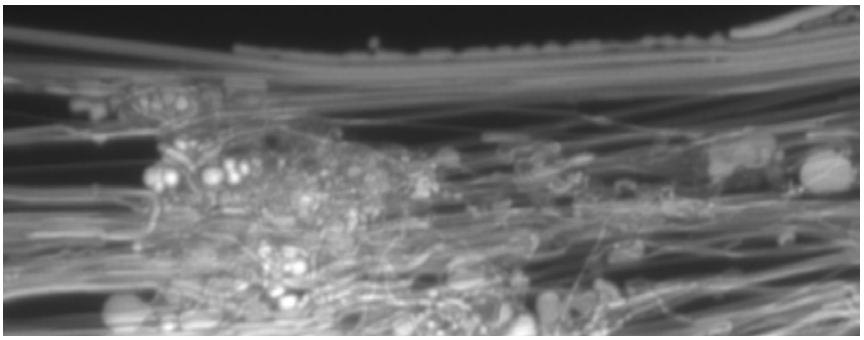


Figure 2. Phospholipid tubes formed in a microchannel under conditions of high shear under flow. Tubes are oriented in a direction parallel to the long axis of the microchannel.

Impact: The work is critical to our efforts in the development of new tools for observing and characterizing single molecule behavior. The characterization of single biomolecules, rather than the study of ensembles of biomolecules, is an important topic in the field of biology since it has been elucidated that the presence and behavior of the biological outlier or the mutant version of the biomolecule can facilitate amplification of that species resulting in catastrophic consequences as highlighted in recent reports on prions. Although our efforts in this area are new, we have made considerable progress toward packaging single molecules, performing controlled reactions with a few molecules, and designing nanochannels through which we will attempt to manipulate and characterize single DNA molecules.

Future Plans: Future work will involve further characterization of the lipid nanotubes to determine stability and lifetime. In the next few years of the competence program, we plan to pursue the incorporation of lipid nanotubes into higher-level microsystems and apply these toward the study of single DNA and RNA molecules.

Technologies for Future Measurements and Standards

Title: Microfluidics: Rapid Mixing in Microfluidic Channels

Authors: W. Vreeland and L.E. Locascio

Abstract: This project deals with the use of bio-inspired vesicles for the spatial manipulation of specific species within a microfluidic environment. Central to microfluidic's implementation for integrated analysis is the ability to precisely control the spatial location of various species. Using a lipid vesicle (or liposome) to encapsulate a species of interest, allows species to be easily maneuvered through a microfluidic chip while being shielded from interactions with surrounding environment. When the species has reached its desired location in the microfluidic system, the liposome can be permeabilized releasing the intravesicular species into the microfluidic environment at a precise and controlled spatial location. Using this strategy we have demonstrated reagent mixing in distances approximately five times faster than standard passive mixing strategies

Purpose: The purpose of this work is to allow for the mixing and delivery of reagents in microfluidics systems with exquisite rapidity and control.

Major Accomplishments: There were two major accomplishments associated with the project during the last fiscal year:

- *Use of liposomes for the rapid mixing of species*
- *Use of liposomes for spatially controlled reactions*

The liposome is a spherical structure composed of a phospholipid bilayer membrane that encapsulates a volume of intravesicular aqueous solution. Liposomes are bathed in an external aqueous solution and are generally ~100 nm to ~1 μm in diameter. The ability to encapsulate a species of interest inside liposomes renders that species inert to chemicals residing outside of the membrane. We have developed a bio-inspired liposome system that allows for the controlled introduction of polar species into a microfluidic system through the modulation of temperature using thermally triggerable liposomes. Thermally triggered liposomes take advantage of the dramatically increased bilayer permeability near the lipid chain melting transition temperature (T_m). Thus at a controlled temperature, a thermally triggerable liposome will release its contents into the extravesicular microfluidic space, allowing for precise delivery of agents to specific regions in the microfluidic environment.

The phenomenon is shown in Figure 1, where a solution of thermally triggerable liposomes (composition 97 % dipalmitoylphosphatidylcholine + 3% cholesterol) encapsulating self-quenched fluorescent sulforhodamine B flow through a microchannel with a lateral temperature gradient. The left end of the channel is thermostated at 20 °C and the right side is thermostated at temperatures ranging from 40 °C to 80 °C in 5 °C increments, please note that due to the low thermal conductivity of polycarbonate, the magnitude of temperature gradient in the channel is slightly less than the nominal temperatures at the termini. As the liposomes flow from the lower temperature region of the channel to the higher temperature region, they pass through the T_m that is specific for this formulation of liposomes (36 ˚C in this demonstration). At the T_m, the liposomes release the fluorescent dye into the extravesicular space causing an increase in

fluorescence at that point and downstream in the channel in all panels except the first, where the temperature gradient does not pass through the T_m of this liposome formulation. Thus, the fluorescent dye, which was initially segregated from the extravesicular space, is effectively "mixed" into the microfluidic environment. Because the liposomes are dispersed throughout the channel, mixing upon release from the liposomes is inherently quite fast. Measurement of the profiles in Figure 1 show the dye goes from "unmixed" to "mixed" state in approximately 200 μm under the experimental conditions shown here, whereas standard diffusive mixing would require almost 9 cm. Since this "mixing" technique is controlled by the T_m of the liposome formulation, rather than the fluid mechanical properties of the channel, this technique should be less sensitive to different operational flow rates than traditional microfabricated mixers. Additionally, because the liposome formulation dictates the T_m, mixed populations of liposomes can be used to control a series of sequential reactions where the temperature in the channel determines reaction timing and sequence.

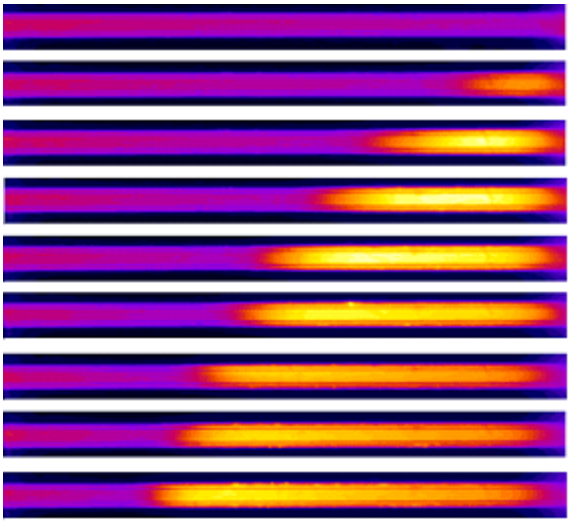


Figure 1. False-color micrographs of the fluorescence intensity of a solution of 97 mol% DPPC, 3 mol% cholesterol liposomes encapsulating self-quenched 100 mM sulforhodamine B in 0.5 M Tris buffer flowing through a polycarbonate microfluidic channel under different applied temperature gradients of 20°C-40°C to 40°C-80°C over 2 mm distance at a flow rate of 5 μL / hour

Impact: This work is the first demonstration of a mixing strategy in microfluidic systems that is not dependent on the operational flow-rate. All other techniques for enhanced mixing efficiency operate optimally in certain fluid mechanical regimes; our liposome-based system, however, depends on the heat transfer and thermodynamic parameters of microfluidic device and thus is limited by a different set of criteria. This is crucial to our effort to design and demonstrate microfluidic systems that are both robust and efficient. Further, we believe this system is ideally suited for biological analyses as the liposome itself mimics the natural environment of the biological cell.

Future Plans: Future work on this project will focus on using liposomes to act as packages to study individual biomolecules, such as single-molecule proteins. Since the permeability of the lipid membrane can be easily modulated through temperature control, various species can be presented to the biomolecule in a controlled fashion to study the effects of that agent on individual, single biomolecules.

Technologies for Future Measurements and Standards

Title: Universal Validation Standard for UV/visible Spectrometry

Authors: D.L. Duewer and J.C. Travis

Abstract: A FY2003 CSTL Exploratory Research Proposal for developing a "universal standard [that] would serve as a replacement for the entire suite of current NIST SRMs [Standard Reference Materials] for chemical spectrometry in the UV/visible spectral region" resulted in the development of a novel whole-spectrum fitting algorithm. This algorithm provides the core functionality for establishing the UV/visible absorption spectra of holmium oxide solution (such as NIST SRM 2034) as an intrinsic standard for the total performance validation of chemical spectrophotometers.

Purpose: To automate the simultaneous calibration of the wavelength axis and validation of the transmittance/absorbance axis of computer-operated chemical spectrophotometers. Traceability will be to NIST-certified data through an artifact standard that can be prepared by end users or commercial producers. This intrinsic standard will perform the function of multiple labor-intensive SRMs currently produced by NIST.

Major Accomplishments: The declaration of dilute holmium oxide solution as an intrinsic wavelength standard was underway at the time the proposal was developed, and is reported elsewhere. Data from that study were used in the successful development of an algorithm to fit the entire (certified) intrinsic spectrum to the spectrum of properly prepared holmium oxide solution taken on an instrument to be validated. The algorithm accounts for the wavelength bias, the absorbance bias, and – most significantly – the spectral bandwidth of the recording instrument. A significant breakthrough in algorithm development was the approach by Statistical Engineering Division collaborators to perform a fit that actually extracts the experimental instrument function ("slit function") in addition to the other expected bias data.

Impact: The traditional suite of UV/visible absorption reference materials designed for research grade instruments has fallen short of meeting the traceability requirements of quality systems associated with "factory floor" instruments. The universal standard will provide simultaneous wavelength calibration and validation of the absorbance scale and spectral bandwidth specification for practical instruments with a single artifact, data acquisition, and automated data analysis. In addition to making life easier for the end user, traceability support will be almost entirely outsourced from NIST. The attention of CSTL can then be turned to emerging measurement technologies while this mature technology is supported by the intrinsic standard.

Future Plans: Work is continuing on the certification of 4% (w/v) holmium oxide dissolved in 10% (v/v) perchloric acid as an intrinsic wavelength standard. With modest additional investment of resources, we will demonstrate that this material is suitable for use as an intrinsic standard for both wavelength and absorbance when spectral baseline artifacts (arising from differences in the surface reflectance of individual cuvettes and minor organic contamination of solvents and cuvettes) are eliminated experimentally. However, we believe that it is possible to mathematically account for these artifacts within the spectral-fit software. This algorithmic development would greatly simplify the necessary experimental burden on the end-user and

would enable routine use of the intrinsic standard to meet most chemical traceability requirements.

Health and Medical Technologies

Title: Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers

Authors: M.J. Welch, D.M. Bunk, M. Satterfield, L.T. Sniegoski, and S.S-C. Tai

Abstract: NIST has a strong commitment to promoting accuracy in health-related measurements and providing measurement traceability to the U.S. in vitro diagnostic (IVD) industry through development of reference methods and SRMs. Work is underway on SRMs for cardiac troponin I (heart attack marker) and homocysteine/folate in serum (risk factor for heart disease/substance that counteracts effects of homocysteine). NIST has completed development of methods for certain thyroid hormones in serum and has participated in international round robins for these analytes. Collaborative work with other NMIs has begun on development of a reference method for C-reactive protein, a risk factor for heart disease. Renewal campaigns are underway for three important clinical SRMs in a frozen serum matrix: SRM 1951b for cholesterol and triglycerides, SRM 956b for electrolytes, and SRM 965a for glucose. Work will begin in FY 04 on a new SRM for steroid and thyroid hormones in serum. These new reference methods and SRMs will help the IVD industry meet new regulatory requirements for traceability.

Purpose: NIST has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. With issuance of the in vitro diagnostic (IVD) device directive by the European Union, it has become even more critical for NIST to develop new reference methods and SRMs to provide traceability for the US IVD industry so that this industry may maintain its strong position in European markets.

Major Accomplishments: Certification measurements have now been completed for SRM 2921 Cardiac Troponin I, an important marker for heart attacks. This work is described in more detail in technical report #. Method development has been completed for triiodothyronine (T3) a marker for thyroid function. This work along with the participation of NIST in an international round robin for thyroid markers is described in technical report #. Work has begun on a new SRM for homocysteine (HCY) and tetrahydrofolic acid (FOL) in serum. This new material will have three levels: (high HCY, low FOL; normal HCY and FOL; and low HCY, high FOL). HCY is considered a risk factor for heart disease and other diseases associated with oxidative damage, while FOL is a strong antioxidant, which is known to reduce the risk of neural defects in fetuses and is believed to counteract the effects of homocysteine. The new material will be ready for certification measurements in late 2003. Research has begun on development of a reference method for another risk factor for heart disease, C-reactive protein (CRP). Modest increases in CRP have been linked to arteriosclerosis and the increased risk of heart attacks. NIST, working with scientists at the Laboratory of the Government Chemist (LGC) in the UK and the Physikalisch-Technische Bundesanstalt (PTB) in Germany, is planning a proteomics approach to isolating characteristic peptides from CRP for measurement by LC/MS. Measurements have begun for the certification of new lots of SRM 1951b Lipids in Fresh-frozen Human Serum, SRM 956b Electrolytes in Frozen Human Serum, and SRM 965a Glucose in Frozen Human Serum, three of the most used clinical SRMs.

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

Future Plans: Development will begin in FY 04 for a human serum-based SRM for T3, T4, cortisol, estradiol, progesterone, and testosterone. The SRM will consist of three levels: a normal adult male pool, a normal adult female pool, and a pool for which levels have been modified to be near medical decision points.

Health and Medical Technologies

Title: Development of SRM 2921, Human Cardiac Troponin Complex: A Primary Calibrator for Assays Used to Diagnose Heart Attacks

Authors: D.M. Bunk and M.J. Welch

Abstract: The clinical measurement of serum cardiac troponin I (cTnI) has become an important tool in the diagnosis of acute myocardial infarction and myocardial damage. Unfortunately, a ten-fold difference in measured concentrations between assays is common, but as much as a hundred-fold difference in response has also been observed. To address the need for standardization of cTnI assays, NIST, the American Association for Clinical Chemistry (AACC), and the International Federation for Clinical Chemistry (IFCC) have worked together to develop SRM 2921, human cardiac troponin complex.

After evaluating several different forms of troponin as candidate reference materials through two round robin studies, a human cardiac troponin complex was chosen as the most suitable reference material. This choice was made after evaluation of the troponin preparation for purity, stability, its ability to provide harmonization of assay results, and the commutability of the material among the twenty commercial cTnI assays used in the study.

Purpose: To reduce variation between the response of clinical cTnI assays, NIST in cooperation with the AACC and IFCC has developed a cTnI reference material, SRM 2921. SRM 2921 will be used by assay manufacturers developing new assays and for validating calibration materials for their current assays.

Major Accomplishments: After evaluation of six different candidate reference materials using two round robin studies, a single reference material has been chosen and used to prepare SRM 2921, the first reference material for cTnI measurements. More than 20 different commercial cTnI assays, made by 12 different manufacturers from the United States, Europe, and Japan were used to evaluate the human cardiac troponin complex chosen for SRM 2921.

The certification of SRM 2921 was accomplished through a combination of analytical methods, both qualitative and quantitative. The values of the concentration of each of the three troponin subunits, including cTnI, were determined using amino acid analysis coupled with a chromatographic separation. The structure of the cTnI protein was evaluated using mass spectrometry coupled with chromatography to determine the extent of post-translational modifications such as phosphorylation and degradation of the protein chain.

Impact: In a recent issue of *Clinical Laboratory News*, the selection of a material to be used a primary cTnI standard was touted as an "important milestone" in the standardization of clinical cTnI assays. Data from the second round robin demonstrates that the use of one common reference material could reduce interassay variability to 10-15%, a substantial improvement compared to the 130% variability observed in the first round robin.

Future Plans: A third round robin study will be carried out to determine fully the ability of SRM 2921 to harmonize the cTnI assay results from patient samples and to establish its

commutability. Future work will also include the development of a reference method for cTnI in serum.

Health and Medical Technologies

Title: Development of Reference Methods and Participation in International Round-Robins for the Determination of Thyroid Markers

Authors: S. S-C. Tai, D.M. Bunk, and M.J. Welch

Abstract: NIST has a strong commitment to promoting accuracy in health-related measurements and providing measurement traceability to the U.S. in vitro diagnostic (IVD) industry through development of reference methods and SRMs. In the past year, NIST has completed development of a reference method for the determination of serum total triiodothyronine (T3*),* a marker for thyroid function. This method is based upon isotope dilution combined with liquid chromatography and tandem mass spectrometry (LC/MS/MS). NIST has also participated in international round-robin comparisons of reference methods for the determination of thyroid markers, total thyroxine (T4) and total T3. Development will begin in FY 04 for a human serum-based SRM for T4 and T3, and several other important hormones.

Purpose: NIST has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. With issuance of the in vitro diagnostic (IVD) device directive by the European Union, it has become even more critical for NIST to develop new reference methods and SRMs to provide traceability for the US IVD industry so that this industry may maintain its strong position in European markets.

Major Accomplishments: A new reference method based upon isotope dilution combined with LC/MS/MS has been developed for serum T3*,* a marker for thyroid function. The major challenges of serum T3 measurement are the low concentration of T3 normally found in serum, and the degradation of serum T4 into T3 during sample preparation. Because the concentration of serum T4 is approximately 100-fold higher than that of T3, even slight degradation of T4 into T3 can have a strong influence on the measured serum T3 values. The T4 degradation in this reference method was minimized to a small percentage, and can be corrected for in the T3 measurement process. This method was critically evaluated and found to produce results of high precision and accuracy for this low level hormone. This LC/MS/MS method for T3 along with LC/MS/MS method for T4 developed at NIST was used in international round-robin comparisons with excellent agreement with laboratories in Europe.

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

Future Plans: Development will begin in FY 04 for a human serum-based SRM for thyroid markers, T4 and T3, and other important hormones including cortisol, estradiol, progesterone, and testosterone.

Health and Medical Technologies

Title: Reference Methods and SRMs for Toxic Species in Body Fluids

Authors: S.E. Long, R.D. Vocke, L.L. Yu, K.E. Murphy, T.A. Butler, and E.A. Mackey

Abstract: We have developed new analytical methods for the determination of trace and ultra-trace levels of toxic elements and cyanide in blood and urine, which have been used to augment the quality and range of reference materials available to the measurement community. A new instrument system featuring a combined liquid chromatography – inductively coupled plasma mass spectrometry system with a Dynamic Reaction Cell for reducing spectral interferences has been installed, and will be used to investigate the role of toxic species in human nutrition and disease.

Purpose: Clinical assessment of the impact of toxic species on human health is critically dependent on the quality of analytical measurements and the availability of suitable reference materials to support these measurements. The most common matrices for such assessments are body fluids such as blood and urine. Measurements on these media are used for establishing dynamic population baseline data, for occupational exposure monitoring and for research. Suitable reference materials certified for the toxic species currently receiving scrutiny have been scarce owing to the considerable analytical challenges in making accurate measurements on specific chemical forms and concentration levels which are often close to the limit of quantitation by traditional methods. To address this, we are developing new analytical methods with improved sensitivity and the capability to generate speciation information and using these methods to produce reference materials that are more closely matched to current needs.

Major Accomplishments: A species-specific method has been developed to accurately determine levels of inorganic mercury in whole blood. The method is based on selective reduction of the inorganic mercury component, which is combined with an ultra-sensitive isotope dilution vapor-generation ICP-MS measurement. The organic mercury present in the sample is not detected. The method has been applied to the determination of inorganic mercury in the elevated level of SRM 966 (Toxic Metals in Bovine Blood). This has been used to upgrade the SRM certificate value for inorganic mercury from a reference value to a certified value, which should be a valuable enhancement for the user community.

Certification of a replacement freeze-dried urine reference material (SRM 2670a, Toxic Elements in Urine) has been completed, mostly using a measurement approach based on isotope dilution combined with the high sensitivity of sector field inductively coupled plasma - mass spectrometry. The new material represents a considerable technical advancement compared with its predecessor, SRM 2670, having ten elements certified at the natural background level and fourteen elements certified at the elevated (spiked) level. Many of these certification measurements were made at the ultra-trace concentration level, which were possible as a result of the availability of new high-quality cleanroom facilities.

We have developed an improved method for the determination of cyanide in whole blood based on headspace gas chromatography with mass spectrometric detection (GC/MS), and employing

$K^{13}C^{15}N$ as the internal standard. Cyanide (CN) is a very fast acting poison, which is rapidly absorbed in the blood and inhibits the cellular utilization of oxygen. Death can result from a blood CN concentration as little as 2.5 µg/mL. Despite its known hazard, CN is extensively employed in a number of chemical processes. Its presence in tobacco smoke and certain foods represent important health issues. The determination of cyanide is analytically challenging, and because it is unstable blood-based standards are not commercially available. The new analytical method is being used to provide value assignment and stability testing data for a pool of baseline and cyanide-spiked blood samples produced by the Emergency Response and Air Toxicants Branch of the Centers for Disease Control and Prevention (CDC).

A new quadrupole ICP-MS instrument, based on Dynamic Reaction Cell technology to reduce or eliminate spectral interferences has been installed in the Analytical Chemistry Division facilities. The instrumentation has been configured with a liquid chromatography separation system and this will be the primary research facility for conducting speciation studies of toxic elements including arsenic, selenium and chromium in biological fluids.

Impact: The development of new state-of-the-art analytical measurement capabilities for toxic elements and other species in biological fluids have provided significant enhancements to ACD measurement services and SRM development. SRM 2670a should prove to be of considerable value for CDC population surveys (such as NHANES) or similar health assessment studies. Similarly, the advent of blood based standards for CN will be useful for exposure screening, which should also prove to be of benefit to homeland security programs.

Future Plans: Development work on analytical methods for speciation studies of selenium, chromium and arsenic in blood and urine will continue, and where relevant, these will be used to develop a new generation of SRMs certified for species specific analytes.

Health and Medical Technologies

Title: Improved Methodology for Determination of Arsenic in Body Fluids

Authors: E.A. Mackey and R. Zeisler

Abstract: The current INAA detection limit for As in typical body fluids is generally between 1 ng/g and 10 ng/g, depending upon the amount of background produced by matrix elements such as Br, P, and Na. Two approaches for decreasing As detection limits were investigated. A simple volatilization procedure did not yield sufficient improvement to detect levels of As present in fractions of biological fluids. Use of a more sophisticated detection system was successful but required longer counting times, thereby limiting throughput. Additional work will involve investigating the utility of simple radiochemical separation procedures in conjunction with the improved detection system.

Purpose: INAA detections limits must be reduced by about an order of magnitude to quantify the lowest levels of As typically found in body fluids. Body fluids contain Na, P, and Br, which elevate the baseline level of radiation next to, and under, the As peak of interest. The potential of improving the As detection limits for biological samples was investigated using improved detection systems and simple radiochemical procedures. Full blown radiochemical separations are time consuming and can result in additional radiation dose to analysts.

Major Accomplishments: The first approach involved the use of a dissolution procedure to volatilize Br. Samples were digested with nitric acid and water and heated to near dryness. This was followed with heating with additional nitric acid and perchloric acids, again until near dryness. The digested material was diluted and counted using a conventional γ-ray spectrometry system consisting of a germanium detector and associated electronics. The γ -ray spectra from these digests were compared with spectra from the intact irradiated samples, acquired prior to digestion. Results indicated that the Br-to-As ratio was reduced by a factor of two using this approach. This reduction is not sufficient to permit quantification of As at the low levels present in materials such as human urine supernatant (SRM 2670a).

> This work demonstrates that the use of more sophisticated electronic signal-processing systems can improve the limit of quantitation for As in biological fluids by a factor 4 or more.

The second approach involved use of electronics capable of processing high count-rates without degraded resolution so that counting could be started earlier in the decay. The combination of a "zero dead-time" spectrometer with the latest gamma-ray spectrometry software has permitted this technical improvement. The As-to-Br ratio in spectra acquired using this system was up to a factor of 4 higher than obtained using a conventional spectrometry system and peaks were well resolved. Using this system the limit of quantitation for a 12 h count of human urine supernatant is about 1 ng/g.

Impact: Simple radiochemistry as well as state-of-the-art γ-ray spectrometry can improve NAA detection limits for As in human body fluids enabling rapid and cost-effective determinations in some benchmark SRMs, with less radiation exposure to analysts.

Future Plans: Additional improvements will be investigating through the use of more aggressive digestion procedures, simple ion-exchange separations, simple liquid-liquid extractions, or a combination of these methods.

Health and Medical Technologies

Title: Radiochemical Method for Benchmarking Silicon Measurements made During Clinical Investigations

Authors: R. Zeisler and R.L. Paul

Abstract: In recent years, the role of trace concentrations of silicon in biological systems, namely in the human body, has become of increasing clinical concern and research interest. Association of elevated silicon levels in blood components and various tissues in patients with chronic renal failure and in women with silicon breast prosthesis was reported, as well as co-localization of silicon with aluminum in senile plaques in the brain tissue of patients with Alzheimer's disease. The role of silicon in these diseases and/or disorders has not yet been elucidated. This is mainly due to analytical difficulties associated with the low-level determination of silicon in biological materials, such as high and variable blank values or analyte losses. This was documented in a recent intercomparison on the element determination in pork liver and pork filet samples using various modes of electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and direct wavelength dispersive X-ray fluorescence analysis (WDXRF). Severe discrepancies were found among results of these techniques, especially at the few mg g^{-1} level. In order to be able to improve the performance of routinely applicable analytical techniques there is a need to develop biological SRMs with certified values of silicon. The developement of this new radiochemical neutron activation analysis (RNAA) method is a critical step towards the development of appropriate benchmark SRMs.

Major Accomplishments: A new RNAA method, utilizing the $^{30}Si(n,\gamma)^{31}Si$ nuclear reaction with thermal neutrons, has been developed for low-level determination of Si in biological materials. The radiochemical separation consists of an alkaline-oxidative decomposition followed by distillation of SiF_4. Nuclear interferences, namely that of the $^{31}P(n,p)^{31}Si$ with fast neutrons, have been examined and found negligible only when irradiation is carried out in an extremely well-thermalized neutron spectrum, such as available at the NIST reactor. The RNAA procedure yields excellent radiochemical purity of the separated fractions, which allows the measurement of the β^- activity of the ^{31}Si by liquid scintillation counting. It was concluded that with the employment of the inherent quality properties of NAA the challenging task of accurate low-level determination of Si in biological materials can be resolved. Interference-free detection limits achieved were 150 μg g^{-1} for irradiation of 200-mg samples in the NIST reactor RT-4 channel for 30 minutes, and 0.5 μg g^{-1} for irradiation of 500-mg samples in the RT-5 channel for 10 h. Initial values have been established for SRM 1549 and SRM 1577b.

Impact: This work demonstrates that the use of this RNAA procedure, using the NIST reactor, can provide detection limits for Si in biological materials that are fit for value assignment of Si in benchmark SRMs.

Future plans: Implement and refine RNAA method to contribute to value assignment of Si content in clinically relevant SRMs such as SRM 1598a.

Health and Medical Technologies

Title: Standards for Fluorescence Microarray Analyses

Authors: G.W. Kramer, A.G. Gaigalas, and P.C. DeRose

Abstract: A group representing all of the U.S. manufacturers of microarray scanners has asked NIST to develop standards for the quantitation of fluorescent microarray analyses. Specifications for a series of physical artifacts have been developed during several NIST workshops and monthly teleconferences. Work continues to find suitable fluorescent materials, to identify appropriate fabrication methods, and to create instrumentation for certifying the fluorescence of such standards.

Purpose: DNA microarrays, also known as DNA or gene chips, have become important tools for gene expression analyses and are poised to revolutionize clinical diagnostics and enable personalized medical care—where treatment can be individually tailored to a specific person through genetics-based diagnoses. The related protein microarrays show great promise for pharmaceutical drug discovery research as well as clinical diagnostic tools. For microarrays, single-stranded genetic or protein material (probes) is bound in an array to a surface the size of a standard microscope slide permitting tens of thousands of molecular reactions to be tracked in parallel when an analyte solution is washed over the array. To accommodate this many sites in such a small area, the individual spots of genetic material or protein must be very small, commonly on the order of 10 to 100 micrometers in diameter. Target DNA or protein in the analyte solution are labeled with a fluorescent dye and allowed to interact with the probes bound on the surface of the array. A favorable interaction leads to fluorescence signal from the appropriate spots. A device called an array scanner or reader detects which spots in the array fluoresce and how much.

Today, there is no consensus method for microarray assays; there are multiple technologies for fabricating microarrays ranging from nano-pipetting to pin-printing to photolithography, and there are several types of scanners. The method variations and uncertainties are so great currently that the biological information obtained from microarray assays is often a function of the method and equipment used, and comparisons of results generated by differing technologies is not possible. If artifact standards for microarray assays can be created and certified, assay quantitation can be improved, results will be intercomparable, and the measurements can be made traceable ultimately back to the SI. The purpose of this project is to develop such standards in collaboration with the manufacturers of microarray readers.

Major Accomplishments: This project began with a request from the scanner industry for NIST to develop standards for this field. After an organizational meeting held at NIST on October 15, 2003, a technical workshop on fluorescence standards for microarray assays co-sponsored by Agilent Technologies and NIST's Biotechnology and Analytical Chemistry Divisions was held on December 10, 2002 and attended by representatives from the major array reader manufacturers. The purpose of the workshop was to develop technical specifications for fluorescence intensity, uniformity, and detection limit standards for the calibrating and validating microarray readers. Several parameters such as the excitation and emission wavelengths of the

fluorescent tags seemed to be common across the industry; however, there were diverging views on other specifications such as spot size and the form factor of the array. Following the initial technical workshop, the participants worked out many issues concerning the types of standards, intensity levels, form factors, etc. in teleconferences. It was agreed that unpatterned artifacts for each of two colors (similar to the dyes Cy-3 and Cy-5 that are commonly used now) would be developed. One set of artifacts with fluorescence intensities in the mid-to-high range would serve as uniformity/homogeneity standards and to measure signal-to-noise ratios for bright features, while a second set of materials would be developed with low fluorescence levels to serve as detection limit standards and to measure signal-to-noise ratios for dim features. To avoid having to accommodate separate sets of standards to serve the two predominant form factors (25 mm by 75 mm microscope slide and ~15 mm square), it was agreed that the artifact would be made oversize with two active areas that could be separated by breaking the glass along a scribed line. A second technical workshop was held at NIST on May 14, 2003 to come to agreement on many of the physical factors and to begin the search for appropriate fluorescent materials and application processes to fabricate the standards. Through monthly teleconferences, the group continues to refine parameters are refined and to search for suitable materials.

Impact: The development of artifact standards for quantitative fluorescence assays on microarrays are a first step in multi-requirement process for standardizing microarray assays. However, it is essential that this process go forward to allow the use of microarrays in clinical settings instead of just research venues. Microarrays have a very promising future, not only in the clinical/biotech/pharmaceutical applications that are being developed now, but also for general chemical analyses. The impact of microarray technologies on the future of analytical chemistry could be huge.

Future Plans: We are currently searching for suitable materials to develop into the artifacts and are building the instrumentation necessary to make the certification and stability measurements. Once these issues are behind us, and we have viable materials, we can concentrate on developing standard protocols for their use. We have discussed these issues with the appropriate ASTM and NCCLS committees. It is likely that we will work jointly with both organizations to develop these documents.

Health and Medical Technologies

Title: Microanalytical Technologies to Support NIH Measurement Programs

Authors: L.E. Locascio, M. Gaitan, D. Reyes (812), N. Morgan (NIH/OD/ORS), P. Smith (NIH/OD/ORS), T. Pohida (NIH/CIT), T. Phillips (NIH/OD/ORS), E. Perruccio (NIH/NEI), P. Becerra (NIH/NEI)

Abstract: We recently established collaborations with NIH scientists for the purpose of enhancing medical research using microfluidics and microengineering. There are two distinct ongoing projects with different teams at NIH whose research involves the following;

- Study of the immune response to Herpes Papilloma Virus (HPV)
- Study of vision-related diseases involving defective neuronal differentiation or cell survival

For the first project, we are developing chip-based microfluidic devices to be used in multi-analyte immunoaffinity capture and detection of proteins related to HPV in cervical secretions. The second project involves patterned immobilization of mammalian retinal cells using microfluidic channels to guide their growth and to facilitate observation of their behavior under different conditions and treatments.

Purpose: The purpose of this collaborative effort is to fabricate microsystems based on MEMS and microfluidics to support and improve the measurement capabilities of medical researchers at the NIH laboratories.

Major Accomplishments:

Project 1. Human Papilloma Virus: This work involves an epidemiological study of the immune response to the Herpes Papilloma Virus (HPV), for which the simultaneous isolation of multiple proteins from microliter samples of cervical secretions is required. For this purpose, we are developing a microfluidic system capable of multi-analyte detection in a single small volume sample by performing multiple sequential heterogeneous immunoassays on chip. Microfluidic

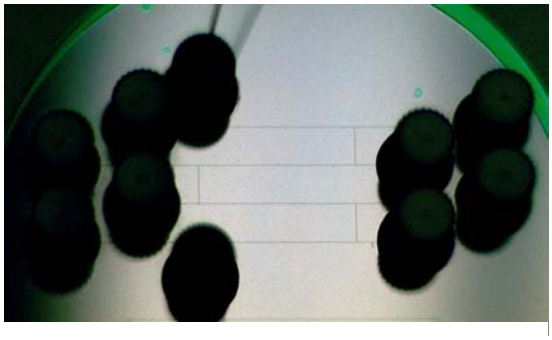


Figure 0a. Microfabricated silicon/glass device with 4 different arms for antibody

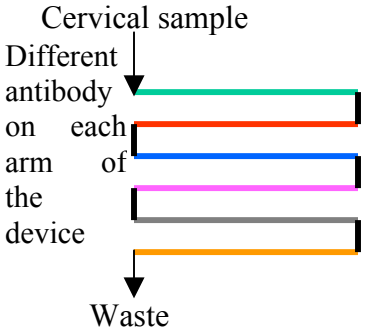


Figure 0b. Schematic diagram of device with 6 arms for measurement of 6 independent analytes.

channels designed in a serpentine pattern are fabricated at NIST using silicon and glass substrates as shown in Figure 1. Different antibodies are then covalently immobilized to each

separate arm of the chip so that the system is capable of measuring a number of analytes equal to the number of arms. The channel device architecture has several advantages over existing array technology including the fact that the device is reusable, and the captured proteins can be extracted after measurement with their biological activity intact. We are designing the system so that the basic methodology can be applied to many different applications relevant to clinical and biological research at both institutions.

Project 2. Vision-related disease: There exist a number of techniques that have been used for patterning cells on surfaces. The strategy often used to adhere single (mammalian) cells involves immobilization of extracellular proteins onto the surface for further adhesion of the cells by interaction with these proteins. In this work, immobilization of rat retinal epithelial cells is accomplished using micropatterned polyelectrolyte multilayer (PEM) -coated surfaces. PEMs were deposited in discrete lines using a (poly)dimethyl-siloxane (PDMS) microfluidic network on top of a flat PDMS slab. The layers were formed by sequentially flowing the polyions throughout the microfluidic network. Retinal cells, seeded on flat PEMs/PDMS surfaces adhered and grew on the PEM areas preferentially as shown in Figure 2. Cells were allowed to grow for up to two weeks showing the pattern delineated by the PEMs. We believe this approach could provide a useful and simple tool to pattern single cells or cell networks in specific locations to monitor neural activity of retinal cells as they are exposed to different stimuli.

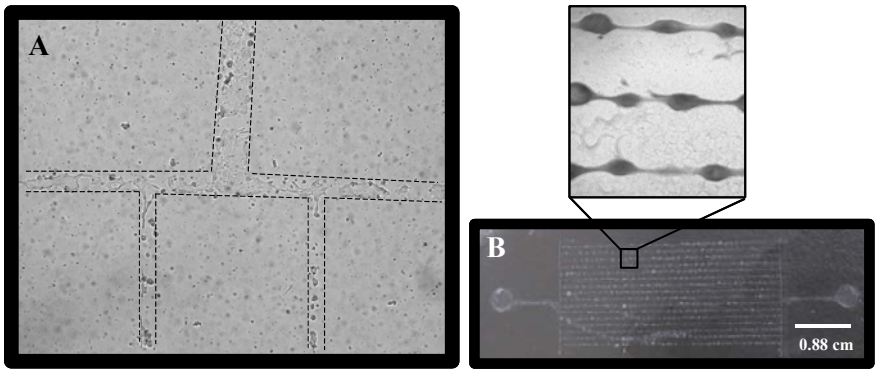


Figure 2. A) Overnight growth (~18hr) of retinal cells on poly(ethyleneimine). Cell growth is observed on the polycation surface areas (within the dotted black lines). B) Two weeks cells' growth on poly(ethyleneimine). Cells are covering all the polycationic area.

Impact: We have found these partnerships with NIH to be very beneficial to our research at NIST. In the last 8 years, we have developed strong microfabrication expertise and capabilities in-house, and have recently been working toward the application of microtechnologies to solving critical problems in environmental monitoring, forensics, nanotechnology, and now, healthcare. We believe that micro- and nanotechnology can be of great use to those involved in clinical research allowing for very elegant small volume sample manipulation and treatment as demonstrated by our successes in the first years of these projects.

Future Plans: The goal of the first project in the next year is to demonstrate multianalyte capability and determine the sensitivity and detection limits of assays performed in this manner. In the next year we also plan to continue work with retinal cells to examine their viability and growth on patterned PEMs surfaces.

Industrial and Analytical Instruments and Services

Title: Development of Critical Benchmark Standards and Methods for the Metals Industry

Authors: J.R. Sieber, A.F. Marlow, M.R. Winchester, R.M. Lindstrom, L.L. Yu, T.A. Butler, M.P. Cronise, and C.N. Fales (230)

Abstract: The Analytical Chemistry Division improves the chemical metrology infrastructure of the US metals industry through method development, training, representation, and SRM development. This fiscal year, the effort on behalf of the metals industry was diversified to include six SRMs for metals and alloys, two SRMs for related materials, and participation in the development of a new ASTM International Standard Method of Test for Ni-base alloys. The new test method for Ni-Co alloys is of critical importance for alloy production control and product quality. With the NIST input, the new method provides options for calibration of X-ray fluorescence (XRF) with fewer reference materials. All of these accomplishments benefit the titanium, steel and aluminium segments of the metals industry, the petroleum industry, the mining industry and their customers in aerospace, automotive, semiconductor, and construction industries. None of this could be accomplished without the expertise and close cooperation of leading metals producers.

Purpose: Metals Industry laboratories need assistance with certified reference materials, improved analytical methods and uncertainty estimates. NIST and ASTM International have a long-standing partnership to develop SRMs and analytical methods. Collaboration is even more important now that NIST is implementing a quality system in compliance with ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*. To meet the challenges of industry-wide compliance with ISO/IEC 17025, it is crucial to have effective industry standard test methods that make efficient use of certified reference materials.

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

» A new ASTM Standard Test Method for Analysis of Ni-Base Alloys by X-ray Fluorescence
» New SRM 2426, Galvalume®, Al-Zn Alloy
» New SRM 2696, Silica Fume
» New SRM 2453, H in Titanium
» Renewal SRM 173c, 6 Al – 4 V Titanium Alloy
» Renewal SRMs 853a and 1240c, Aluminum Alloy 3004
» Renewal SRM 1d, Argillaceous Limestone
» Restocked SRM 2165 Low Alloy Steel E
» Participated in CCQM P34 Pilot Comparison on Analysis of Aluminum Alloy 5182.

Additional accomplishments included analyses of four more proposed Al alloy SRMs for packaging and casting, and an Al-Ti-Nb-W aerospace alloy under development as an SRM for microanalysis.

Impact: The NIST contribution to a new ASTM XRF method for Ni-base alloys gives industry labs the option of using a full fundamental parameters (FP) calibration. FP methods require fewer calibration standards than conventional XRF methods for calibration of matrix corrections.

This is one way to reduce demand for reference materials while concurrently broadening the scope of the method.

The wide range of reference materials developed this year, demonstrates the breadth of the US metals industry, its suppliers, and its customers. It also demonstrates the need to renew existing SRMs that have gone out of stock. Feedback from ASTM International makes it clear that the industry requires a steady supply of both new and existing SRMs to maintain analytical methods that satisfy its own and its customers' high expectations for product quality.

Future Plans: In the coming year, the level of interaction between NIST and the US metals industry will be increased when ASTM International Committee E01 prepares to hold their semi-annual meeting at NIST in Gaithersburg. The meeting will bring together industry, NIST, commercial reference materials producers, and quality system accreditors to focus on the problem of high demand for an ever-growing variety of new certified reference materials versus high development costs, long lead times, and low sales prospects for any single reference material.

Industrial and Analytical Instruments and Services

Title: Elucidation of Systematic Effects in Primary pH Measurements

Author: K.W. Pratt

Abstract: Research in FY03 yielded significant improvements in the Type A uncertainty ("scatter"), associated with measurement replication, of the primary measurement of pH as realized at NIST. Transient mixed potential phenomena arise within Ag|AgCl electrodes, used in the primary measurement, following their transfer from the storage solution (0.01 mol kg^{-1} HCl) to the pH buffer being certified. These transient phenomena decay to a negligible level if the electrodes are stored in the given buffer solution for at least 12 h prior to performing the primary measurement. This pre-equilibration step has been incorporated into the NIST procedure for primary pH measurements. The quality of primary pH measurements obtained at NIST now equals or exceeds that of other NMIs that currently perform similar measurements.

Purpose/Justification: NIST supports the certification of seven pH SRM buffers covering a range of roughly pH 1.7 to 12.4. These SRMs provide the primary benchmarks to establish the traceability of pH measurements. International recognition of these SRMs is supported by NIST participation in Key Comparisons (KC), administered by the Conseuil consultatif pour la quantité de matière (CCQM). NIST performance in prior pH KCs has been sufficient to ensure international recognition of the pH SRM suite. However, the reproducibility of the NIST primary pH measurements in these KCs and in prior SRM certifications has been degraded by larger-than-expected Type A uncertainties ("scatter") of the measurement replication in the primary pH measurements. The primary measurement yields the standard pH values, designated pH(S), of the given buffer. The Type A uncertainties in these pH(S) values have been larger than expected, based on analysis of the set of expected Type B (systematic) components of the overall uncertainty in the primary pH(S) measurement. This state of affairs suggested that an additional, uncontrolled factor, hitherto not considered in the uncertainty analysis, limited the reproducibility of primary pH metrology as realized at NIST. A fundamental study was initiated to elucidate this unknown factor.

Major Accomplishments: The investigation rapidly focused on transient effects at Ag|AgCl electrodes as the main source of the Type A uncertainty. The Ag|AgCl electrode constitutes one half-cell in the primary electrochemical cell without liquid junction (Harned cell) used in the primary pH measurement. These Ag|AgCl electrodes are stored in 0.01 mol kg^{-1} HCl between measurements but are transferred to buffer solution (with added Cl^-) for the given pH(S) measurement. Transient potentials result from incomplete equilibration associated with this transfer. Electrode-to-electrode variations are directly reflected in the Type A uncertainty for a set of pH(S) measurements. A significant contributing factor toward these transient potentials is the formation of an internal liquid junction within the porous sponge of the Ag|AgCl mass following the transfer and subsequent diffusion of buffer into the initially HCl-filled pores of the Ag|AgCl electrode.

These transient effects were found to decay to negligible levels (ΔE < 30 μV, roughly corresponding to 0.0005 pH) if the Ag|AgCl electrode is equilibrated for 12 h (overnight) in the

given solution. The corresponding "easy fix" for pH SRM certifications was to incorporate an overnight equilibration period for each Ag|AgCl electrode in the exact solution that it was scheduled to "see" in its Harned cell the following day. This new protocol yielded a 3-fold improvement for the carbonate buffer (pH 10). Smaller improvements were noted for the phthalate buffer (pH 4). Typical Type A uncertainties were less than 0.0005 pH for each buffer with overnight equilibration.

Fundamental studies were then undertaken with a 2-compartment cell (filled with buffer and HCl, each compartment containing an equilibrated Ag|AgCl electrode) that modeled the HCl-filled "core" and buffer-filled "shell" of a non-equilibrated Ag|AgCl electrode. When disconnected, the respective electrodes represented the isolated "core" and "shell" of the modeled electrode: the resulting potential difference corresponded to the open-circuit liquid junction potential of the buffer vs. 0.01 mol kg^{-1} HCl. These measurements yielded an estimated liquid-junction potential of 25 mV for carbonate buffer and 10 mV for phthalate buffer, both vs. 0.01 mol kg^{-1} HCl. The mixed potential present in a single non-equilibrated Ag|AgCl electrode was then simulated by short-circuiting these two electrodes together. The resultant mixed potential was determined by comparison against a third, equilibrated Ag|AgCl electrode in the buffer solution. This "modeled mixed potential" result semi-quantitatively agreed with the transients previously observed with measurements of freshly-transferred electrodes. The greater liquid junction potential in the carbonate buffer, and its electrode-to-electrode variation, explains, in part, the larger Type A uncertainties obtained with the alkaline pH(S) buffers.

Impact/Future Plans: These changes in the NIST protocol for primary measurements of pH(S) have already yielded significant improvements in the uncertainty obtained in certifications of pH SRMs. Based on these pH SRM certifications, it is expected that the NIST performance in future CCQM pH KCs, as evaluated by the Type A uncertainties that are attained, will surpass the corresponding "best" level attained by the set of NMIs participating in the given CCQM pH KC.

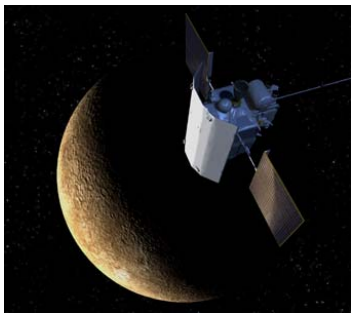
The results of the fundamental study into the transient behavior of Ag|AgCl reference electrodes provided new information regarding the systematic uncertainties and behavior of Ag|AgCl electrodes in primary pH measurements. These fundamental studies will be continued, yielding explanations of transient phenomena in Ag|AgCl electrodes based on modern electrochemical theory.

Industrial and Analytical Instruments and Services

Title: Calibration of a Gamma-ray Detector for a Mission to the Planet Mercury

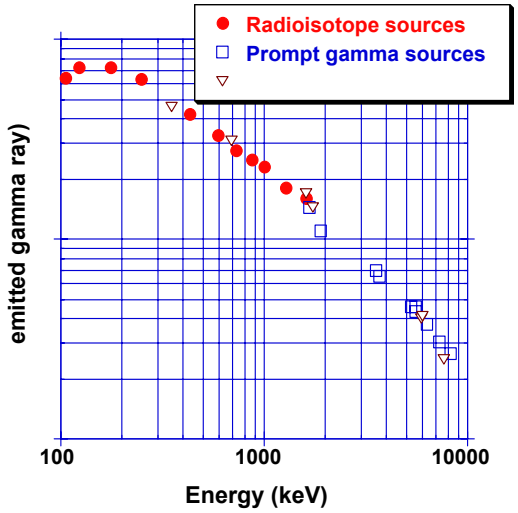
Authors: R.L. Paul, G.P. Lamaze, R.M. Lindstrom, and E.A. Rhodes (John Hopkins University)

Abstract: The NASA MESSENGER spacecraft will soon become the first mission to orbit the planet Mercury. The spacecraft will map the composition of Mercury's crust by measuring gamma rays produced by cosmic ray induced neutrons interacting with elements in the crust. In order to accomplish this task, the gamma-ray detector must be calibrated for efficiency vs. energy for gamma rays up to 9 MEV over the spatial orientation angles associated with the orbits around the planet. Because isotopic sources do not cover this range of energies, the only viable method for performing the calibration was to use prompt gamma rays from targets mounted in a high intensity neutron beam. Calibrations were performed by scientists from Johns Hopkins University in collaboration with NIST using the unique beam facilities of the NIST Center for Neutron Research. An absolute efficiency calibration was performed by measuring prompt gamma rays from NaCl and Cr targets irradiated in a high intensity neutron beam and gamma rays emitted by calibrated radioisotope sources of ^{56}Co, ^{228}Th, and ^{226}Ra mounted in the same configurations as the targets. The calibrations, performed in a timely manner, will allow MESSENGER to yield a relatively accurate compositional map of Mercury.



Purpose: The NASA MESSENGER spacecraft, designed and built by scientists and engineers at the Johns Hopkins University Applied Physics Laboratory, will become the first mission to orbit the planet Mercury. Once in orbit, a gamma-ray spectrometer aboard MESSENGER will map the gamma-ray emission spectra over the planet resulting from capture of cosmic ray induced neutrons by nuclei of elements in the planet's crust. Analysis of these spectra will allow determination of the composition of Mercury's crust, which will shed light on the evolution of this planet and our solar system. In order to get an accurate assay map, the gamma-ray detector must be calibrated for efficiency vs. energy over the spatial orientation angles associated with the orbits around the planet. This calibration is usually performed using calibrated radioisotope sources. However, radioisotope sources have weak or nonexistent gamma-ray emission at energies above 3 MeV, while the gamma-ray energies of importance for planetary assay extend up to energies of around 9 MeV. An additional problem facing JPU scientists was that the germanium detector must be cooled by a small cryocooler that has an estimated lifetime not much longer than the mission. Therefore, the calibration measurement could not be allowed to consume much time.

Major Accomplishments: Because prompt gamma-rays span the energies needed for the calibration, the only viable method for obtaining a timely and accurate calibration of this detector was to use gamma-rays produced by neutron capture of a target in a high intensity neutron beam. Measurements were made using the unique beam facilities of the NIST Center for Neutron Research. Targets made of NaCl and Cr, irradiated at the NG0 beam port, provided the necessary gamma rays for calibration of the MESSENGER detector, which was rotated to the



angular orientations required for the space flight. In order to obtain an absolute efficiency calibration, radioisotope sources of ^{56}Co, ^{228}Th, and ^{226}Ra were calibrated at NIST and were measured in the same configurations as the targets. An illustration of an efficiency calibration determined in this manner is shown.

Impact: The spectrometer calibration was performed in a timely manner, allowing the scientists to meet the mission deadline while using a minimum of cryocooler time. The spectrometer has been loaded aboard the spacecraft. This calibration procedure will allow MESSENGER to yield a relatively accurate compositional map of Mercury, and will likely set a new standard for space-flight gamma-ray spectrometers.

Future Plans: The detection of neutron capture gamma rays from planetary surfaces is likely to be an integral part of future space missions. We would gladly welcome any future collaborations.

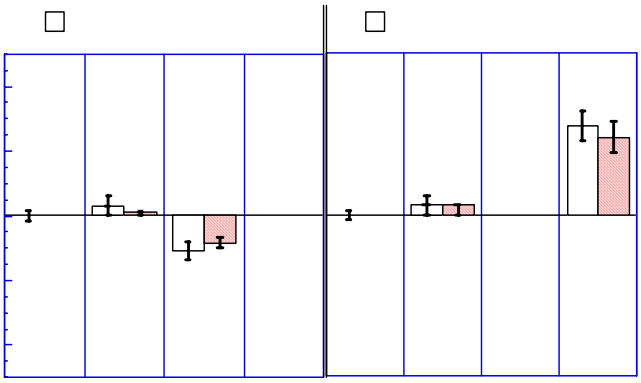
Industrial and Analytical Instruments and Services

Title: Determination of Hydrogen in High-Purity Niobium for Particle Accelerators

Authors: R.L. Paul, H.H. Chen-Mayer (846), and G.R. Myneni (Jefferson Laboratory)

Abstract. Niobium RF superconducting cavities form the basic component of particle accelerators. The presence of interstitial hydrogen in niobium is believed to have a detrimental effect on its mechanical and superconducting properties. It is suspected that chemical polishing (acid treatment) used to remove surface defects from the niobium introduces hydrogen, while vacuum heating may be used to remove it. We have used prompt gamma-ray activation analysis (PGAA) and neutron incoherent scattering to study the effects of these two processes on the hydrogen content of the niobium. The measurements indicated that both processes could significantly alter the metal's hydrogen content. The results of these and future studies should lead to significant improvement in the methods for manufacturing and processing the high purity niobium used in these cavities.

Purpose: Niobium RF superconducting cavities are fast becoming the basic accelerator structures of a new breed of particle accelerators. It is suspected that the presence of interstitial elements in the niobium used to build these cavities may result in degradation of mechanical, thermal, and electrical properties. Prior to use, the high-purity niobium is chemically polished (treated with acid) to remove surface imperfections that could degrade the superconducting properties. This chemical polishing is believed to introduce trace hydrogen. A significant drop in the superconductor's Q-value has been observed if the temperature of the niobium is held at 75 K $\leq$ T $\leq$ 130 K for more than about an hour before being returned to its superconducting state below 9 K, and has been attributed to hydride precipitation of interstitial hydrogen. Hydrogen may be removed by vacuum degassing at high temperature but the effectiveness is of this procedure is not known.



Changes of H content as a result of degassing or loading on two samples (#4 and #5) as determined by PGAA and NIS.

Accomplishments: In this investigation we set out to answer two questions: Does acid treatment of the niobium introduce a measurable amount of hydrogen into the material, and can the hydrogen be removed by vacuum heating? Hydrogen measurements were made at the Center for Neutron Research using cold neutron prompt gamma-ray activation analysis (PGAA), which measurements gamma-rays emitted upon neutron capture, and neutron incoherent scattering (NIS), which measures neutrons scattered from the sample. The latter technique takes advantage of the anomalously high neutron scattering cross section for hydrogen. Hydrogen measurements were made on 10 g samples of high purity niobium that had been vacuum degassed from 6 to 8 hours at temperatures from 600 °C to 800 °C. The samples were then acid treated with applied voltage for 0.5 to 5 minutes, and again analyzed. The measurements indicated no change in H concentration after the acid treatment. To see if more rigorous treatment could induce changes, two samples were again vacuum degassed at 800 °C for 8 hours, and one sample was again acid treated for 5 minutes. Both techniques measured a significant decrease for the degassed sample, and a significant increase for the acid treated sample, indicating that both processes can affect the H content of the niobium. One possible explanation for the observed results is that heat treatment at 800 ˚C causes removal of strongly bound hydrogen, thereby activating sites where hydrogen can be picked up by the niobium upon subsequent acid treatment.

Impact: The results of this and future research will lead to newer and better ways to manufacture and dehydrogenate high purity niobium. The ultimate benefit will be a significant decrease in the cost of building accelerators to study fundamental physics. Since many more accelerators are planned for the future, the studies we are doing on niobium will have a wide impact on the entire landscape of accelerator related science and technology.

Future Plans: Further research is needed to fully understand the mechanism by which H is introduced into niobium and its effect on superconducting properties. Future work will center on studying both the quantity of hydrogen present and the nature of H bonding in niobium. The latter may be studied by a combination of NIS and internal friction methods in collaboration with the NIST Metallurgy Division.

Title: Characterization of the New Thermal Neutron Prompt Gamma Ray Activation Analysis Instrument

PGAA limits of detection have been greatly reduced compared with those of the original instrument due to reduced gamma-ray backgrounds and increased element sensitivities.

Authors: E.A. Mackey, D.L. Anderson[1], P.J. Liposky[2], R.M. Lindstrom, H.H. Chen Mayer, G.P. Lamaze

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[2]NIST, Center for Neutron Research, Gaithersburg MD, 20899-8561

Abstract: A new thermal neutron PGAA instrument was built at the NIST Center for Neutron Research. It replaced the original PGAA instrument that was built in the late 1970's. This new instrument provides greater sensitivities, better detection limits, and much lower background radiation. The new instrument is also easier and safer to assemble for use.

Purpose: The new thermal neutron prompt γ-ray activation analysis instrument was designed to provide greater sensitivities, better detection limits, and much lower background radiation. (See Figure 1.) Experiments were performed to measure the improvements in element sensitivities and background count rates.

Major Accomplishments: A sapphire filter was placed in the neutron beam shutter assembly to reduce the fast neutron and low energy γ-ray components of the beam. The fast neutron component of the beam was reduced by a factor of five and the low energy background γ-radiation by factors of 5 to 10. A new external beam tube, sample chamber, beam stop, and support structure were built and a new detection system installed. The new beam tube is made of two cylindrical aluminum sections lined with a lithiated polymer. The sample chamber was also fabricated from aluminum and lined with lithiated polymer, and may be evacuated to minimize the number of neutrons scattered and absorbed by air. The beam tube and sample chamber assembly is suspended from the aluminum support structure. The detection system consists of a 40% efficient germanium detector (resolution 2.0 keV at 1332.5 keV) and bismuth germanate Compton suppressor. The detection system shield consists of lead surrounded by borated and lithiated polyethylene, placed on a table attached to the support structure. The new, more compact beam stop is welded to the support structure. Capture gamma-ray photopeaks from H, B, C, N, Na, Al, Fe, Ge, I, and Pb in the background spectrum were either of lower intensity or eliminated with the new PGAA instrument. The improved detection system, positioned closer to the sample, increased element sensitivity by 5% to 50%.

Impact: The new PGAA instrument is safer to assemble for use and provides greater sensitivities, better detection limits, and much lower background radiation. Analyses of SRMs and other materials will benefit from these improvements.

Future Plans: Additional work is in progress to fully characterize and quantify all sources of uncertainty associated with analyses performed using this new instrument.

ORTEC

Industrial and Analytical Instruments and Services

Title: New Series of SRMs for Raman Intensity Correction

Authors: S.J. Choquette, E.S. Etz (837), W.S. Hurst (836)

Abstract: SRMs 2241, 2242, and 2243 are new relative intensity standards for Raman spectrometers that employ 785 nm, 532 nm , and 514 nm laser excitation sources respectively. These standards were developed to correct a samples' spectrum for instrument response and to validate spectrometer performance. These standards are primarily intended for use by the pharmaceutical community and other industries that require traceabilitiy**.** These new standards allow the calibration of Raman spectrometers at a fraction of the cost of other methods. The use of these standards will enable the development of standardized Raman spectral libraries, the direct comparison of different instruments, and the transfer of calibration methods between labs and instruments.

Purpose: To provide users of Raman instrumentation with a set of performance validation standards for traceable measurements and to establish "corrected" Raman spectra that will enable the compilation of Raman spectral libraries.

Major Accomplishments: Standardized Raman spectral libraries do not exist because of the lack of adequate calibration methods and standards. To date, Raman spectra are instrument dependent and are a convolution of the spectrometer instrument response and the scattering characteristics of the sample**.** The series of SRM 224x standards is intended largely to solve this problem. These SRMs are glasses that luminesce when irradiated with the spectrometer's laser. The shape of this luminescence emission is certified by NIST and described by a simple mathematical expression. This certified expression, together with a measurement of the luminescence spectrum of the SRM, can be used to determine a spectral response correction that is unique to each instrument. This will enable the correction of a sample Raman spectrum so that it is system independent. SRM 2242: Raman intensity correction standard for systems operating with 532 nm excitation was certified this year and will be released in November. SRM 2243, a standard for argon ion laser wavelengths (488 nm/514.5 nm) is currently in production. Additionally, candidate materials for the SRM useable with 1064 nm laser excitation (SRM 2244) have been identified and SRM production is scheduled for FY '04.

Impact: The introduction of SRM 2242 and the next sets of glasses for 1064 nm and 488-514 nm excitation sources will further enable the use of Raman spectroscopy in regulated industries where the lack of performance validation and system correction standards have prevented Raman spectroscopy from being widely used in the pharmaceutical, clinical, environmental, and forensics communities.

Future Plans: We are currently examining candidate materials that could be used for the 633 nm excitation laser systems. This standard (SRM 2245) will complete our planned suite of Raman intensity correction standards providing the basis for developing validated and system independent Raman libraries. In addition to their use in regulated industries, standard spectral

libraries will put Raman spectroscopy into the toolkits of first responders dealing with Hazmat and homeland security situations.

Industrial and Analytical Instruments and Services

Title: Fluorescence Standards Suite for Spectral Correction of Fluorometers

Authors: P.C. DeRose, D.H. Blackburn, and G.W. Kramer

Abstract: To prepare, characterize and certify a set of fluorescent glass SRMs that will enable spectral emission to be calibrated on fluorometers in the near UV and visible regions of the spectrum.

Purpose: Luminescence measurements have become the detection methods of choice for new clinical and biochemical assays, and related high-throughput techniques, due to their extraordinary selectivity and sensitivity. These new analytical methods are becoming increasingly more quantitative, requiring standards to calibrate the luminescence measuring instruments that they utilize and aid in the validation of the methods. Ideally, users would like to employ the same organic dye probes used for analyte detection as standards for fluorescence intensity and spectral correction. Unfortunately, organic dyes photobleach quickly, do not have long shelf lives in solution, have environment dependent fluorescence and are expensive to produce at high purity.

Major Accomplishments: After studying the characteristics of the different types of fluorescent materials, we found metal oxide doped (MOD) glasses to be the best choice for use as fluorescence standards for spectral correction. These glasses are photostable, robust, relatively inexpensive and can be made to suit most detection formats. Our research-grade fluorometer is being used to characterize the spectral characteristics of a series of MOD glasses to determine which will have the correct spectral shape and intensity for appropriate standards. A UV light chamber and a laser are also being used to irradiate the candidate glasses over a timed period. The fluorescence intensity of each glass is measured before and after irradiation to determine its rate of photobleaching.

Results and Future Plans: Spectral emissivity standard candidates have been identified with UV/violet, blue, green and yellow/orange fluorescence, corresponding to CeO_2 or PbO, CuO, U_3O_8 and MnO_2 dopants, respectively. At this point, the U_3O_8 and MnO_2 glasses have been most thoroughly studied. They are both highly resistant to photobleaching and possess all of the desired characteristics for spectral emissivity standards. They are presently in the prototype stage of development and are expected to be certified within the next year. These standards are being made in the form of a standard sized cuvette with three polished long faces for 90 degree detection and one frosted long face for front-face detection. The CeO_2, PbO and CuO glasses have all been shown to have desirable spectral characteristics and are in various stages of development leading up to prototype production. Once certified, the U_3O_8 and MnO_2 glasses can be used in combination with SRM 936a quinine sulfate dihydrate; a blue spectral emissivity standard that is the only standard presently sold for spectral correction of fluorescence; to cover the visible region from 400 nm to 700 nm. The CeO_2 or PbO glass will be used to cover the near UV/violet from 350 nm to 450 nm. We hope to replace SRM 936a with the CuO glass, as the present standard is an organic dye that has many of the stability problems associated with organic fluorophores. We have not yet found a suitable MOD glass for a red emission standard

that would cover the region from approximately 650 nm to 750 nm, and beyond. In the future, we intend to work with a nanocrystal manufacturer to help us to develop a nanocrystal-based material with the desired red fluorescence.

Industrial and Analytical Instruments and Services

Title: SpectroML—An Extensible Markup Language for UV-Visible Spectroscopy Data

Authors: A.D. Nguyen, A. Arslan, M.V. Smith, J.C.Travis, and G.W. Kramer

Abstract: We have created an extensible markup language (XML) for UV/visible data called SpectroML and are using it to interchange data between our spectrometers and our optical filters database. SpectroML is now an integral component in certification and recertification process for optical filter SRMs. We are working with the ASTM Subcommittee E13.15 on Analytical Data Management, representatives from instrument companies, and the IUPAC committee responsible for JCAMP-DX to create a unified XML-based approach for interchanging molecular spectrometry and chromatography. Based in part on SpectroML, this effort is being called the Analytical Information Markup Language (AnIML).

Purpose: The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. Over the past 15 years, several different interchange formats have been developed that allow data generated by different vendor systems of a given technique to be exchanged. For example, using JCAMP-DX, users of most commercial FT-infrared and NMR systems can interchange their respective data. However, in the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange beyond the capabilities of these protocols. Today's analytical chemists need not only to interchange data generated within a specific technique, they need to interchange, import, export, store, and combine all their data from multiple sources at multiple sites. Users are now no longer content with just having "pictures" of their data (i.e., data plots). They need real access to their data from within a report itself—they want to be able to expand plots, re-integrate peaks, overlay spectra, etc. without having to revert to the instrument or computer system that generated the original data. And, they don't want to have to purchase, learn, or even load proprietary programs to do this. In industrial settings, experimental data belong to and must be accessible to the entire organization, not just the laboratory personnel that generate it. Proprietary data formats and existing interchange programs simply cannot fill such corporate needs for data accessibility

Major Accomplishments: Over the past two years, we developed an XML-based mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectroscopy data. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectroscopy and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers with our optical filters database. When our optical filters database was created as a replacement for paper records, we could import data only from the High Accuracy Spectrometer (HAS) used to certify the SRM optical filters. The complex and archaic data importation process badly needed to be modernized, and we wanted to be able to import data from our other spectrometers as well.

This year we completed the SpectroML drivers/translators for the HAS-II, the PerkinElmer Lambda 900, and the Hewlett-Packard HP 8453 spectrometers. The data from these instruments

can now be converted into a single format SpectroML file. We have also completed the application that reads the SpectroML format file and imports its data into our optical filters database. Now that we can easily import data into the database from any of our spectrometers, SpectroML has become an integral part of the process for certifying and recertifying optical filter SRMs; plus, we can import research data taken on machines other than the HAS for comparison purposes.

Even before we developed SpectroML, we began to proselytize both instrument vendors and the ASTM E13 Molecular Spectrometry and Chromatography Committee about the merits of an XML-based approach to data interchange. In 2001, the ASTM E13 committee established a task group to support a markup language for molecular spectrometry based in part on SpectroML. This year at PittCon, the task group was made into a full ASTM subcommittee—E13.15 on Analytical Data Management. E13.15 was created principally to develop an XML-based markup language for spectroscopy and chromatography data; however, ASTM E01.25 voted to transfer its membership and standards to E13.15; standards transferred included LECIS, ANDI, and LIMS standards.

E13.15 has been working with instrument vendors and the IUPAC Committee responsible for JCAMP-DX to develop a unified XML-based approach for interchanging molecular spectrometry and chromatography data called the Analytical Information Markup Language (AnIML). AnIML is based on a hierarchical model that calls for the initial development of a core markup language containing the elements common to all applicable analytical techniques. The core schema defining the common notions of sample information, measurement data, system information, quality assurance, etc. will be handled by ASTM E13.15. Technique-specific schema will build on the core and will be the responsibilities of the respective E13 subcommittees in conjunction with other interested expert groups such as those from the IUPAC, American Society for Mass Spectrometry, etc. Data/instrument vendors are responsible for implementations of the instrument/origin specific schema that will build on both the core and the technique specific standards. Higher-level specifications could include organization-specified schema that are company-specific and even end-user or application-specific mark up languages. The core is being built as much as possible to bridge previous data exchange standards work (e.g., ANDI and JCAMP-DX), and efforts are being made to collaborate with and include other organizations with similar interests and as much as possible to reuse terminology and concepts from existing standards.

Impact: SpectroML has served its dual purposes well. As a model implementation, it paved the way for AnIML. At NIST, SpectroML has become an integral part of the optical filters program. The development of AnIML as a universal molecular spectrometry and chromatography mark up language will revolutionize data interchange, eliminate long standing barriers to data exchange, enable new data manipulation software, facilitate new web-based notions for data usage, and permit the development of realistic schemes for long-term data archival. When coupled with parallel efforts in developing XML approaches to the manipulation of other chemical data, it will pave the way for the fully electronic chemistry data archive, where any known chemical fact or information is but a simple query away on your wireless Personal Digital Assistant from anywhere in the world.

Future Plans: Currently, work is underway in our Group to implement the AnIML core schema and to develop an example implementation of a technique-specific AnIML schema for liquid chromatography photodiode array spectra. If these projects are successful, we can begin implementing AnIML for other techniques. The IUPAC committee has already converted their JCAMP-DX terminology to XML so progress should be rapid for those techniques supported by JCAMP-DX. The AnIML project also dovetails nicely with another IUPAC project to convert the IUPAC units and terminology documents (Green and Gold books) to XML.

Industrial and Analytical Instruments and Services

Title: Molecular Simulation of Alkylsilane Stationary Phases in Liquid Chromatography

Authors: K.A. Lippa, L.C. Sander, and R.D. Mountain (838)

Abstract: The molecular-level structural features of monomeric and polymeric alkylsilane stationary phases of varying chain lengths, surface coverages, bonding chemistries and temperature conditions were investigated using molecular dynamics simulation techniques. Results of alkylsilane chain structural analyses (i.e., length, conformational order, *gauche* dihedral angle defects) are consistent with previous experimental observations. In addition, simulation models that represent chromatographic columns that have been characterized as highly shape-selective (through use of the SRM 869a Column Selectivity Test Mixture) are correlated with an observed region of alkyl chain order with primarily *trans* dihedral angle conformations. The design of future RPLC phases through the use of such computational models will enable the analyst to intelligently design surface-modified materials that will possess specific structural characteristics for the targeted utility.

Purpose: "Shape-selective" polymeric alkylsilane stationary phases (typically C_{18} chains) are routinely employed over the more common monomeric alkylsilane stationary phases in reversed-phase liquid chromatography (RPLC) to improve the separation of geometric isomers of constrained solutes, such as polycyclic aromatic hydrocarbons, carotenoids, steroids and polychlorinated biphenyls. Critical to describing such shape-selectivity processes in RPLC is the elucidation of the molecular-level structural features of the alkylsilane stationary phase that promote such separations. Previous molecular dynamic simulation models that illustrate the structure and dynamics of alkyl chromatographic stationary phases have been focused on only monomeric-type materials.[1-4] Therefore, we have investigated the molecular dynamics of chromatographic models that represent both monomeric and polymeric stationary phases with alkylsilane lengths, surface coverages, bonding chemistries and temperature conditions that are typical of actual materials prepared in the laboratory.[5,6] The resultant structural features of the alkylsilane chains (i.e., length, conformational order, *gauche* dihedral angle defects) of these simulation models were related to the observed shape-selectivity properties of corresponding LC stationary phases.

Major Accomplishments: The structural characterization of these computational models (**Figure 1**) are consistent with previous experimental observations: 1) alkyl chain order increases with increased surface coverage; 2) monomeric and polymeric phases with similar surface coverages give rise to similar degrees of alkyl chain order (although subtle differences exist); 3) longer chain length phases (e.g., C_{30} chains) exhibit significantly more order than C_{18} phases; and 4) alkyl chain ordering is significantly enhanced at lower temperature. In addition, a significant portion of the alkyl chain closest to the silica substrate surface is disordered (primarily *gauche* conformations) and the opposite end is most ordered (*trans* conformations). For simulation models that represent RPLC columns that have been characterized as highly shape-selective (through use of the SRM 869a Column Selectivity Test Mixture), a significant region of alkyl chain order with primarily *trans* dihedral angle conformations is observed. This is consistent with the view that the alkyl chains comprising the polymeric stationary phase contain a series of

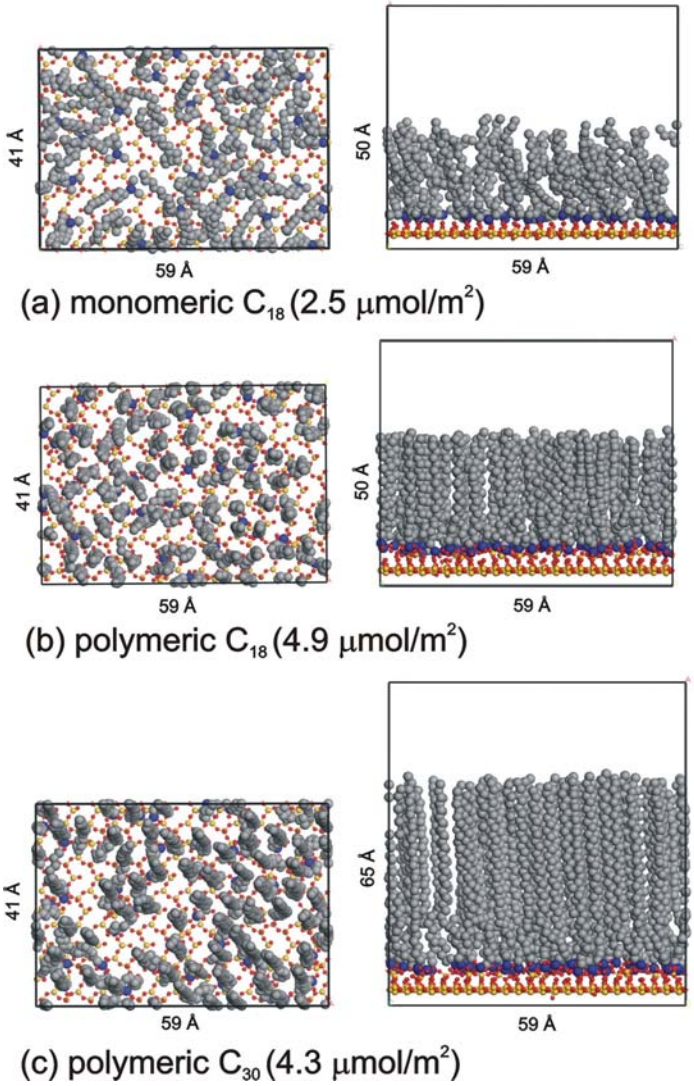

Figure 1. Top and side view snapshots of molecular dynamic simulated LC phases at experimentally relevant surface coverages (μmol/m^2). Alkylsilane chains are displayed in gray (C), blue (Si) and red (O). The silica surface is displayed in red (O) and yellow (Si). Hydrogen is not displayed for clarity.

well-defined and rigid slots in which shape-constrained solutes can penetrate and hence be selectively retained.

Impact: The application of molecular dynamic simulation techniques for the design of future RPLC phases will enable the analyst to intelligently design surface-modified materials that possess specific structural characteristics that will result in a particular retention mechanism. For instance, the results of this recent simulation work have promoted the synthesis of low- to moderate-surface coverage polymeric alkylsilane RPLC stationary phases that are anticipated to possess unique shape-selectivity behavior.

Future Plans: The work described herein represents the initial, non-solvated component of a comprehensive molecular dynamic study of RPLC monomeric and polymeric stationary phase models. A corresponding study of selected monomeric and polymeric models with chromatographically relevant solvent systems (water-acetonitrile mixtures)[7] as a mobile phase is currently underway to gain insight into the molecular level differences in solvation structure that can influence the shape-selective process.

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International Standards

Title: Identification and Provision of "Higher Order" Certified Reference Materials and Reference Measurement Procedures Required for U.S. Industry Compliance with the EU IVD Directive

Authors: W.E. May and V.L. Vilker

Abstract: The recently enacted European Directive 98/79/EC on in vitro diagnostic medical devices (IVD MD) requires, among other things, that "the traceability of values assigned to calibrators and control materials for in vitro diagnostic devices must be assured through available reference measurement procedures and/or reference materials of higher order". At present, neither reference materials nor reference methods are available for more than 300 different chemical or biochemical species that are measured in medical laboratories using IVDs. Reference methods and/or materials exist for only about 30 worldwide

Purpose: Excluding home diagnostics, the overall world-wide invitro diagnostic market is approximately $20 billion. The total IVD market in Europe was about $5.6 billion in 1998 and has shown growth of about 4% per year over the past five years (data from www.edma-ivd.be). Approximately 60% of the IVD products currently on the European market are imported from the US. The U.S. IVD industry (ADVAMED) has asked NIST to work with our counterparts in Europe and the Asia-Pacific to provide the reference materials and methods of "higher order" that are urgently needed to comply with the requirements of the EU IVD Directive. Without timely completion of these standards, the U S. IVD industry's access into the European market will be seriously jeopardized.

Major Accomplishments: To facilitate the identification of "higher order" reference methods and reference materials that are currently available, the Joint Committee on Traceability in Laboratory Medicine (JCTLM) was created at a meeting held at the International Bureau of Weights and Measures (BIPM) in early June 2002. NIST (Willie E. May) is leading the activities of JCTLM Working Group-I, charged with establishing the process for identifying the "higher order" Certified Reference Materials and reference measurement procedures that will be required for IVD industry compliance with the EU IVD Directive.

Three meetings were held this year to review nominations for more than 80 Reference Methods and 435 Reference Materials for Electrolytes, Enzymes, Drugs, and Metabolites and Substrates Coagulation Factors, Non-Peptide Hormones, Nucleic Acids, and Proteins. The higher order standards endorsed by this process will be published in a database maintained by the International Bureau of Weights and Measures (BIPM) and will be publicly available on the BIPM website in February 2004 with links to the NIST CSTL and other relevant websites. See example below for Reference Measurement Procedures and Reference Materials for Cholesterol:

Reference Measurement Procedure					
Procedure Name and/or ID #	Analyte Name	Applicable Matrices	Measurement Principle	Reference Procedure Citation(s) or Document(s)	Reference Procedure Comparability Assessment Studies
NIST definitive method for serum cholesterol	cholesterol	lyophilized, fresh, or frozen serum	ID/GC/MS	Anal Chem 61, 1718-1723 (1989)	CCQM-K6; http://kcdb.bipm.org/appendixB/appbresults/ccqm-k6/ccqm-k6_final_report.pdf; Clin Chem 36, 370-375 (1990)
U. Of Ghent reference method for cholesterol	cholesterol	lyphilized, fresh, or frozen serum	ID/GC/MS	Clin Chem 39,1001-6 (1993) [=part II of Clin Chem 39,993-1000 (1993)]; Eur J Clin Chem Clin Biochem 34, 853-60 (1996); Clin Chem 42, 531-5 (1996)	EUROMET 563
DGKC definitive Method for Serum Cholesterol	cholesterol	lyophilized, fresh, or frozen human serum or plasma	ID/GC/MS	Siekmann et al., Z. anal. Chem. 279, 145-146 (1976)	PTB - National Key Comparison for Accredtation
CDCAbell-Kendall method for cholesterol	cholesterol	lyophillized, fresh or frozen human serum	Spectrophotometry	Cooper, GR, et al, Clin Chem 32: 921-929, 1986	Clin Chem 36, 370-375 (1990)

Reference Materials								
Information about Material				Contact Information	References			Comments
Analyte	Matrix	Material Name and/or ID #	Estimated * Availability (months, as of Jan 2004)	- Producer - Country - Website - Email Address - Phone Number - Fax Number	Commutability Study Information and/or Citations	Other Relevant Publications	Hyperlink to Comparability Assessment Studies	Comments
cholesterol	cholesterol	GBW09203b	60	NRCCRM China Tel: 086-10-64221811 Fax: 086-10-64213149 Email: crmservice@nrccrm.com.cn	Primary calibrator for higher order reference methods			
cholesterol	cholesterol	SRM 911b	21	NIST USA http://ts.nist.gov/ts/htdocs/230/232/232.htm Email:srminfo@nist.gov Tel:(301)975-6776 Fax: (301)948-3730	Primary calibrator for higher order reference methods			
cholesterol	human serum	JCCRM 211		HECTEF Japan http://www.in8.co.jp/hectef/starte.htm Tel: 81-44-813-0055 Fax: 81-44-813-0224			NIST study presented at JCTLM Meeting, June 20, 2003, BIPM, Sevres, France	
cholesterol	human serum (frozen)	SRM 1951b	60	NIST USA http://ts.nist.gov/ts/htdocs/230/232/232.htm Email:srminfo@nist.gov Tel:(301)975-6776 Fax: (301)948-3730	Material prepared following NCCLS Document C37-A "Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for	Previous lot (1951a) was measured in NIST study presented at JCTLM Meeting, June 20, 2003, BIPM, Sevres, France		
cholesterol	human serum (lyophilized)	SRM 1952a	60	NIST USA http://ts.nist.gov/ts/htdocs/230/232/232.htm Email:srminfo@nist.gov Tel:(301)975-6776 Fax: (301)948-3730		Method used for certification: Anal Chem 61, 1718-1723 (1989)	NIST study presented at JCTLM Meeting, June 20, 2003, BIPM, Sevres, France	
cholesterol	human serum (lyophilized)	SRM 968c	38	NIST USA http://ts.nist.gov/ts/htdocs/230/232/232.htm Email:srminfo@nist.gov Tel:(301)975-6776 Fax: (301)948-3730		Method used for certification: Anal Chem 61, 1718-1723 (1989)	NIST study presented at JCTLM Meeting, June 20, 2003, BIPM, Sevres, France	
cholesterol	human serum (lyophilized)	SRM909b	60	NIST USA http://ts.nist.gov/ts/htdocs/230/232/232.htm Email:srminfo@nist.gov Tel:(301)975-6776 Fax: (301)948-3730		Certification process described: Fresenius' J. Anal. Chem. 361:2 71-80 (1998). Method used for certification: Anal Chem 61, 1718-1723 (1989)	NIST study presented at JCTLM Meeting, June 20, 2003, BIPM, Sevres, France	

Based on this work and input from the medical professional and IVD manufacturing communities, JCTLM WG-I will also establish a list of highest priority needs for new reference procedures/ reference materials. NIST, EU and AP Laboratories have also begun a dialogue regarding sharing the work-load involved in developing and maintaining the more than 100 standards that are currently needed for IVD industry compliance with the EU IVDD. NIST has been asked to provide about 40% of the standards based on our current capabilities and U.S. market share.

Staff in the Analytical Chemistry and Biotechnology Divisions are developing "higher order" reference materials and methods urgently needed for US IVD industry compliance with the requirements of the EU IVD Directive including:

Health Stautus Marker	Disease State
• Cardiac Troponin-I	heart attack occurrence and damage
• Cadmium and Mercury	toxic metal poisoning
• Folates	neural tube defects
• HER-2 Neu	breast cancer
• Homocysteine	heart disease risk
• Glycated Hemoglobin	diabetes status
• Prostate-Specific Antigen	prostate cancer
• Triiodothyronine and Thyroid Stimulating Hormone	thyroid function
• Trinucleotide Repeat	mental retardation
• Speciated Iron	hemochromatosis, anemia

In 2003, work will be completed for standards for Toxic Metals in Urine, Cardiac Troponin-I and Electrolytes in Human Serum.

Impact: Clinical measurement results that are reliable and comparable over both space and time are essential for optimal patient care, most efficient use of available healthcare funds, and full utilization of the potential of new information technology tools. Incorrect interpretation of measurement results by the physician can lead to incorrect diagnosis and treatment, additional unnecessary tests and medical procedures, and increased pain and suffering for the patient. Measurements are responsible for 10%-15% of the $1.5 T annual costs of healthcare in the United States. A significant portion (25% - 30%) of health-related measurements is performed for non-diagnostic reasons (re-tests, error prevention and detection). The "German Health Report of 1998" states explicitly that "the costs of repeat measurements amount to 1.5 billion US $ per year in Germany." If normalized to the U.S. GDP for that year, these costs would be $7.4 B. Even modest improvements in measurement accuracy and quality assurance will result in multi-billion dollar savings in health-care costs. In addition to measurements reliability and related cost issues, timely completion of these standards will also assure U S. IVD industry's continued access to the European market.

Future Plans: Work will continue on the high priority list presented above. SRMs will be completed for Homocystein, Folates, Thyroid Markers in 2004. Work will begin on standards for gene expression including RNA standards and a fluorescence standard for microarray scanning devices. JCTLM Review Teams for Blood gases, Blood Groupings, Microbial Serology, Non-Electrolyte metals and Vitamins will be established for providing lists of "higher order" methods and materials by December 2004.

International Standards

Title: International Benchmarking of NIST Capabilities for Chemical Measurements

Authors: W.E. May, G.C. Turk, S.A. Wise, F.R. Guenther, G.W. Kramer, R.R. Greenberg, and R.M. Parris

Abstract: Traceability to stated references and global confidence in this realization are the basis for mutual recognition and confidence in data used to facilitate and underpin international trade and decisions regarding health, safety, commerce, and scientific studies In October 1999, the Directors of National Metrology Institutes (NMIs) for the thirty-eight member states of the Meter Convention, and representatives of two international organizations signed a Mutual Recognition Arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). The NIST Analytical Chemistry Division has ongoing major activities to meet the MRA requirements for NMIs:

1. Declaring and documenting calibration and measurement capabilities
2. Evidence of successful participation in formal, relevant international comparisons
3. Demonstration of system for assuring quality of each NMI's measurement services

Purpose: Traceability to stated references and global confidence in this realization are the basis for mutual recognition and confidence in data used to facilitate and underpin international trade and decisions regarding health, safety, commerce, and scientific studies In October 1999, the Directors of National Metrology Institutes for the thirty-eight member states of the Meter Convention, and representatives of two international organizations signed a Mutual Recognition Arrangement on national measurement standards and calibration and measurement certificates issued by national metrology institutes (MRA). This MRA provides an open, transparent, and comprehensive framework for obtaining reliable quantitative information on the comparability of metrological services needed for mutual recognition of national measurement standards and measurement certificates issued by national metrology institutes around the World. This Arrangement also provides governments and other parties with a secure technical foundation for wider agreements related to international trade, commerce, and regulatory affairs.

Major Accomplishments: For implementation of this MRA, the signatory NMIs agreed to:

1. declare and document their calibration and measurement capabilities (CMCs)[Appendix C]
   - CSTL's Analytical Chemistry Division has over 1100 CMCs for Chemical Measurements included in ~3000 CMCs for Chemical Measurements published in the CIPM MRA Appendix C (http://kcdb.bipm.org/AppendixC/default.asp)

2. participate in relevant international comparisons to support their CMCs [Appendix B] (http://kcdb.bipm.fr/BIPM/KCDB/, http://icdb.nist.gov)
   - CSTL's Analytical Chemistry Division has participated in >75 international comparisons to meet requirements of the CIPM MRA. In FY03, the comparisons in which ACD participated were for the determination of a variety of organic and

inorganic components in matrices such as gaseous mixtures, sediments, food, water, fuel, and serum. Results of selected recent comparisons are shown below.

3. implement and document the existence of a system for assuring the quality of the measurement services provided.
   - CSTL's Analytical Chemistry Division's Quality Manual that summarizes and formalizes policies and approaches for addressing quality-related issues concerning the services that it provides is being updated to assure appropriate compliance with ISO/EC 17025 and ISO Guide 34.

The implementation of the CIPM MRA is carried out by a Joint Committee of Regional Metrology Organizations (RMOs) and the BIPM (JCRB). The JCRB is made up of representatives from each RMO and the BIPM, and provides oversight for results included in the Key Comparisons Database (Appendix B of the MRA), as well as the determination of the degree(s) of equivalence of results from individual NMIs. RMOs have the responsibility for carrying out supplementary comparisons and other actions within their regions to support mutual confidence in the validity of calibration and measurement certificates through the Joint Committee of the RMOs and the BIPM (JCRB). They are also responsible for approval of calibration and measurement capabilities (CMCs) of their member NMIs.

Systema Interamericano de Metrologia (SIM) is the metrological regional organization (RMO) for the Americas. CSTL provides the Chair for the Chemical Metrology Working Group and SIM representative to the JCRB. In order to assure the effective, fair and metrologically sound implementation of the MRA. CSTL staff have led the critical review of both SIM and international chemistry CMC data for Appendix C of the BIPM.

CSTL staff also play a leadership role the International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM). The CCQM has formed seven working groups: (1) Gas Analysis, (2) Organic Analysis, (3) Inorganic Analysis, (4) Electrochemistry (5) Biometrology, (6) Surface Analysis and (7) Key Comparisons and CMC Quality. These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. CSTL staff are active in all seven and has provided formal leadership for the Organic Analysis, Biometrology and Key Comparisons Working Groups.

Impact: NIST and other National Metrology Institutes around the world have the responsibility for establishing, maintaining, and disseminating the highest level of metrological references for a given country or economy. The calibration and measurement services that these NMIs provide must be of high quality and delivered to our customers in a consistent and transparent manner.

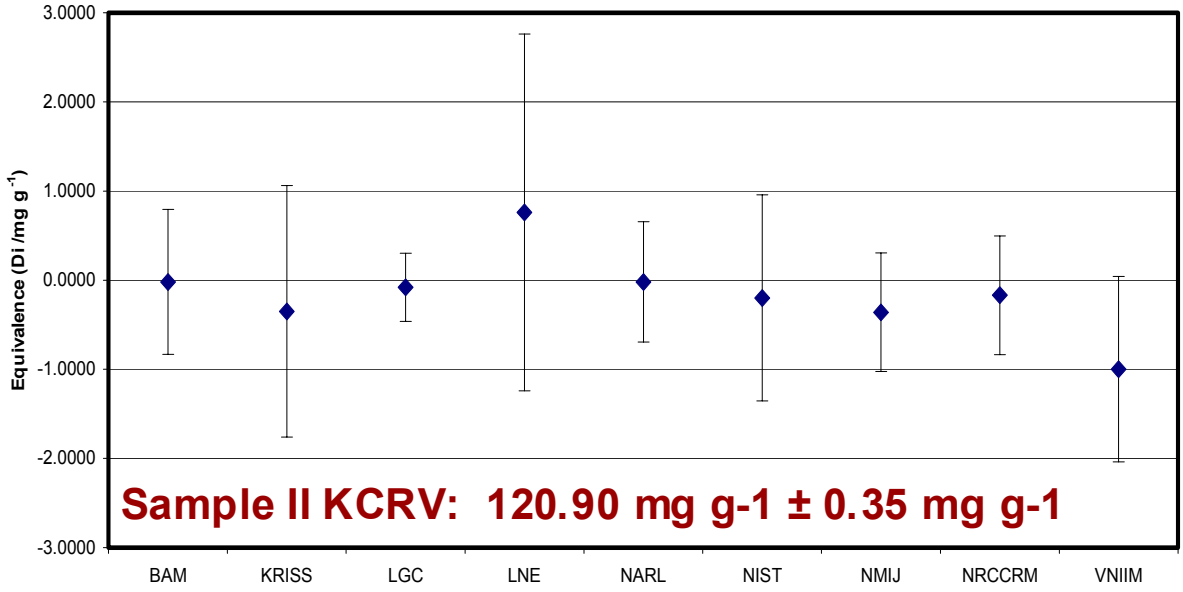
Future Plans:
Over 25 additional CCQM studies are planned to be conducted over the next two years and the Analytical Chemistry Division has already committed to coordinate at least 7 of these. In addition, the Division's Quality System for its measurement services will be presented to and assessed by the SIM Quality System Task Force. The results of this review will be reported to

the CIPM JCRB the complete the requirements for maintaining our CMCs in the CIPM Database.

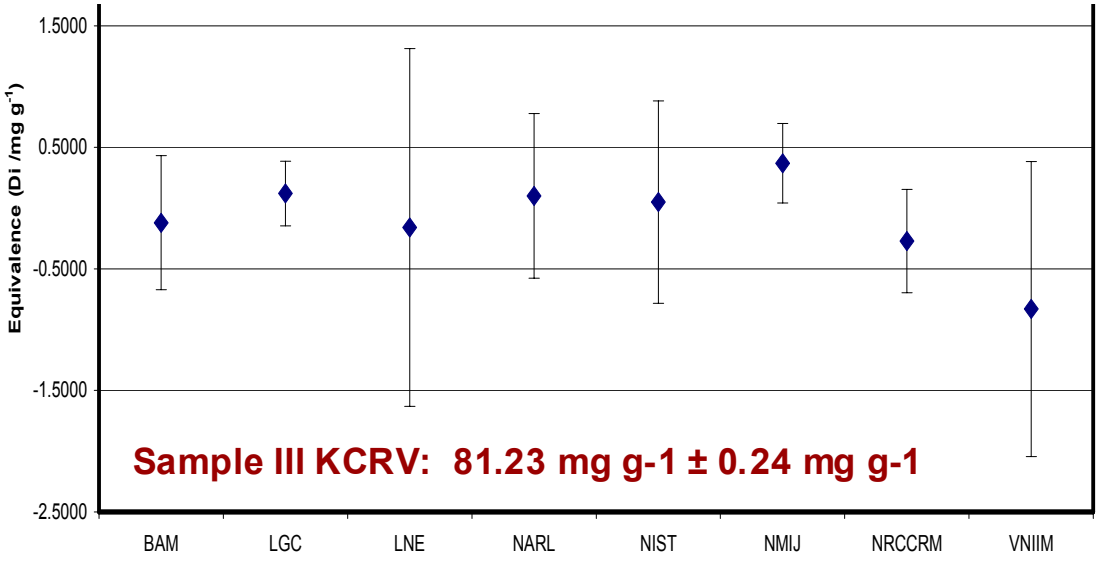
Examples of NIST participation in recently completed international comparisons

CCQM-K27: Determination of Ethanol in Aqueous Matrices
Study Period: 2002

CCQM-K27a Forensic level
Samples II (aqueous solution spiked gravimetrically with ethanol)



CCQM-K27b Commodity level
Sample III (commercial red wine stabilized by irradiation)



Determination of Ethanol in Aqueous Matrix

Study Period: 2003

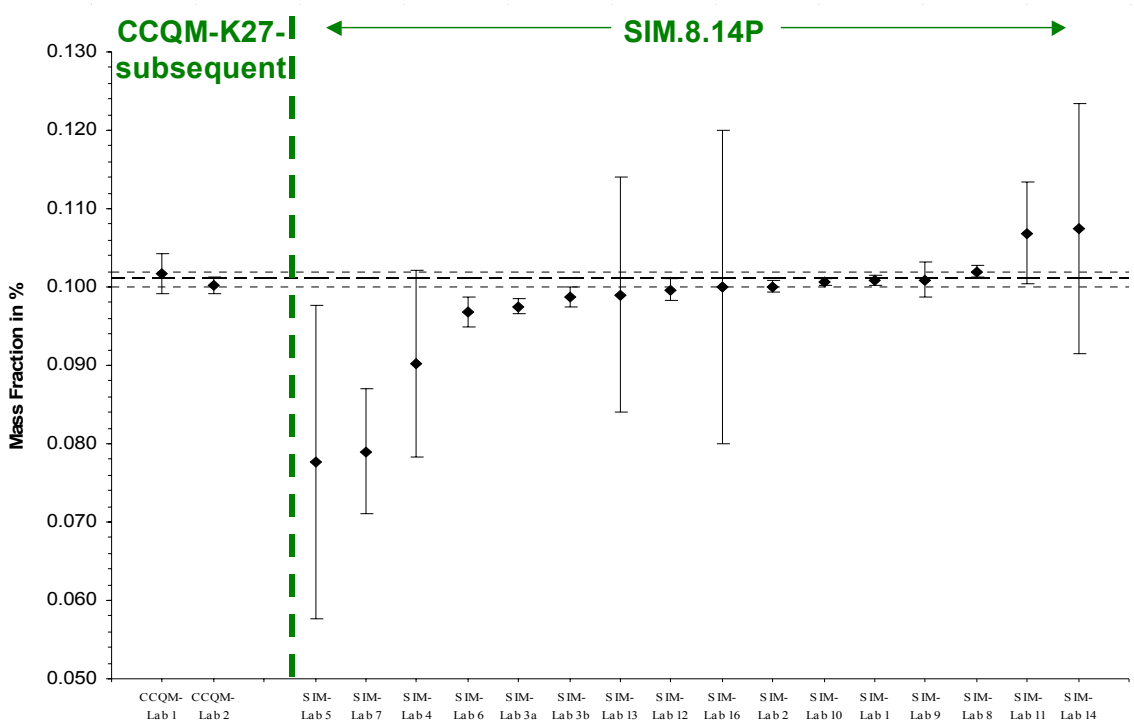
As SIM Pilot Comparison: SIM.8.14P (16 participants)

As Key Comparison: CCQM-K27-subsequent (4 participants)

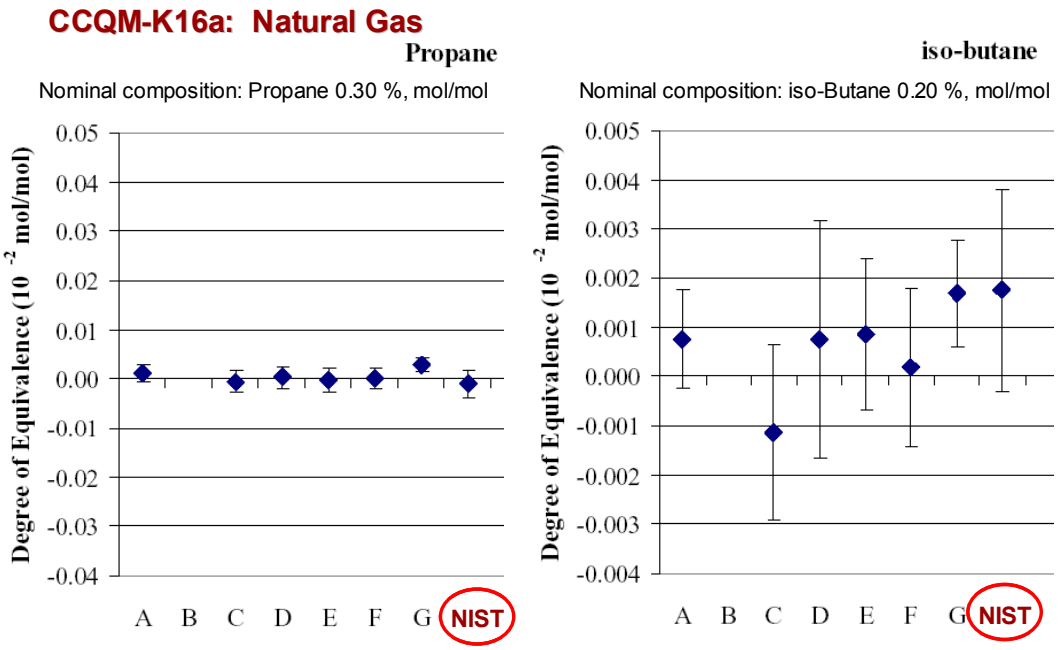
Ethanol in Aqueous Matrix

Sample SII: nominal concentration 0.1% ethanol in water

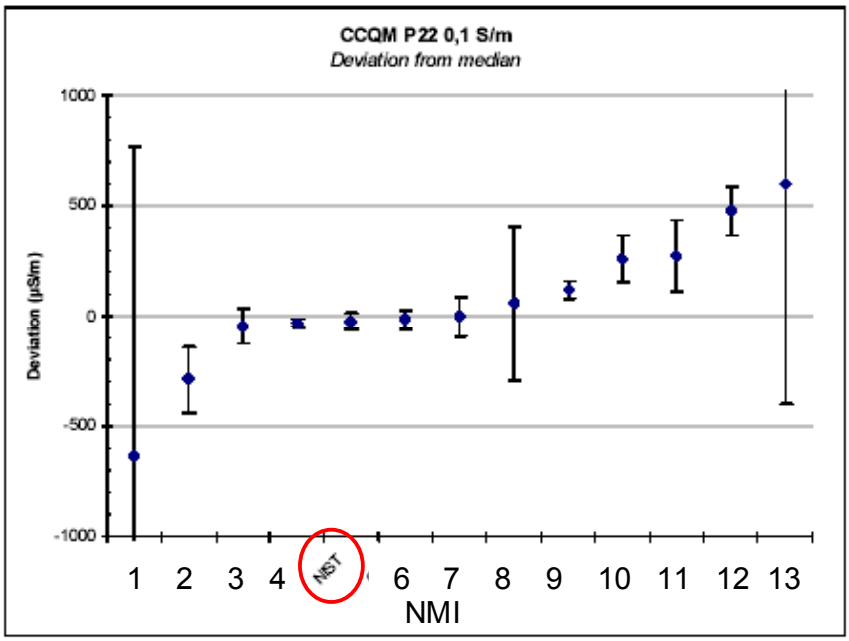
(showing gravimetric value and upper and lower limits of the 95% CI of the gravimetric value based on the CCQM-K27a study)



Determination of Components of Natural Gas



CCQM-P22:
Electrolytic Conductivity
(primary and secondary measurements)

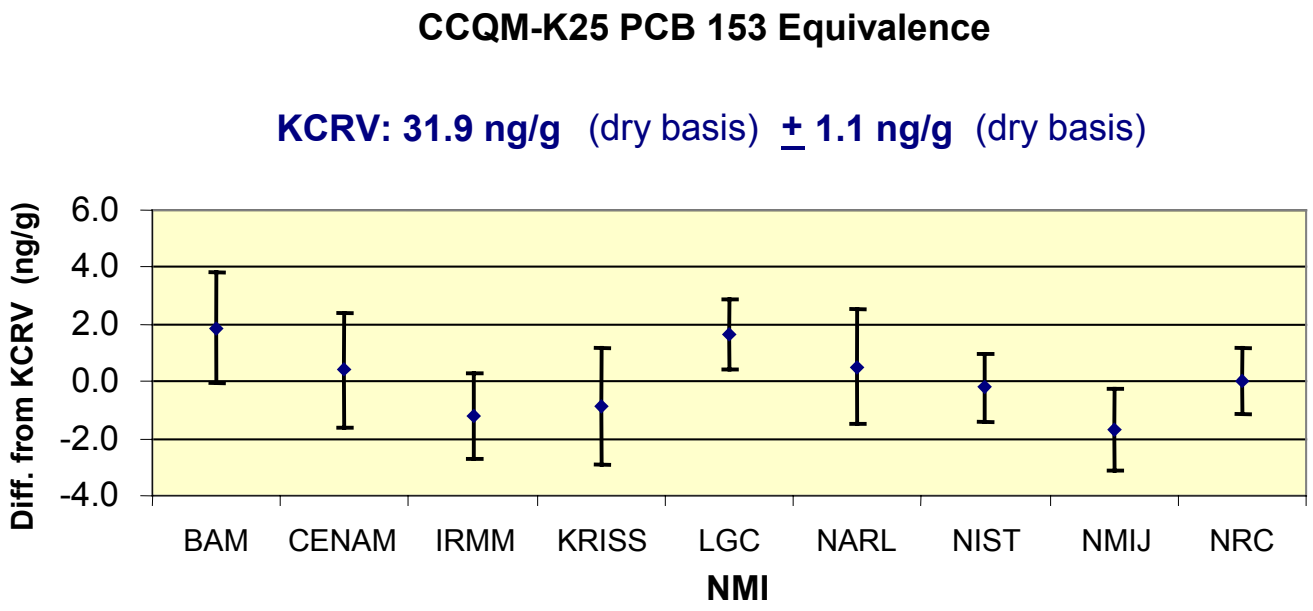


Nominal 0.1 S/m (1000 µS/cm)

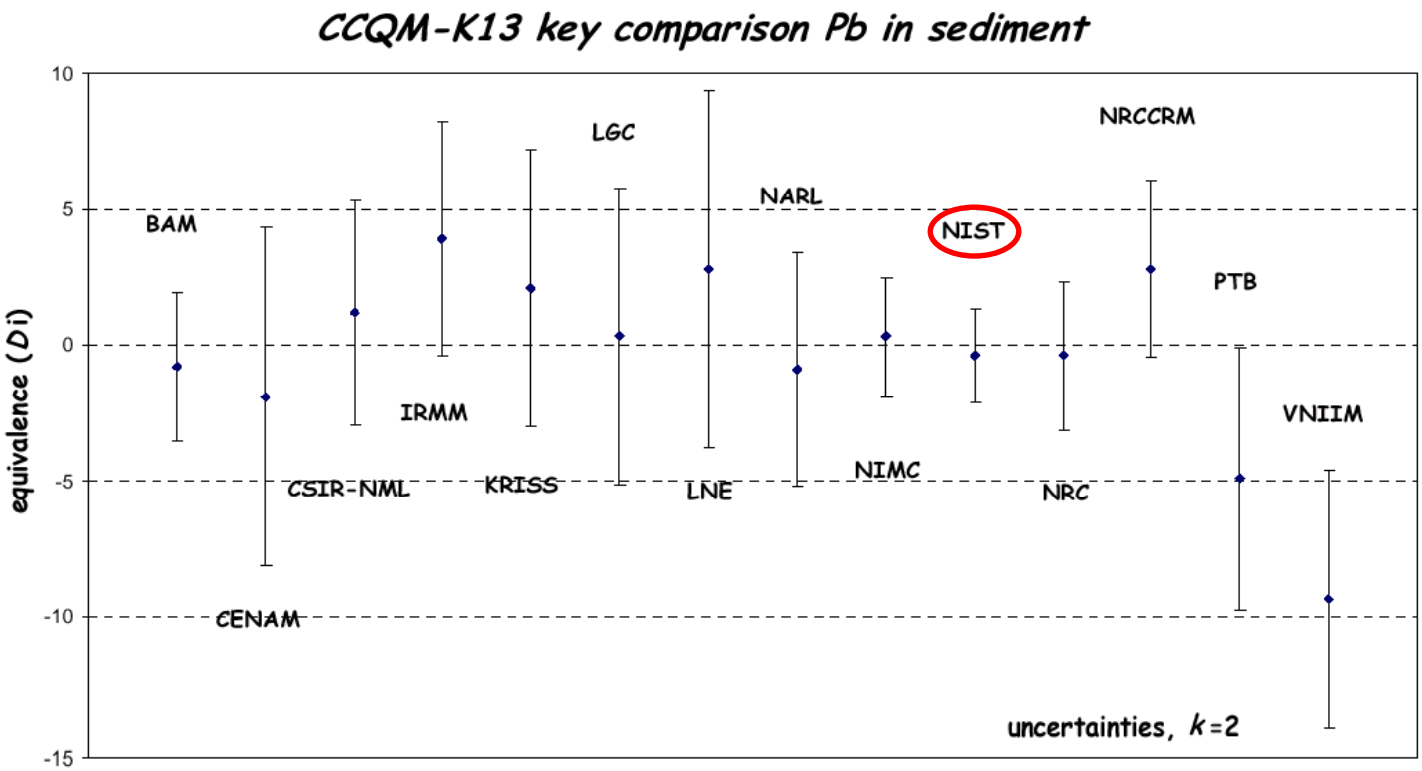
CCQM-K25: PCB Congeners in Sediment

The five PCB congeners measured in CCQM-K25 were selected to be representative of the approximately 150 congeners found in environmental samples. These five congeners also provided the typical analytical measurement challenges encountered including problematic GC separations and a wide volatility range and concentration range for the individual congeners. The results for PCT 153 are shown below.

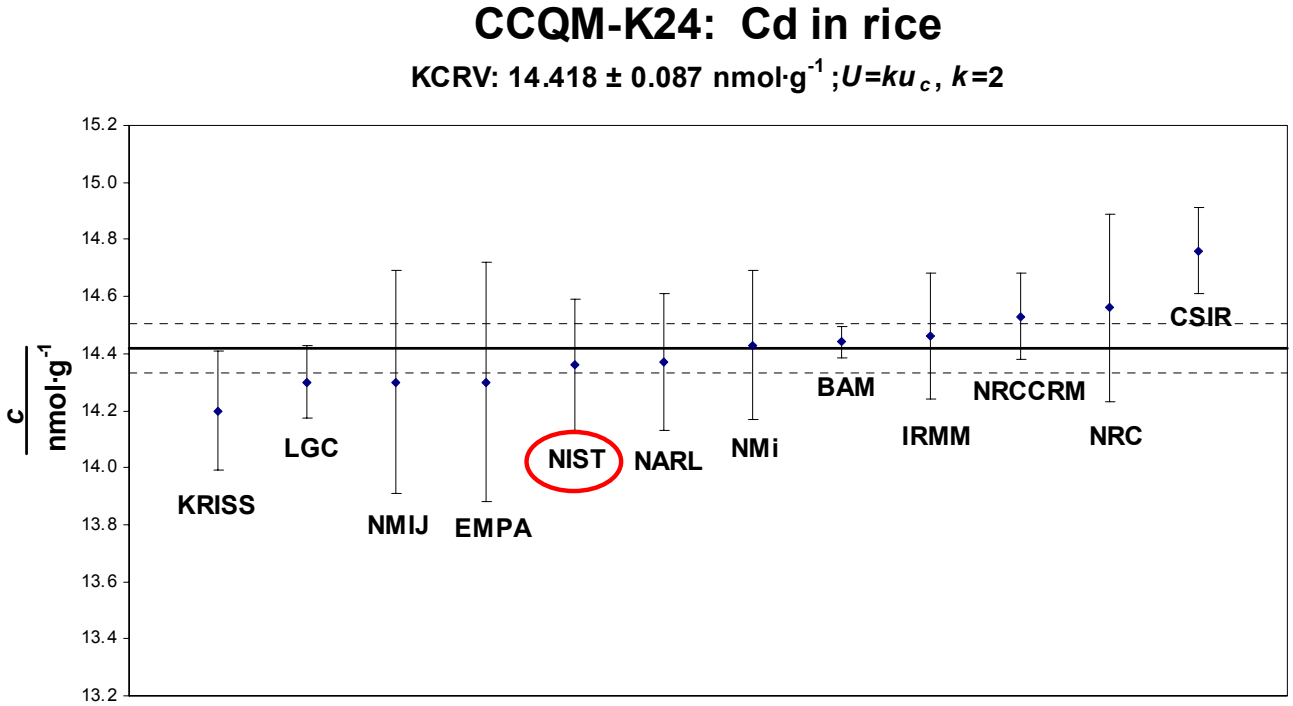
- PCB 28 - volatile and potential coelution with PCB 31
- PCB 101 - potential coelution with minor congener, PCB 90
- PCB 105* - lower concentration and potential elution order changes with PCB 153 and/or PCB 132
- PCB 153 - potential coelution with PCB 132
- PCB 170 - lower concentration and potential coelution with PCB 190



Determination of Cadmium and Lead in Sediment



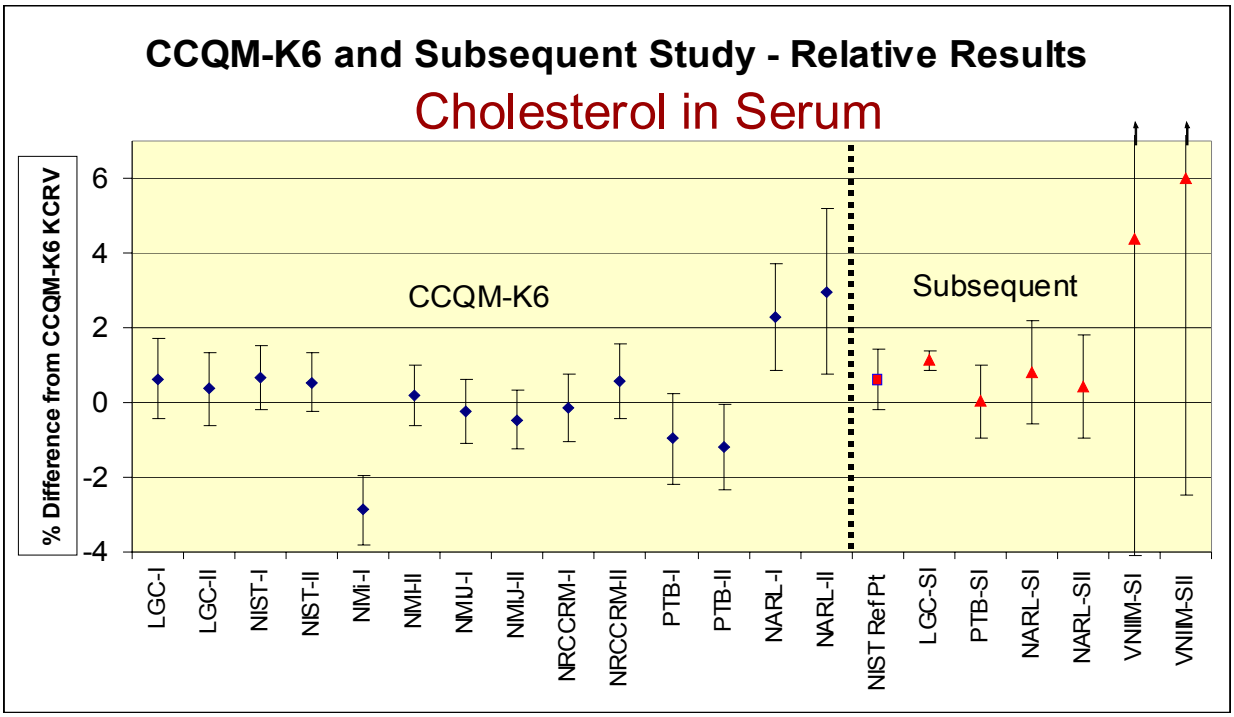
Determination of Cadmium in Rice



Expanded uncertainties with coverage factor of k=2

Uncertainty of prescribed drying protocol was major component of NIST expanded uncertainty.

Determination of Cholesterol in Serum



K6 results are plotted as % differences from KCRVs

Subsequent results are plotted relative to NIST results in K6S and are offset by average (NIST-KCRV) result from K6 (NIST Ref Pt)

International Standards

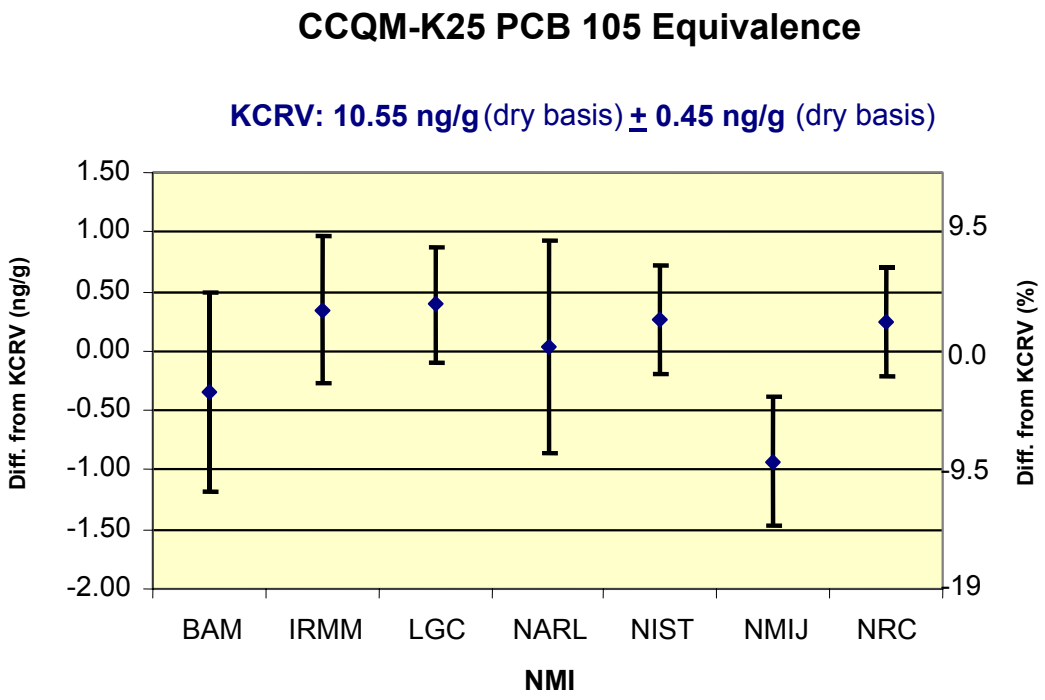
Title: Results of CCQM-K25 Key Comparison for Polychlorinated Biphenyl Congeners in Sediment

Authors: M.M. Schantz and S.A. Wise

Abstract: Polychlorinated biphenyls (PCBs) consist of 209 possible congeners depending on the substitution of the chlorine atoms around the biphenyl molecule. PCBs have been widely used as industrial fluids, flame retardants, diluents, hydraulic fluids, and dielectric fluids for capacitors and transformers. As a class of compounds, they are environmentally stable and tend to bioaccumulate. Of the 209 possible congeners, approximately 150 congeners have been reported in the environment. A CCQM key comparison for PCB congeners in sediment was conducted with NIST serving as the coordinating laboratory. For the key comparison, five target PCB congeners were selected as representative of the measurement of individual congeners in environmental samples. The target congeners included some potential problematic gas chromatography (GC) separations, and they spanned the volatility range and the typical concentration range for the 150 congeners found in environmental samples. Nine laboratories participated in the key comparison. All but one of the participants used gas chromatography/mass spectrometry (GC/MS) with carbon-13 labeled PCB congeners as internal standards/surrogates (ID-GC/MS).

Purpose: The five PCB congeners chosen were PCB 28, PCB 101, PCB 105, PCB 153, and PCB 170. PCB 28 (2,4,4'-trichlorobiphenyl) is volatile and under certain conditions coelutes with PCB 31 (2,4",5-Trichlorobiphenyl). PCB 101 (2,2',4,5,5'-pentachlorobiphenyl) has the potential coelution with a minor congener, PCB 90 (2,2',3,4',5-pentachlorobiphenyl). PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl) is typically one of the most abundant congeners and potentially coelutes with PCB 132 (2,2',3,3',4,6'-hexachlorobiphenyl). PCB 105 (2,3,3',4,4'-pentachlorobiphenyl) is a congener with a lower concentration than the other congeners selected and which may change elution order with PCB 132 depending on the analytical conditions. Finally, PCB 170 (2,2',3,3',4,4',5-heptachlorobiphenyl) is one of the less volatile congeners, is typically found at lower concentrations, and can potentially coelute with PCB 190 (2,3,3',4,4',5,6-heptachlorobiphenyl). The ability of a laboratory to measure these five congeners should indicate their ability to measure the suite of 150 PCB congeners found in sediments.

Major Accomplishments: This Key Comparison study demonstrated a high level of equivalence in capabilities of the participating NMIs to successfully measure five PCB congeners (congener numbers 28, 101, 105, 153, and 170) in a moderately contaminated sediment using ID-GC/MS-based methods. The uncertainties of the key comparion reference values (KCRVs) ranged from 2% to 5% indicating excellent agreement among the participants. The following figure of equivalence for PCB 105 shows the excellent agreement among the laboratories and the comparison to the KCRV. Similar degrees of equivalence were demonstrated for the other four congeners.



Impact: The five PCB congeners measured in CCQM-K25 were selected to be representative of the approximately 150 congeners found in environmental samples. These five congeners also provided the typical analytical measurement challenges encountered in the measurement of individual congeners, including problematic GC separations and a wide volatility range and concentration range for the individual congeners. The ability of the laboratories to measure these five congeners should translate to the ability to measure the typical suite of PCB congeners found in sediments at concentrations encountered in moderately contaminated sediments (> 5 ng/g dry basis).

Future Plans: To help understand differences among the laboratories in calculating uncertainties, a pilot study will be conducted using a calibration solution, an extract, a spiked extract, and a tissue sample with the same five PCB congeners as the targets. The primary focus of this study will be to identify sources of uncertainties for each step of the analytical method.

International Standards

Title: Results from International Key Comparison for Calcium in Serum

Authors: S.E. Long and K.E. Murphy

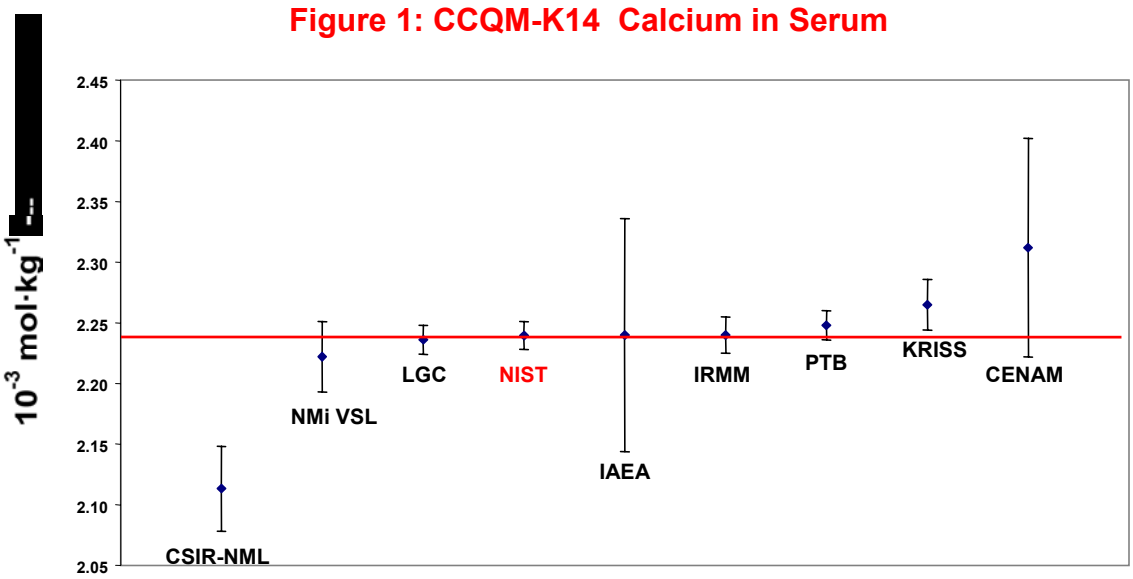
Abstract: The Analytical Chemistry Division successfully participated in a key comparison of calcium in blood serum (CCQM-K14), which was conducted by the Inorganic Analysis Working Group of the CCQM. The calcium was determined using isotope dilution inductively coupled plasma – mass spectrometry. The result was in excellent agreement with the other participating laboratories and almost identical to the key comparison reference value chosen to represent the amount content of calcium in the serum material.

Purpose: Successful participation in CCQM key comparison studies is an important requisite for the demonstration of analytical measurement competence by national metrology institutes (NMIs). Key comparisons are an important component of the CIPM Mutual Recognition Arrangement and establish a quantitative measure of the degree of equivalence of national measurement standards of signatory NMIs.

Major Accomplishments: Under the auspices of the CCQM Inorganic Analytical Working Group (IAWG), the key comparison, CCQM-K14, was aimed at addressing the need for better measurement methodology for routine clinical measurements of total calcium in blood serum. It is evident that the current measurement system is not satisfactory from a clinical diagnostic standpoint, and as a result, the impact on health-care costs may be considerable. Following successful participation by ACD in the pilot study, which was conducted during FY 01, calcium was determined in an un-modified frozen human serum, which was distributed by European Commission's Institute for Reference Materials and Measurements (IRMM) as the pilot institute. Eight other national metrology institutes also participated. Calcium was determined using an isotope dilution "cold-plasma" inductively coupled plasma – mass spectrometry (ICP-MS) measurement. The result (2.2395 ± 0.0115 mmol.kg^{-1}) was in excellent agreement with the other laboratories and also the proposed value for the key comparison reference value, which was 2.240 ± 0.008 mmol.kg^{-1}. The analytical data from the key comparison are shown in Figure 1. It is interesting to note the shift from using thermal ionization mass spectrometry (TIMS) to ICP-MS for making such measurements, which has been made possible by the advent of systems for reducing or eliminating spectral interferences. Of the nine participants in the study, only two used TIMS. The remainder used a combination of high-resolution sector field, collision cell and cold-plasma ICP-MS, while one laboratory used ICP optical emission spectrometry.

Impact: The veracity of the analytical measurement system for calcium in blood serum has been demonstrated. Such measurements provide documented performance for the NIST quality system which is an integral component of its measurement services. The final report for CCQM K-14 concludes that the analytical procedures developed during the study are also relevant to other analytes in serum, so the comparison study has extended benefits. The material will be used in the future for external quality assurance programs.

Future Plans: Participation in appropriate CCQM / IAWG comparison exercises will continue to demonstrate the quality of the analytical measurement capabilities of NIST which underpin measurement services for its customer base.



International Standards

Title: Results from International Comparisons for Selected Components in Natural Gas

Authors: G.C. Rhoderick and F.R. Guenther

Abstract: NIST participated in the CCQM-K16 Natural Gas Key Comparison in which the final report was released in October of 2002. The study evaluated the degree of equivalence for the given measurand in natural gas for National Metrology Institutes (NMI). Unlike past comparisons, this natural gas included heavier hydrocarbons and was designed to emulate real natural gas at both high and low calorific value. Due to shipping problems, NIST was able to analyze only the cylinder containing the low calorific natural gas mixture. NIST's results show excellent agreement with the gravimetric key comparison reference values. A similar Pilot Study was organized by NIST for the SIM countries. Results of the SIM 8-P8 pilot study on natural gas reveal much larger deviations than those of the CCQM key comparison.

Purpose: These comparisons were designed to evaluate the capabilities of National Metrology Institutes to certify natural gas standards, and are intended to provide evidence for calibration and measurement capabilities listed by each NMI in the BIPM MRA Database.

Major Accomplishments: NIST completed the analysis of the low calorific value natural gas standard in FY03 and released the report to the pilot laboratory. The high calorific valued natural gas standard was not analyzed by NIST, as the cylinder was lost in shipment and there was not enough time to replace it before the Key Comparison ended. Table 1 lists the NIST results of the comparison.

Table 1. Agreement between NIST value and Preparation Value (given as % mol/mol).

Compound	Reference Value	Reference Value	NIST Value Uncertainty	NIST Value Uncertainty	Absolute Difference Δ_x	Relative Difference $\Delta_{x/x}$
Methane	81.6826	0.0027	81.77	0.46	0.087	0.11 %
Nitrogen	12.1205	0.0007	12.11	0.16	-0.010	-0.09 %
CO_2	4.0446	0.0005	4.055	0.026	0.010	0.26 %
Helium	0.50813	0.00016	0.5158	0.0026	0.0077	1.51 %
Ethane	0.7489	0.0002	0.7493	0.0046	0.0004	0.05 %
Propane	0.29144	0.00010	0.2905	0.0026	-0.0009	-0.32 %
iso-Butane	0.20165	0.00019	0.2034	0.0019	0.0018	0.87 %
n-Butane	0.20260	0.00007	0.2024	0.0018	-0.0002	-0.10 %
iso-Pentane	0.04861	0.00011	0.0483	0.0006	-0.00031	-0.64 %
n-Pentane	0.04982	0.00011	0.0500	0.0007	0.00018	0.36 %
neo-Pentane	0.04972	0.00006	0.0495	0.0007	-0.0002	-0.44 %
n-Hexane	0.05061	0.00011	0.0502	0.0008	-0.0004	-0.81 %

A similar Pilot Study was organized by NIST for the SIM countries. Results of the SIM 8-P8 pilot study on natural gas reveal much larger deviations than those of the CCQM key comparison. As this was a pilot study, the results are not to be made publicly available. All the participating labs submitted methane values that were within ≤ 1.0 % of the certified

concentration. However, the agreement among labs for all of the remaining compounds was poor. One lab was consistently within ± 1.5 % of the certified values with the exception of carbon dioxide (4.6 %) and n-butane (1.7 %). Therefore much work remains to be done in SIM to bring the comparability among SIM NMIs in line with the CCQM.

Future Plans: There will be another Natural Gas CCAM Key Comparison in FY2004. However since NIST has participated in the CCQM-K16 natural gas comparison with good results, NIST has decided to forgo this comparison. A SIM pilot study on automobile emission gases, piloted by NIST, is ongoing in FY2004, and will be completed by March 2004. A new SIM pilot study on industrial gas emission (sulfur dioxide) will be piloted by NIST, and initiated in late FY2004.

International Standards

Title: Development and Dissemination of Intrinsic Standards for Chemical Measurements

Authors: P.M. Chu, D.L. Duewer, M.L. Salit, and J.C. Travis

Abstract: Intrinsic standards "based on well characterized laws of physics, fundamental constants of nature, or invariant properties of materials" (ANSI/NCSL Z540) have many uses in physical and chemical metrology. Such standards can reduce the need for (1) labor-intensive artifact standard production by National Metrology Institutes (NMIs) and (2) repeated costly laboratory measurement comparisons associated with mutual recognition agreements.

Purpose: Intrinsic standards enable NMIs to concentrate efforts on metrology rather than artifact production or calibration, and offer end-users of commercial standards ready access to traceability through data. Economy of scale encourages users to purchase the physical realization of an intrinsic standard (an artifact transfer standard) rather than constructing it themselves. Thus, the production of artifact standards devolves from the NMI to the commercial sector, which is better suited to production.

Major Accomplishments: Three separate efforts within the Analytical Chemistry Division report significant progress. (1) In collaboration with the NIST Physics Laboratory (NIST/PL), we have prepared the first of three manuscripts reporting improved spectral line positions for mercury and argon atomic lines in low-pressure discharges. These positions serve as secondary length standards and support higher accuracy in the determination of atomic line positions in such analytical plasmas as the Inductively Coupled Plasma (ICP). (2) SRD 79 Quantitative Infrared Database is an on-going project providing quality-assured quantitative infrared absorption reference data to support open-path Fourier transform infrared (FT-IR) measurements, such as those described in EPA Method TO-16. New spectra based on gravimetrically prepared samples are added to the database as they are acquired. Comparisons with Pacific Northwest National Laboratories continue to play a key role in validating the data. (3) Samples of SRM 2034 (dilute holmium oxide solution sealed into fused silica cuvettes) were distributed to fifteen NMIs. Results from these laboratories, NIST/PL, and our own reference instruments will form the basis for an internationally supported intrinsic wavelength standard for UV/visible chemical spectrophotometry.

Impact: Intrinsic standards provide a passive means of support for our customers, requiring attention only at wide intervals to improve uncertainties or respond to emerging needs. End users or standards producers may construct artifacts deriving from these standards – such as atomic pen lamps, bottled gas standards, and sealed solution wavelength standards – with traceability provided by data. This provides economic benefits and improved responsiveness to NIST and other NMIs, commercial standards producers, and end users alike.

Future Plans: The atomic wavelength project is essentially complete, awaiting only the publication of the three manuscripts. The database for molecular gas cross sections is a continuing project, with improvements planned including Internet distribution. Long-term plans for the database include investigating calibration transfer issues and international comparisons of

the data. The intrinsic wavelength standard should be completed in FY04, with the possible extension to simultaneous coverage of wavelength and absorbance in the future.

Microelectronics

Title: Neutron Transmutation Doping of Compound Semiconductors

Author: R.M. Lindstrom

Abstract: The selective addition of small amounts of doping elements to semiconductors can be done by using neutron capture, followed by beta decay, to transmute elements. New compounds are being explored, to exploit the experience gained in industry with silicon.

Purpose: Transmutation of silicon by nuclear reactions has become well established for the production of high-power rectifiers and other electronic devices. In this application, phosphorus is created by the capture of neutrons by ^{31}Si to make ^{32}Si, which decays to ^{32}P. Because silicon is transparent to neutrons, the phosphorus dopant is created uniformly through a large Si crystal.

Major Accomplishments: The same process as used for elemental silicon is applicable to 1:1 compound semiconductors. Recently, neutron transmutation doping (NTD) has been explored in two additional systems. In collaboration with North Texas State University and the NIST Center for Neutron Research, samples of $Hg_{0.8}Cd_{0.2}Te$ films on $Cd_{0.95}Zn_{0.05}Te$ substrates have been subjected to neutron irradiation. The goal is to transmute both Hg to Au and Te to As. A first experiment using neutron fluences of 1-2 x 10^{16} cm^{-2} showed that transmutation is feasible. The results of this experiment were reported at the Narrow Bandgap Conference in 2003, and will be published in *Physica E*. To study the effects of irradiation quantitatively, additional samples have been exposed to a pure thermal neutron flux for fluences from 3 x 10^{16} to 1.3 x 10^{17} cm^{-2}; these specimens are now under study at NTSU.

Gallium nitride is of great current interest for high-temperature devices, and is the subject of a large national research project coordinated by DARPA. The opportunity exists for uniform doping of this material, by transmuting both ^{69}Ga and ^{71}Ge to the corresponding Ge isotopes. In collaboration with IEEL, a sample of bulk GaN was obtained from ATMI, Inc. and irradiated with 1.0 x 10^{18} neutrons/cm^2, for a calculated uniform doping of 2.5 x 10^{16} atoms Ge/g Ga, or 1.3 x 10^{17} atoms Ge/cm^3. This material is has been returned to ATMI for characterization.

Impact: Experiments so far are preliminary, but the ability to dope GaN uniformly would presumably be as useful as NTD has proven to be in Si. The opacity of Cd to neutrons restricts NTD in this system to thin films, but this is precisely where the interest lies.

Future Plans: The future applications of this technology depend on the results of characterization measurements now underway in our partner institutions.

Microelectronics

Title: Chemical Characterization of Thin Films to Facilitate Understanding and Improvement of Manufacturing Processes

Authors: G.P. Lamaze, H.H. Chen-Mayer (846), and K. Soni (Corning Laboratories)

Abstract: Neutron depth profiling (NDP) has been employed to examine manufacturing processes and starting materials for several high technology applications. The method is both quantitative and non-destructive. Samples were prepared at the Corning Laboratories and the analyses were performed at the Center for Neutron Research at the NIST. Lithium profiles of $LiNbO_3$ specifically addressing the problem of lithium stoichiometry have been made. Studies of boron volatility in boro-silicate glasses were also made by NDP. Nitrogen profiles of GaN/GaAs bilayers were also made.

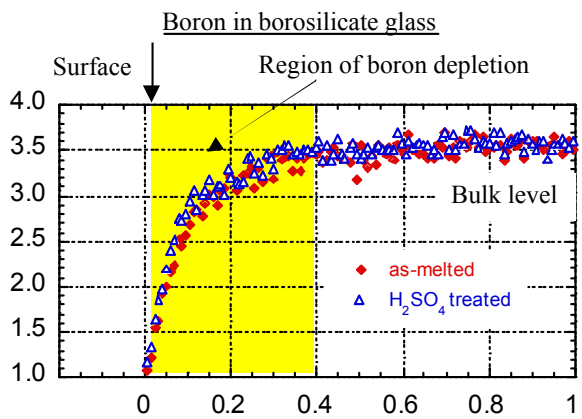
Purpose: To investigate starting materials and manufacturing processes for high technology applications of interest to Corning Laboratories. Three types of samples have been analyzed: 1) Boron profiles are measured in glasses to determine B loss due to its volatilization during manufacturing. Surface depletion of B is a key characteristic of borosilicate materials for both chemical vapor deposition and conventional melting processes. 2) For lithium niobate, a quantitative measure of Li concentration can differentiate congruent and stoichiometric compositions and any surface depletion in commercial wafers. 3) Nitrogen concentrations are measured in GaN/GaAs bilayers. The addition of N lowers the bandgap and enables design of long-wavelength lasers. However, there are processing issues in the introduction of N at high levels without causing phase separation. An important need towards that goal is a reliable, accurate method for N concentration as a function of depth in GaAs-based alloys.

Major Accomplishments: Sulfuric acid-cleaned borosilicate glass shows a similar depth profile to untreated samples indicating that this surface treatment does not alter the depletion of boron in the glass (Fig.1 upper panel). Sulfuric acid is typically used to clean the glass surface to remove any organic contamination and sodium. In all cases, the surface concentration of boron is reduced but is non-zero, indicating that it will play a role in surface reactivity, though not at the same level as exhibited by the bulk composition. Lithium concentrations were determined for two lithium niobate wafers: one commercial Z-cut wafer of congruent composition and one of stoichiometric composition prepared by vapor transport equilibration method (Fig.1 lower panel). The average lithium concentration for the congruent sample agrees with the known value of congruent line at 48.4 mol.% Li_2O. The processed sample shows a value close to the calculated stoichiometric value of 1.89 ± 0.4 (1σ) x 10^{22} at/cm^3. These analyses clearly show the ability to identify these wafers on the basis of composition directly. A paper has been submitted to Applied Surface Science on the volatilization of boron in borosilicate glass and the stoichiometry of $LiNbO_3$. In addition, N concentration profiles of two N-doped GaAs films deposited on GaAs substrates have been obtained with adequate depth resolution and sensitivity for N. The film thickness is in good agreement with measured values using profilometry. This experiment may be the first study of N in GaN/GaAs by NDP.

Impact: Direct quantitative measurements often provide greater insight to material properties than relative measurements. The boron study of surface treatment demonstrated that current-cleaning techniques do not affect material properties. The lithium niobate studies showed that

NDP could distinguish between stoichiometric and non-stoichiometric samples. The continuing study of GaN/GaAs has potential implications for design of quantum well devices.

Future Plans: We plan to continue the collaboration between CSTL and Corning. A paper is being prepared on nitrogen concentrations in GaAs. We will explore the possibility of using NDP for the analysis of N-doped GaAs quantum wells in devices. We also plan to provide a nitrogen-in-GaAs artifact standard for use in calibrating in-house SIMS measurements.



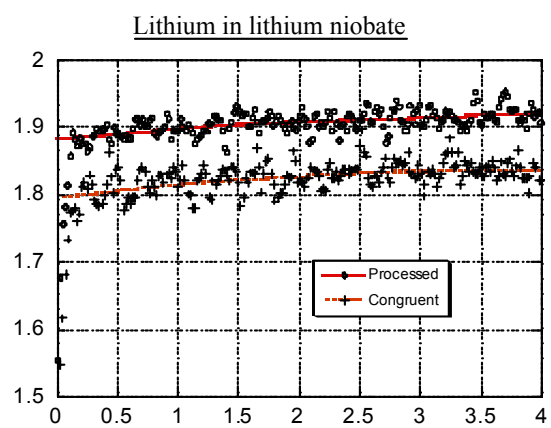


Fig. 1. NDP of boron in borosilicate (top) and lithium in lithium niobate (bottom), two substrates of technological importance.

Pharmaceuticals and Biomanufacturing

Title: Critical Evaluation of Capillary Electrophoresis for the Determination of Enantiomeric Composition of Ephedra Alkaloids

Authors: K.W. Phinney, T. Ihara (National Metrology Institute of Japan, AIST), and L.C. Sander

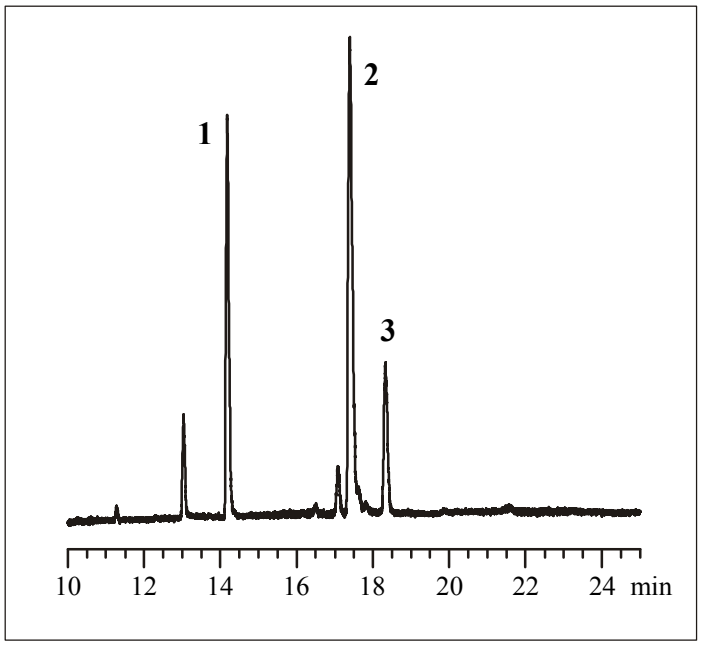
Abstract: The high efficiency and ease of method development in capillary electrophoresis (CE) make CE attractive for enantioselective separations. However, CE has not been widely used for the determination of enantiomeric purity because the sensitivity of the technique is viewed as being inferior to that of liquid chromatography (LC). In this work, three CE methods were developed for the separation of ephedrine and pseudoephedrine enantiomers found in plant material, plant extracts, and dietary supplements containing ephedra. Neutral cyclodextrins or mixtures of neutral and charged cyclodextrins were used as the chiral additive to achieve enantioselective separations. Sample preparation involved a simple methanol extraction, and a high sensitivity UV detection cell allowed detection and quantification of these analytes in a variety of complex matrices. The identity of the enantiomers was confirmed through a standard addition method. These methods have been applied to the certification of Standard Reference Materials (SRMs) containing ephedra.

Purpose: Dietary supplements containing ephedra have generated considerable interest because of their proposed ability to promote weight loss and increase athletic performance. However, ephedra has also been associated with adverse health effects. Ephedrine and pseudoephedrine are two of the major alkaloids found in ephedra, but these compounds are also found in traditional prescription and over-the-counter medications. Both compounds exist as pairs of enantiomers, but only (-)-ephedrine and (+)-pseudoephedrine are found in ephedra plants. Detection of (+)-ephedrine, (±)-ephedrine, or (-)-pseudoephedrine in a product containing ephedra would suggest that the content had been altered through the addition of synthetic alkaloids. Hence, the enantiomeric composition of these compounds is important in verifying the natural (plant) origin of the alkaloids.

Major Accomplishments: Three different CE methods were developed for the separation of ephedrine and pseudoephedrine enantiomers. Each of the three methods provides slightly different selectivity, thereby reducing the likelihood of undetected peak overlap. The use of a high sensitivity UV detection cell dramatically improved detection capabilities by increasing peak areas and signal-to-noise ratios. The methods were utilized for the certification analysis of five different SRMs containing ephedra (SRMs 3240-3244).

Impact: This work has demonstrated that CE can be used to determine the enantiomeric composition of chiral analytes in complex matrices. The resulting methodology provides new measurement capabilities that complement existing LC and LC/mass spectrometry methods. The analysis of ephedra-containing materials is important from a regulatory standpoint to confirm the natural origin of the ephedra alkaloids and to determine the levels of ephedrine and related alkaloids in dietary supplements.

Future Plans: The CE analysis of five ephedra SRMs has been completed. Successful demonstration of the improved sensitivity achieved with the high sensitivity UV detection cell will likely lead to additional applications of this approach.



Analysis of a solid oral dosage form of a dietary supplement. Peaks are: internal standard (1), (-)-ephedrine (2), and (+)-pseudoephedrine (3).

Pharmaceuticals and Biomanufacturing

Title: New Glass Filter Reference Materials for Validating the Performance of UV/visible Spectrophotometers in the High Absorbance Range

Authors: J.C. Travis, M.V. Smith, and G.W. Kramer

Abstract: SRM 2930 is a set of three neutral density glass filters and a blank, used to verify the accuracy of the transmittance/absorbance scales of molecular absorption spectrometers (spectrophotometers). The filters are individually certified at five visible wavelengths using the Reference Spectrophotometer in the Analytical Chemistry Division. The 0.1, 0.3 and 92 percent transmitting filters of SRM 2930 bracket and thus extends the range of transmittance coverage of SRM 930 (10, 20 and 30 percent transmitting) and SRM 1930 (1, 3, and 50 percent transmitting). The extension of absorbance coverage to three absorbance units permits a dynamic range of three orders of magnitude in chemical concentration, reducing the need to dilute highly absorbing samples for spectrophotometric analysis and avoiding needless handling and additional uncertainty. With the 92 % transmitting filter (plain glass) included in the new set, the total neutral density glass coverage of the three SRMs is now consistent with the transmittance calibration range supported by most NMIs who support transmittance scales.

Purpose: The nine neutral density glass filters of the new set of three coupled with the older SRMs provide coverage of absorbance consistent with chemical needs and with the measurement capabilities of modern instruments.

Major Accomplishments: The software of the ACD Reference Spectrophotometer (HAS-II) was extended to include a stepwise procedure to extend the range of qualification of the instrument to three absorbance units. The initial twelve sets of SRM 2930 have been assembled and initial stability, homogeneity, and uncertainty determination measurements have been made.

Impact: In appropriate instances, the new filters will permit pharmaceutical and chemical producers to validate and quantitate a product or constituent without an intermediate dilution step, subject to introducing needless bias and uncertainty. The existence of the new SRM also adds a traceability link for standards producers to provide a similar product and leverage NIST's measurement capability.

Future Plans: Completion of the certificate, aging of the filters, and final certification will be accomplished in FY 04, and the filters will be available for sale. Production of these filters will not be considered continuous, and renewal will be on a case-by-case basis. Secondary producers will be encouraged to use these filters to provide traceability. ACD will provide recertification services for a few years, after which all SRM absorbance filters may be recertified through the NIST Calibration service for continued traceability support.

Pharmaceuticals and Biomanufacturing

Title: Use of SRM Based Column Performance Tests to Guide LC Column Selection in the Pharmaceutical Industry.

Authors: L.C. Sander and S. Wise

Abstract: Although reversed-phase liquid chromatography (LC) is a mature analytical technique, analytical methods are often developed by trial and error, without full understanding of the fundamental mechanisms that make separations possible. Chromatographic columns provide the basis for chemical measurements in LC. Columns from different manufacturers or different manufacturing lots may exhibit a broad range of properties even though the columns are nominally similar (e.g., C_{18} columns). Such differences in retention behavior provide opportunities for method development and optimization; however, variability in LC column manufacture may invalidate existing methods and necessitate method redevelopment. Between-column reproducibility is an important aspect that must be considered for the implementation of methods for routine use. Changes that occur in column performance with usage can also directly affect system suitability. In 2002, the U.S Pharmacopeia announced the formation of a Working Group on HPLC Columns. This group was charged with "creating categories in order to subclassify the USP designation L1 for C_{18} (octadecylsilane) HPLC columns." Several classification schemes are currently under consideration in this effort, including approaches based on Standard Reference Material column performance tests.

Purpose: A clear need exists to distinguish among the hundreds of commercial LC columns that are available to the analyst. Details of column preparation and other descriptive information are usually considered proprietary by manufacturers; however, performance based tests provide a way of characterizing column properties without disclosing such trade secrets. The availability of unbiased tests for the assessment of LC column performance are required to facilitate: 1) the development and optimization of chromatographic methods by providing a rational basis for column selection, 2) evaluation of changes in column performance that occur over the life of the column; 3) evaluation of the suitability of columns for specific applications; and 4) characterization and classification of column properties to permit selection of similar columns among multiple manufacturers.

Major Accomplishments: Three Standard Reference Materials have been developed specifically to address the needs of column characterization. SRM 869a Column Selectivity Test Mixture, SRM 870 Column Performance Test Mixture, and SRM 877 Chiral Selectivity Test Mixture offer independent tests to assess column effectiveness for the separation of compounds based on differences in solute shape ("shape selectivity"), general aspects of column performance (efficiency, retentiveness, activity toward bases and metal chelators), and column effectiveness for the separation of various classes of optically active compounds ("chiral selectivity"). The U. S. Pharmacopeia is currently considering the usage of SRM 869a and SRM 870 to augment their L1 designation for C_{18} columns.

Impact: The availability of SRMs to support column characterization will greatly facilitate column selection in pharmaceutical and clinical applications. Utilization of these SRMs by

column manufacturers will result in the production of more reproducible LC columns, which in turn will improve the ruggedness of methods for routine use.

Future Plans: Although SRMs 869a, 870, and 877 provide considerable information about column performance, other tests may be required to characterize recently introduced columns with new bonding chemistries. Several classification schemes have been considered, and an approach by Lloyd Snyder and John Dolan (LC Resources) has considerable merit. This approach utilizes 10 carefully selected solutes to compare column selectivities. An algorithm has been developed to calculate a single numerical descriptor, which provides an indication of the relative similarity of two columns. On this basis, decisions can be made concerning column choice in method development by selection of a column that is similar to one used in an existing method, or alternatively, selection of a dissimilar column to one that failed to provide adequate separation. A large body of data already exists for commercial columns characterized by Snyder and Dolan (~ 150 columns to date). Future work at NIST may be directed towards the development of an SRM for LC column classification based on this Snyder/Dolan approach.