Analytical Chemistry Division -Table of Contents

Reference Methods and Standards for Food and Nutrition

CSTL Program: Healthcare Measurements
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Abstract: The Analytical Chemistry Division (ACD) is working in several areas within the broad category of "food and nutrition" to facilitate compliance with nutritional and dietary supplement labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices. The areas in which ACD is working include development of reference materials and analytical methods for nutrition labeling applications, dietary supplements including botanical materials, and genetically modified organisms. A number of food-matrix reference materials with values assigned for nutrient concentrations are now available across a wide spectrum of food compositions. NIST has begun to focus on more specific interests in the food area such as dietary supplements (particularly botanicals) and genetically modified foods to impact manufacturers and consumers of these products.

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

Major Accomplishments:

Nutrients

The composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed. AOAC (Association of Official Analytical Chemists) International has developed a nine-sectored triangle in which foods are positioned based on their fat, protein, and carbohydrate content. AOAC's belief is that one or two reference materials within each sector should be representative of other foods within that sector and could be used for quality assurance and method validation when analyzing those other foods. NIST has been working with other government agencies and the food industry over the past several years to provide an increased array of SRMs with values assigned for proximates (procedurally defined values for fat, protein, carbohydrate, etc.), fatty acids, cholesterol, vitamins, elements of nutritional interest, etc. Concentration values in the food-matrix SRMs are assigned based on a combination of data from NIST, from interlaboratory comparison exercises involving approximately 20 member laboratories of the National Food Processors Association's (NFPA's) Food Industry Analytical Chemists Subcommittee (FIACS), and from additional collaborating laboratories.

Value-assignment of two food-matrix SRMs was completed this year. Their positions, along with those of the other food-matrix reference materials available from NIST, are shown below. SRM 1946 Lake Superior Fish Tissue is a cryogenically homogenized fish fillet collected from adult lake trout (*Salvelinus namaycush*). This material has values assigned for nutrients including proximates (fat, protein, etc.) and individual fatty acids, including several omega-3 fatty acids. SRM 2384

Baking Chocolate has values assigned for proximates, individual fatty acids, total dietary fiber, several vitamins, and elements of nutritional interest.

Contaminants

NIST has SRMs available for cod liver oil (>90% lipid), mussel tissue (<1% lipid), and whale blubber (~70 % lipid) but has received a number of requests for a fish tissue (~10% lipid), particularly from programs that routinely monitor different species of fish, both for environmental concerns and nutritional value, as described above. Thus, SRM 1946 Lake Superior Fish Tissue was produced. Levels of organic contaminants in this material are indicative of urban contamination levels and will be beneficial to both the environmental and food/nutrition measurement communities. Certified values will be available for 30 polychlorinated biphenyl (PCB) congeners, including 3 non-ortho congeners, 13 chlorinated pesticides, methyl mercury, and total mercury. This is the first SRM available with certified concentrations for non-ortho PCB congeners that are considered the most toxic, similar in structure to polychlorinated dibenzo-*p*-dioxins.

Nutraceuticals

The U.S. 1998 market for nutraceuticals (i.e., herbal supplements and functional foods) was estimated at nearly $26 billion; botanicals accounted for about $3.5 billion of this market, and a continued growth rate of 10% to 20% was expected. However, in 1998 sales of botanical products began to level off, and by the end of 1999, overall sales had dropped 3% compared to 1998. Part of this decrease in sales may be attributed to a loss in consumer confidence in botanical products as a result of negative publicity. Reports of inaccurate labeling, adulteration, contamination (pesticides, heavy metals, or toxic botanicals), and drug interactions have all contributed to the decline in sales. Taxonomically authentic botanical reference materials with assigned values for active and/or marker compounds, as well as for contaminants such as pesticides, mycotoxins, and heavy metals, are necessary for verification of manufacturers' label claims and quality control during the manufacturing process.

NIST representatives have been working with the AOAC's Dietary Supplement Task Group to identify nutraceuticals for which validated analytical methods are needed. It is useful to have a reference material when validating an analytical method. FDA representatives also providing funding to this task group, therefore the supplements identified as those for which methods are needed are likely to be the same as those for which reference materials are needed.

The Analytical Chemistry Division has developed a collaborative program with the Food and Drug Administration's (FDA's) Center for Food Safety and the National Institutes of Health's (NIH's) Office of Dietary Supplements to produce a number of authentic, well-characterized reference materials of botanical raw materials to support the needs of the dietary supplement community. ACD, FDA, and NIH plan to collaborate to prepare six to eight selected botanical-matrix SRMs from authentic raw materials during the next several years. NIH has provided funding and FDA has committed to provide funding starting in FY02. FDA, with input from other agencies and organizations, will consult with NIST to identify the materials of high priority.

Genetically modified organisms

Crops have been genetically modified to resist pests and disease, to tolerate chemical herbicides, to resist poor soil and weather conditions, or to possess improved nutritional content, food processing characteristics, ripening, texture, or flavor. Genetically modified seeds accounted for $1.5 billion in sales in 1998, and genetically modified seeds were used to plant more than 70 million acres around the world (excluding China) – 50 million of these in the U.S. Agronomists have been breeding plants for centuries, and the FDA recognizes no difference between this and current practices. No labeling of genetically modified products is required in the U.S. unless the resultant product's nutritional content or allergenicity differs from the "normal" product; FDA is currently developing *voluntary* guidelines for labeling. However, labeling is currently mandated in the European Union, Japan, Australia, and New Zealand, and labeling regulations in South America are pending. The GMO issue is becoming a significant barrier to trade. Market disruptions in the EU have impacted U.S. corporations attempting to do business there.

Currently, the USDA's Grain Inspection, Packers, and Stockyards Administration (GIPSA) is establishing a reference laboratory that will evaluate and accredit PCR laboratories that test for GMOs in grains and oilseeds. These laboratories provide qualitative analyses. GIPSA is also evaluating the test kits that are available to determine the presence or absence of GMOs at certain levels. These test kits include ELISA (enzyme-linked immunosorbent assay)-based kits, which may be quantitative, as well as lateral flow strips, which provide a yes/no answer, much as a home-pregnancy test kit does. These test kits provide rapid results, but cannot adequately sample entire truckloads of grain. NIR spectroscometers can be modified to examine as many as 1000 kernels or beans per test, and would be more appropriate for truckside testing.

GIPSA has been working with the Institute for Reference Materials and Measurements (IRMM) for production of GMO reference materials. Reference materials for many of the GMOs in GIPSA's proficiency testing program are now available from IRMM, and IRMM is currently constructing clean facilities for production of additional materials. Representatives from IRMM appear to be willing to collaborate with NIST in the production and value-assignment of additional materials to ensure that results worldwide are traceable to just one set of materials and to avoid further complicating an already complicated issue. Production of a series of GMO reference materials in conjunction with IRMM would allow GMO measurements in the US and Europe to be traceable to a single standard recognized by the governments of all parties, and could facilitate international trade by demonstrating the absence of GMO grains at the regulated threshold levels.

ACD has organized a workshop to be held at the Interamerican Metrology System's General Assembly Meeting to discuss the legislation that is currently in place within the Americas and in other geographic regions, the analytical methods that are currently available to address the legislation, and whether there is a gap between the two that needs to be addressed.

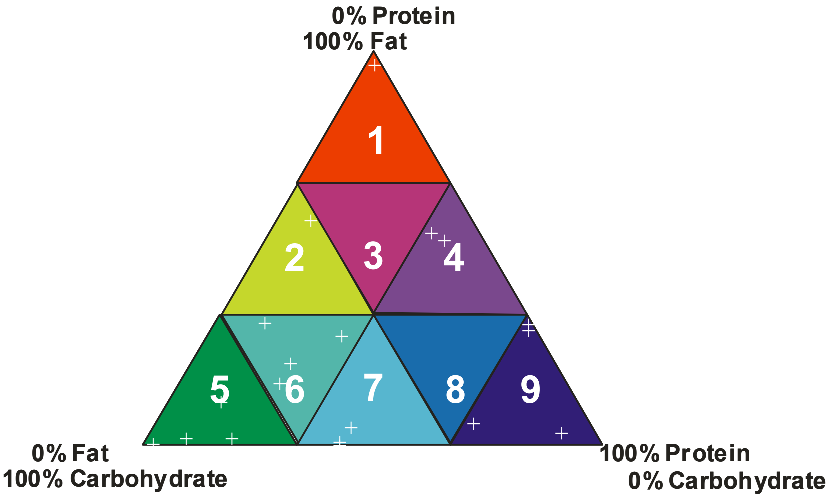
Impact: A number of food-matrix reference materials with values assigned for nutrient concentrations are now available across a wide spectrum of food compositions. These materials will ideally improve the accuracy of nutrition information provided on product labels. NIST has begun to focus on more specific interests in the food area such as dietary supplements (particularly botanicals) and genetically modified foods to impact both manufacturers and consumers of these products.

Future Plans: Two food-matrix SRMs are currently in production: SRM 2385 Spinach and 2387 Peanut Butter. Values are expected for nutrients, carotenoids, and pesticides in the spinach. Values are expected for nutrients, allergens, aflatoxins, and amino acids in the peanut butter. Following the release of these two materials, nutrition information will be available for at least one reference material in each sector of the AOAC triangle. This particular aspect of the food/nutrition component of NIST's programs can be expected to decrease. Focus will then shift to more specific analytes such as individual amino acids, allergens, and mycotoxins in foods, as well as botanical materials.

Many of the materials in the suite of food-matrix SRMs were developed as a result of needs assessments workshops in which participants from the food industry and regulatory agencies were asked to identify priority reference materials. These workshops, held in 1997 and 1999, led to the development of SRM 2384 Baking Chocolate, SRM 2385 Spinach, and SRM 2387 Peanut Butter; the forth-coming addition of amino acid information to SRM 1546 Meat Homogenate; and the inclusion of allergen and aflatoxin information with SRM 2387. Many members of the food industry are involved in the production of "functional foods," a category of nutraceuticals, and NIST's involvement with dietary supplements has also been identified as a high priority, and work in this area is ramping up with the support of FDA and NIH.

Food Triangle Sector	SRM Coverage of Food Triangle
1	SRM 1563 Cholesterol and Fat-Soluble Vitamins in Coconut Oil
2	SRM 2384 Baking Chocolate
3	SRM 2387 Peanut Butter
4	SRM 1546 Meat Homogenate *(recently completed)*
5	SRM 2383 Baby Food Composite *(recently completed)*
6	SRM 1846 Infant Formula *(recently completed)* SRM 1548a Total Diet *(recently completed)* SRM 1544 Fatty Acids and Cholesterol in a Frozen Diet Composite
7	SRM 1566b Oyster Tissue *(recently completed)* SRM 1570a Trace Elements in Spinach Leaves SRM 2385 Spinach
Junction of 8, 9	SRM 1974a = Mussel Tissue
Junction of 4,8,9	SRM 1946 Lake Superior Fish Tissue SRM 1947 Lake Michigan Fish Tissue

Fig. 1. Populated AOAC food triangle with food matrices chosen by stakeholder



Methods and Standards for Commodities Characterization

CSTL Program: Chemical Characterization of Materials
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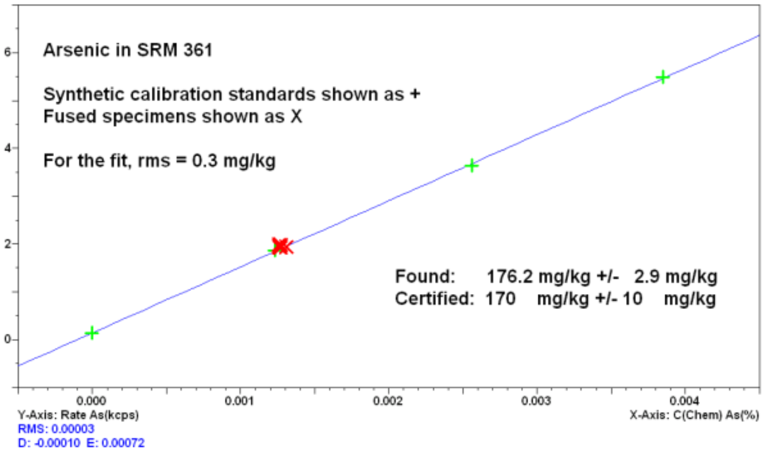
Abstract: The industrial engine of the United States runs on the production and commerce of many very basic commodities, including fossil fuels, steel (and other metal alloys), and cement. In fact, these three commodities represent the top three categories of Standard Reference Materials certified for chemical composition by NIST. The value and function of these commodities are strongly related to their chemical composition and chemical analysis is the basis of their trade. Since they are produced and traded in great volume, these chemical analyses tend to be done by high throughput, inexpensive instrumentation that rely on high accuracy standards for their calibration and validation. The importance of standards and standard methods for these materials is reflected in the prominence of the American Society for Testing and Materials (ASTM) committees for these industrial sectors. NIST works closely with ASTM, as well as other industrial groups to help define the highest priority measurement and standard needs for these basic commodity sectors. For instance, ASTM Committees C01 Cements, D02 Petroleum Products and Lubricants, D05 Coal and Coke, and E01 Analytical Chemistry for Metals, Ores, and Related Products, and others work with NIST for new standards development.

CSTL has developed new methods and standards to respond to these industries' needs. These include new hydrogen, sulfur, phosphorus, and nitrogen in metals standards. A new matrix independent method X-ray Fluorescence method has been developed that has been used for metals and cements, as well as a definitive method for Hg in Coals. Titanium dioxide has been recertified using an absolute coulometric method.

Purpose: To develop and apply methods and develop standards that underpin accurate and traceable commodity analysis in the United States.

Major Accomplishments:

Metals:
1) <u>Matrix-independent X-ray fluorescence (XRF):</u> This method provides direct traceability to primary reference materials in combination with the speed and repeatability of XRF. The complete matrix-independent concept was implemented for low alloy steel as a demonstration case. There are several parts to the method: 1) sample preparation by acid reaction and borate fusion; 2) demonstration of performance for repeatability, sensitivity and detection limits; and 3) calibration using primary reference materials. Chip form, low alloy steel SRMs 361 – 365 were fused after reaction with HNO_3 and H_2O_2. Relative standard deviations of replicate fusions



were less than 1 %. The same alloys are available in disk form as SRMs 1261 – 1265. Calibrations for 20 elements were created using a straight-line model for both fused and disk forms. Borate fusion does not sacrifice sensitivity equal to the dilution factor because bulk Fe absorbs X-rays about three times more strongly than Li borate beads. Fusion diluted the chip form SRMs by a factor of 13.4, but sensitivity loss was only a factor of 4, at worst, and detection limits (original sample basis) were higher by a factor of only 3, at worst. Calibration by synthetic, bracket standards (shown) was used to analyze SRM 361 and resulted in excellent performance even for trace arsenic (plot), normally considered a volatile element.

This method provides direct traceability to primary reference materials for a segment of the metals industry that has few certified reference materials. In general, XRF is no longer limited to disk form specimens, which is especially important for alloys that do not form homogeneous solids. In fact, the superior speed and repeatability of XRF can be used to greater advantage for metals, alloys, ores and related materials with fewer certified reference materials.

2) Certification of SRM 2453 Hydrogen in Titanium. SRM 2453, the first of a projected series of three Standard Reference Materials of trace hydrogen in titanium alloy has been completed. The certified hydrogen concentration will be 114 mg/kg, with an expanded uncertainty of 5 mg/kg. This Standard Reference Material (SRM) is intended for use in the evaluation of methods and the calibration of equipment used in the determination of hydrogen in titanium alloy. Plans for the FY02-03 call for the production of two additional materials at nominal concentrations of 50 and 200 mg/kg.

This SRM was prepared and analyzed at NIST. Sheets of Ti90/Al6/V4 alloy were cleaned, weighed, and degassed at temperatures up to 800°C in a high-vacuum furnace system. The degassed material was sampled, reweighed, and returned to vacuum. A measured quantity of hydrogen was added to the system from a calibrated volume, and hydriding accomplished at elevated temperature. The samples were analyzed by cold-neutron prompt-gamma activation analysis (PGAA) and neutron incoherent scattering (NIS). The batch was chipped to a particle size of approximately 15 mg to improve homogeneity. The chipped material was cleaned by extraction with methanol, dried, blended, and bottled. Cold-neutron prompt-gamma activation analysis (PGAA) was used to determine the hydrogen concentration. The certified value is the sum of the residual concentration in the blank material (measured by PGAA and NIS) and the quantity of hydrogen added as determined by volumetric measurements.

3) Low-Level Phosphorus in Metals: A radiochemical neutron activation analysis (RNAA) procedure has been developed to measure low-level phosphorus in metals. Samples are first irradiated at a neutron flux of 3×10^{13} $cm^{-2}s^{-1}$. During sample irradiation, ^{31}P undergoes neutron capture to form ^{32}P, a pure beta emitter with a half life of 14.28 days. After irradiation, samples are mixed with inactive phosphorus carrier, then dissolved in a mixture of acids. Phosphorus is separated from the matrix and purified of other radionuclides by cation exchange, followed by precipitation of phosphorus as ammonium phosphomolybdate, then as magnesium ammonium phosphate. The yield (fraction of recovered carrier) is determined gravimetrically as $MgNH_4PO_4.6H_2O$. ^{32}P is measured using a beta proportional counter. Comparison of sample count rates and carrier yields with those of standards processed in a similar manner allows calculation of P mass fractions. The detection limit for a 0.1 g sample irradiated for 30 minutes is 5 µg/kg. The procedure was originally developed for and critically evaluated through analysis of low alloy steels with P mass fractions ranging from 100 to 400 mg/kg. A slightly modified version of the original procedure was used to value assign phosphorus in two low phosphorus metal alloy Standard

Reference Materials. SRM 2175 MP35N Refractory Alloy had a measured phosphorus mass fraction of 6.40 mg/kg ± 0.56 mg/kg, and SRM 861 Nickel-based Superalloy with phosphorus measured at 12.71 mg/kg ± 0.71 mg/kg.

4) <u>Sulfur in SRM 861 Nickel-based Superalloy.</u> The U.S. aerospace industry is a leading technology driver in R&D spending and the nation's leading net exporter of manufactured products. The aircraft industry and supporting foundries and their Consortium on Casting of Aerospace Alloys brought to NIST the need of the industry to produce turbine blades with sulfur concentrations near 1 mg/kg, a critical level between acceptable and unacceptable materials. The ACD has investigated and applied its primary isotope dilution mass spectrometric method to the low concentration required, and certified sulfur at 0.561 mg/kg ± 0.078 mg/kg, almost an order of magnitude lower than any previous sulfur in metal standard. The limiting source of uncertainty in this measurement was the blank.

5) <u>Nitrogen in SRM 1755 Nitrogen in Low Ally Steel:</u> Nitrogen is an important element that is monitored in the steel manufacturing process as it contributes to the hardness of the resulting steel. A high nitrogen content makes steel unsuitable for many applications. Nitrogen is typically determined in industry by matrix-dependent techniques that require calibrating standards. To produce standards requires an absolute technique for nitrogen. ACD applied the classical Kjeldahl method to the determination of nitrogen in this standard, and certified the nitrogen amount content by combining the results using this method with independent results from cooperating laboratories—vacuum gas fusion with volume measurement and combustion with infrared detection. The availability of the SRM will allow steel manufacturers to calibrate their optical emission spectrometers (OES) on the production floor and monitor the nitrogen levels during the manufacturing process. Nitrogen is certified at 118.4 mg/kg ± 1.8 mg/kg.

Metal SRMs Certified in FY 2001:

SRMs 1775/2175	MP35N Refractory Alloy	10/10/2000
SRM 1755	Nitrogen in Low Alloy Steel	12/8/2000
SRM 861	Nickel-based Superalloy	3/28/2001
SRM 131g	Low Alloy Silicon Steel	5/20/2001
SRMs 361/661	AISI 4340 Steel	5/29/2001(revision)
SRMs 363/663	Chromium Vanadium Steel	5/29/2001(revision)

Cements:

The cement industry is a significant component of the U.S. economy, with annual shipments in excess of $4 billion. This industry is regional in nature. Because of the cost of shipping cement quickly overtakes its value, customers traditionally purchase cement from local sources. In the U.S. 50 companies operate 120 cement plants in 38 states. The vast majority of cement produced in the United States is shipped less than 500 kilometers. The dispersed nature of the cement industry, coupled with the vast quantities used in commerce, results in extremely large numbers of cement analyses made by analytical service laboratories. The cement industry uses X-ray Fluorescence (XRF), a rapid and cost effective method of analysis. XRF is effective as long as there are primary reference materials for calibration. NIST cement SRMs are the primary reference materials used throughout the U.S. and the world, and are the basis of all of the standard methods used by U.S. industry.

CSTL initiated a plan in 1998 to address the recertification and perpetuation of cement SRMs. This plan includes certification measurements made at NIST by XRF, but uses the Portland Cement

Association's premier laboratory to perform the bulk of the certification work. The matrix independent XRF technique as described above for metals has been successfully applied in the NIST certification effort.

Cement SRMs Certified in FY 2001:

SRM 1884a	Portland Cement	7/13/2001
SRM 1885a	Portland Cement	7/13/2001
SRM 1887a	Portland Cement	8/9/2001

Fossil Fuels:

1) Certification of Mercury in SRM 1632c Trace Elements in Coal: NIST has had to respond quickly to the U.S. Environmental Protection Agency's very rapid move to regulate the emission of mercury by coal-fired electric power plants. On December 14, 2000, EPA announced its intention to regulate utility mercury emissions and force reductions by coal-fired power plants.

Obviously, accurate and reliable coal analysis results are required to make meaningful estimates of mercury emissions. The Electric Power Research Institute, questioning the adequacy of the test methods that would be used by the numerous commercial and utility laboratories responding to the need to measure Hg emissions, initiated a round robin study to evaluate the adequacy of the test methods. The objective of the study was to compare the performance of the various test methods against recognized benchmarks of laboratory performance. This study was done in cooperation and collaboration with the ASTM D5 committee. Several new test methods developed by ASTM were also evaluated. The lack of coal SRMs certified for mercury content was of immediate concern to the D5 committee, EPRI, and the utilities at the start of this time period.

Two method development research efforts were pursued in parallel in ACD to improve the accuracy for mercury measurement. A new method based on isotope dilution, cold vapor, inductively coupled plasma mass spectrometry (ID-CV-ICP-MS) and an updated method based on radiochemical neutron activation analysis (RNAA) minimized previous sources of measurement uncertainty. Their analyses of SRM 1630a resulted in a five-fold reduction in the uncertainty and the certification of Hg content (93.8 ng/g ± 3.7 ng/g). The certification of SRM 1630a was timely, as it allowed the evaluation of bias of methods used in the EPRI round robin study. Neither of the two main ASTM methods, Bomb Combustion CVAA and Acid Extraction CVAA showed significant bias, although the interlaboratory variability was very high (30 % and 20 %, respectively).

2) Development and Application of CV-ICP-MS for Mercury Certification: The CV-ICP-MS method is based on isotope dilution mass spectrometry (IDMS), combined with a sample pretreatment step and a matrix separation step to form a unique measurement process. There are two parts to the method, both of which are necessary for accurate results:

- The sample is combusted at high temperature in a closed system (Carius tube), which ensures equilibration of the natural Hg atoms with those in the enriched spike. The sample is placed in the Carius tube with a known amount of a ^{201}Hg enriched isotope, and high-purity nitric acid. The tube is flame-sealed, and heated to 240°C in a laboratory oven for approximately 6 hours. This is about 100 degrees higher than conventional microwave digestion systems. This process completely oxidizes the coal to CO_2, and equilibrates the enriched ^{201}Hg atoms with the Hg atoms in the sample. At this point the $^{201}Hg/^{202}Hg$ ratio is fixed and needs only to be measured accurately to determine the mercury in the original sample. Subsequent loss of sample does not affect the final accuracy. After combustion, the Carius tube is opened and the sample simply diluted with high-purity distilled water.

- Accurate measurement is made of the perturbed ratio using an ICP-MS instrument. The sample is mixed with tin (II) chloride, which reduces the oxidized mercury to volatile mercury vapor. The Hg is separated from the matrix using a cold-vapor matrix separation cell, and transferred to the ICP-MS instrument using argon carrier gas. The $^{201}Hg/^{202}Hg$ ratio is measured using conventional ion counting techniques.

The method is now considered by NIST to be a primary method and has been applied to the certification of mercury in the existing suite of coal SRMs. This suite represents a number of types of coal (chosen principally for the range of sulfur content). The mercury content certified is this suite is from 11 to 146 ng.

Coal SRMs certified for Mercury in FY2001

SRM 1632c	Trace Elements in Coal	1/11/01
SRM 1635	Trace Elements in Coal	1/4/01
SRM 2682b	Sulfur and Mercury in Coal	2/6/01
SRM 2683b	Sulfur and Mercury in Coal	1/23/01
SRM 2684b	Sulfur and Mercury in Coal	2/6/01
SRM 2685b	Sulfur and Mercury in Coal	2/6/01
SRM 2692b	Sulfur and Mercury in Coal	2/6/01

3) Determination of Sulfur in Diesel Fuels and Gasoline: The primary method for sulfur determination is based on isotope dilution thermal ionization mass spectrometry (ID-TIMS) and has been used to certify over 50 SRMs since its development in 1984. The fossil fuel sulfur SRMs are in extremely high demand because sulfur content affects their quality and price. In addition, new regulatory requirements for lower sulfur emissions have resulted in the demand and development for new materials. The analytical work in FY 2001 reflects the need to meet these new regulatory requirements. SRM 1620c Sulfur in Residual Fuel Oil 4 % was certified to provide a high-level sulfur standard for the electric power industry. SRMs 2298 and 2299 Sulfur in Gasolines were analyzed to benchmark new low-level S in gas regulations. And, BCR 104R, BCR 671, and BCR 672 were three new reference materials (CRMs) of the European Union’s Community Bureau of Reference (BCR). NIST collaborated with the Institute for Reference Materials and Measurements (IRMM) in the certification process of these CRMs. This collaborative work between National Measurement Institutes reflects the need to provide comparability and traceability for measurements and standards on a global basis.

New EPA regulations go into effect in 2007 that will limit sulfur in gasoline to 30 mg/kg (ppm). SRMs 2298 and 2299 are new low-level sulfur in gasolines that anticipate these regulations and will allow the development of new methods and instrumentation to make measurements at this level. With indicated concentrations at 4.5 and 13.6 mg/kg, respectively, these measurements were especially challenging—sample sizes had to be minimized to achieve controlled digestion and blanks at the ppm level are significant. Nonetheless, these standards should meet the more stringent analytical requirements. The development of homogeneous materials for sulfur at such low levels and reducing sulfur contamination in the measurement process are challenges to be faced in the future.

The BCR CRM "gasoils" are heating oil and two diesel fuels; they have indicated sulfur levels between 0.02 % to 0.10 %. These standards anticipate the EEC regulations that limit the maximum sulfur in gasoil to 0.10 % by the year 2008. Former BCR gasoil standards were certified by interlaboratory exercises. However, the IRMM proposed to certify these standards by methods more traceable to the SI and, consequently, they developed the NIST ID-TIMS procedure. (The one difference, however, was the use of closed microwave dissolution instead of NIST's Carius tube dissolution practice). Since this method was new and unproven at IRMM, NIST collaborated in the certification process, which is scheduled to be completed in Spring 2002. Two of these standards were also distributed in a Pilot Study of CCQM (P-26 Sulfur in Fuel).

Fossil Fuel SRMs Certified for Sulfur in FY 2001

SRM1620c	Sulfur in Residual Fuel Oil 4 %	3/22/01

Titanium Dioxide:

Assay of Titanium Dioxide by Coulometric Back-Titration**:** Titanium dioxide is a commodity that is basic to the paint and paper industries, as well as a major component in many ceramic materials. SRM 154 Titanium Dioxide is the benchmark for purity measurement and an accurate assay value is essential for product quality control in these industrial sectors. In the re-certification of SRM 154c Titanium Dioxide, the target uncertainty for the certified value was less than 0.1%. A coulometric procedure of primary nature was developed and applied; the total uncertainty achieved was 0.062 %.

Titanium dioxide assay techniques rely on the oxidation-reduction chemistry of titanium (Ti). In titanium dioxide, Ti is present as 4-valent Ti(IV). Following dissolution, this Ti(IV) can be reduced in a Jones reductor to 3-valent Ti(III). To obtain the assay value, this Ti(III) must be quantitatively reoxidized to Ti(IV) adding excess oxidizing agent which itself has an accurately-known assay value. It is also necessary to establish that the reduction of the Ti(IV) to Ti(III) is quantitative. In addition, it is necessary to take into account any impurities in the titanium dioxide that react with the oxidizing agent in a similar manner to Ti(III) and to make corrections for these systematic sources of bias. The final certified uncertainty must include the contributions from all component uncertainties arising from each of these possible sources.

The analytical approach used a dissolution procedure adopted from state-of-the-art industrial practice, followed by reduction of Ti(IV) in the resulting solution to Ti(III) by a Jones reductor. The reduced Ti was then reoxidized to Ti(IV) by addition of an accurately known amount of the solid potassium dichromate ($K_2Cr_2O_7$), NIST SRM 136e, whose assay was determined by coulometry at NIST. The oxidizing agent was added in solid form to avoid any uncertainty from solution handling and evaporation. The amount of potassium dichromate added was greater than that required to oxidize the Ti(III) present and, hence, excess potassium dichromate remained after all the Ti was reoxidized back to Ti(IV). The excess potassium dichromate remaining in the solution was then determined by a separate coulometric titration identical to the assay procedure for the SRM 136e. The difference corresponded to the Ti(IV) present.

The coulometry used for both these titrations is an absolute method (i.e. one not requiring calibration with a chemical standard). Hence, the final assay value for SRM 154c is not dependent on another chemical standard, but rather it is based on fundamental laws (Faraday's Laws of Electrolysis) and a fundamental constant (the Faraday constant). Efficiency of the Jones reductor was assessed by analysis of Ti metal of known purity. The reduction of the dissolved Ti solution and the subsequent titrations were procedurally identical to the corresponding titanium dioxide assays.

A glow-discharge mass spectrometric analysis of the new SRM 154c was performed to account for possible interfering elements. This analysis yielded the trace contents of virtually all elements in the periodic table. Corrections were made to account for the known oxidation-reduction chemistry of those elements that interfere with the titanium dioxide assay. The final certified value for SRM 154c is 99.591 % with an uncertainty of 0.062 %. This uncertainty includes the component uncertainties from each of the sources discussed above and, in contrast to the previous certification, the present result conforms to the revised NIST policy on the treatment of uncertainty.

Titanium Dioxide SRM certified in FY 2001

SRM 154c	Titanium Dioxide	6/1/01

Impact: In February 2000, the Economic Impact of SRMs for Sulfur in Fossil Fuels was published that quantified a portion of the economic benefits associated with sulfur SRMs, which were tremendous. The conclusions of this study can be generalized to other commodities as well. The development of methods and certification of SRMs result from NIST's unique expertise in measurement. SRMs play a key role in the National Measurement System for analytical chemistry. They serve as national primary standards and are used as calibrants and as quality assurance materials to evaluate measurement accuracy, to intercalibrate laboratories in measurement programs, and to provide compatibility of measurement data. NIST SRMs greatly improve measurement accuracy by reducing the uncertainty of measurement. These improvements enhance the products and services of the measurement industry, such as testing laboratories and analytical instrument manufacturers. More accurate elemental content information also reduces the likelihood of disputes between buyers and sellers.

Future Plans: The ACD will continue the difficult task of balancing the need to develop and benchmark new measurement capabilities, while at the same time maintaining core capabilities. The ACD will continue to address industry's need to have the best calibration materials so that they can make fast, inexpensive, and accurate measurements. We will address this need not only by the development of new reference materials, but also by the development of new instrumental matrix-independent and high performance methods that should reduce the dependence upon these matrix-matched reference materials.

Methods and Standards for Advanced Materials Characterization

CSTL Program: Chemical Characterization of Materials
Authors: *G.P. Lamaze, H. H. Chen-Mayer, R.L. Paul, and M. Salit*

Abstract: The Analytical Chemistry Division maintains an active program of advanced materials characterization through the application of a wide variety of measurement techniques. These techniques are used to determine elemental concentrations in SRMs for producers of advanced materials, as well as standards for calibration of surface and other analytical techniques. This year we have developed a radiochemical procedure to determine the amount of ion-implanted phosphorus in a silicon wafer by neutron activation analysis. This method will be used in conjunction with Division 837 to certify an SRM to meet a high-priority request of the semiconductor industry. We have also been working with EEEL and Division 837 to characterize sub-samples of epitaxial thin-film prepared AlGaAs. The remaining portions of which would then be used as primary standards to calibrate non-destructive probe techniques, ultimately in the preparation of a new suite of SRMs for Al:Ga ratios in AlGaAs. The Al:Ga substitution ratio is critical for the optoelectrical properties of devices fabricated in thin films of AlGaAs. Non-destructive probe techniques, such as x-ray fluorescence, while precise, have been demonstrated to show a bias in these ratios.

In addition, ACD maintains and operates two cold neutron beam facilities and have access to a large number of other neutron techniques through our collaborations with the Center for Neutron Research. Customers for these services include industry, universities and other government organizations. Proprietary research is on a cost recovery basis. During the past year, we have made a number of measurements that have helped solve problems in the areas of semiconductor technology and thin film science. One example of semiconductor research is a study of nitrogen content in GaN/GaAs bilayers with Corning. The material system GaN/AlGaN holds promise for the production of blue light emitting lasers. The N concentration is a crucial parameter for establishing the device characteristics. SIMS can measure the N profile of the grown GaAsN layers if provided with a calibrated GaAs/GaN standard. Corning scientists have come to us for quantitative nitrogen determination in a GaN/GaAs system and to establish an in-house calibration capability. Another example is the correlation of manufacturing conditions with the lithium distribution of lithium phospho-oxynitride layers used in lithium batteries. This is being done in conjunction with Tufts University as part of a long-term research program in battery materials.

Purpose: The purpose of our efforts is to transfer NIST accuracy and to industry, academia and other government agencies. In addition, we provide our measurement capabilities directly to industry to solve important measurement problems when the required capabilities do not exist outside of NIST. The cold neutron instruments at NIST are operated as part of a national user facility. These instruments have been established with the intent of furnishing the US scientific community (industry, academia, and government) with state of the art facilities for research with neutrons. Our purpose is to maintain the facilities, make measurements and perform data analysis in order to improve measurement capabilities. The two facilities operated by the ACD (NDP and PGAA) cover a wide range of applications with semiconductor materials making up a large part of the materials analyzed. The neutron depth profiling instrument, by its nature, makes measurements only on thin films and surfaces (up to about 10 μm), while the PGAA instrument measures elemental concentrations in bulk samples.

Major Accomplishments:

- Developed a radiochemical activation analysis procedure to certify phosphorus in implanted silicon. See Figure 1.
- Developed a digestion procedure for epitaxial thin-film prepared AlGaAs, and established that the analytical scheme would have sufficient signal-to-noise ratio to perform the requested measurements with uncertainties in the AL:Ga ratio of less than 1% relative
- Studied of GaN/GaAs bilayers (Corning). See Figure 2.
- Evaluated lithium depletion in $LiNbO_3$ (Corning).
- Determined lithium in thin films for battery applications (Tufts University). See Figure 3.
- Studied neutron induced soft upsets in semiconductor devices (Texas Instruments)
- Evaluated boron mobility in ion implanted boron in Tungsten (Aberdeen Proving Grounds and USMA).
- Characterized thin films using Neutron Depth Profiling and Neutron Reflectometry (with Center for Neutron Research). See Figure 4.
- Assessed long term stability of primary boron and lithium thin film standards (with Physics Lab).

Impact: Our measurement capabilities are unique in the US and are not available as in-house capabilities for individual industries. We provide a series of tools to allow industrial researchers to calibrate, understand, and evaluate production processes. Because our work is both quantitative and of high accuracy, our customers use measurements made at NIST to calibrate their own instruments. This is especially true of NDP measurements that are used to calibrate SIMS instruments.

Future Plans: The emphasis on semiconductor materials and thin films continues. The new cold source now being installed at the NIST reactor will approximately double the effective neutron flux. In the near future (FY04?), both the NDP and PGAA instruments will be moved to a new curved neutron guide that will greatly improve the signal-to-noise of both instruments. We continue to invest in improvements to both instruments so future customers will have the best available resources for their experiments. We will also perform basic research, such as stopping power studies of charged particles to provide more accurate parameters for NDP analysis.

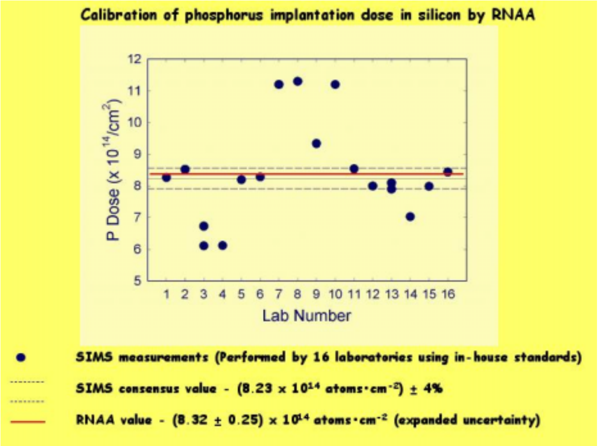


Figure 1. A new phosphorus implant in silicon SRM, certified by radiochemical neutron activation analysis (RNAA), is in development. An RNAA procedure has been developed and tested through analysis of 12 samples of silicon implanted at a nominal dose of 8.5 x 10^{14} atoms cm^{-2}. A mean and standard deviation of (8.32 ± 0.11) x 10^{14} atoms cm^{-2} (relative standard deviation = 1.3 %) was obtained, with an expanded uncertainty of about 3 %. A comparison with SIMS measurements is shown.

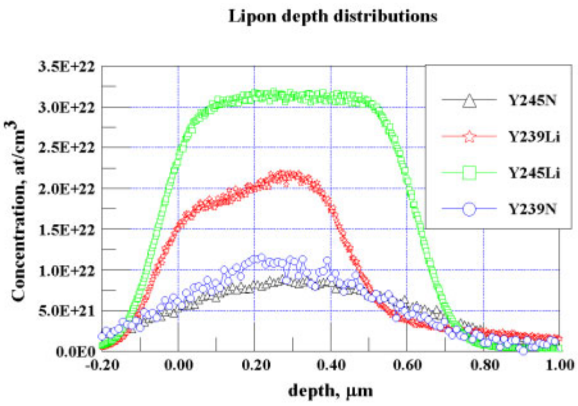


Figure 3. Depth profiles of lithium and nitrogen for two different lithium phospho-oxynitride samples. Results show that sample Y245 has the more desirable lithium to nitrogen ratio.

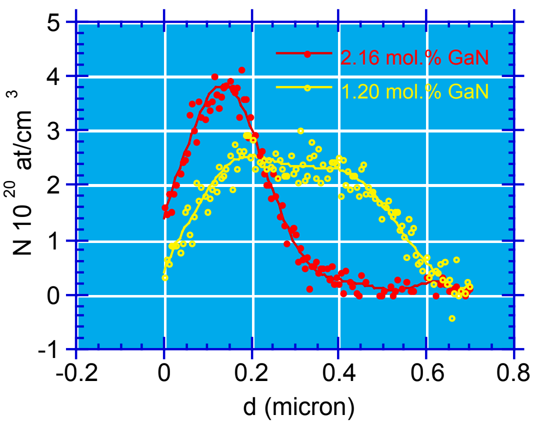


Figure 2. Distribution of N in the GaAs matrix. Both samples were about 10% thicker than expected giving important feedback to the manufacturing process.

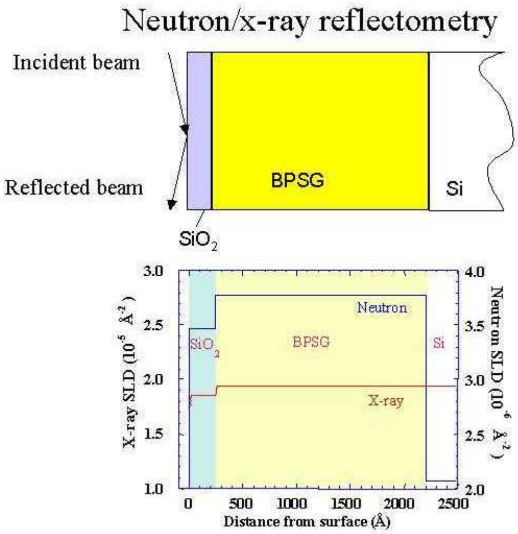


Figure 4. Schematic showing experimental reflectometry arrangement and determined density variations. When combined with NDP spectra, depth scaled is determined unambiguously.

The Investigation of Green Chemical Cleaning Processes

CSTL Program: Chemical Characterization of Materials
Authors: *L.L. Yu; and C.S. Grant (North Carolina State University)*

Abstract: The use of calcium ion sequestrants provides an environmentally benign alternative to strong acids for the cleaning of mineral deposits, typically calcium phosphate, calcium oxalate and calcium carbonate, on stainless steel surfaces, which is the current industrial practice. Basic research to characterize the mechanism and kinetics of this green chemical cleaning process has been initiated at North Carolina State University (NCSU). The focus of the current work is on the use of sodium polyaspartate (pAsp), because it is non-toxic and biodegradable in the range of molecular weights (~10,000) considered. The research requires the identification and quantification of calcium and its species on-line under various mechanical cleaning conditions; however, the measurement of these species is a major challenge. NIST and NCSU have collaborated in the development of techniques for calcium species quantification. A tandem technique has been developed that incorporates size-exclusion chromatographic (SEC) separation of the species, spectrophotometric (UV/VIS) determination of pAsp, and inductively coupled plasma optical emission spectrometric (ICPOES) determination of each calcium species. Free calcium ions and calcium-pAsp complex species have been determined. The next stage in this collaboration is the transfer of the technique to NCSU to study the mechanism and kinetics of the cleaning process during various test conditions.

Purpose: The purpose of this research collaboration is to apply the unique chemical measurement technology and expertise that resides at NIST to a problem with both major industrial and environmental consequences. This green chemical cleaning process addresses the industrial problem of scale formation, which can result in the serious loss of heat exchange efficiency and the obstruction of fluid flow. It is an especially important problem for the chemical industry.

Major Accomplishment: We have studied the properties of calcium and its pAsp complex and have developed SEC-UV/VIS-ICPOES tandem technique for the on-line measurement of calcium and its pAsp species.

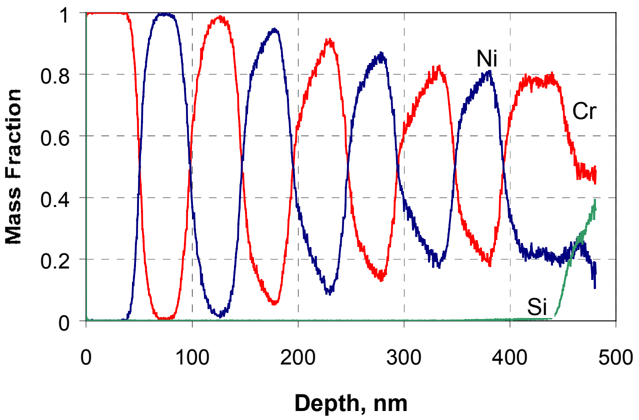
Impact: The development of environmentally friendly cleaning processes will save industry money while at the same time reducing the need for chemical disposal/treatment.

Future Plans: The on-line measurement technique is being implemented at NCSU for on-line monitoring of the calcium species in the study of cleaning mechanisms.

Studies of Quantitative Depth Profiling Using Glow Discharge Optical Emission Spectrometry

CSTL Program: Chemical Characterization of Materials
Authors: *M.R. Winchester; and D.S. Simons (837)*

Abstract: The quantitative determination of elemental composition as a function of depth on surfaces is an important analytical problem in a wide variety of modern technologies. Examples include the characterization of galvanneal layers on steel, the measurement of the composition and thicknesses of paint layers on various substrates, and the evaluation of the depth distributions of dopants in Si wafers. While the quantitative determination of elemental composition as a function of depth is likely to become even more important in the future, this remains a difficult, time consuming, and expensive metrological problem. Consequently, there is a need for better, faster, and more economical characterization methods. At NIST, glow discharge optical emission spectrometry (GDOES) is being developed and critically evaluated for this purpose. GDOES is inherently much faster and much less expensive than most competing methods of surface analysis. The current major thrust of the research is on evaluating and improving the quality of the quantitative depth profiling (QDP) data provided through GDOES. A GDOES QDP method for SRM 2135c (Nickel-Chromium Thin Film Depth Profile Standard) is being developed and the GDOES QDP results can eventually be compared to existing AES and SIMS data for this material. When fully developed, GDOES QDP may provide United States industry with the ability to make these important measurements more rapidly and less expensively than is currently possible. These developments may be expected to affect industry sectors that rely upon thin films, coatings, and other types of surface layers, such as the microelectronics and advanced materials industries.



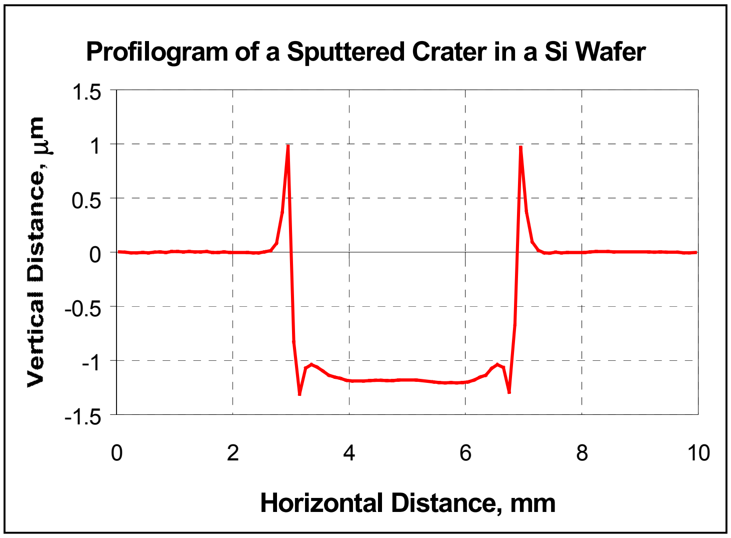
Purpose: The quantitative determination of elemental composition as a function of depth on surfaces is an important analytical problem in a wide variety of modern technologies. Examples include the characterization of galvanneal layers on steel, the measurement of the composition and thicknesses of paint layers on various substrates, and the evaluation of the depth distributions of dopants in Si wafers. While the quantitative determination of elemental composition as a function of depth is likely to become even more important in the future, this remains a difficult, time consuming, and expensive metrological problem. Consequently, there is a need for better, faster, and more economical characterization methods. At NIST, glow discharge optical emission spectrometry (GDOES) is being developed for this purpose. GDOES is inherently much faster and much less expensive than most competing methods of surface analysis. A major thrust of the research is on evaluating and improving the quality of the quantitative depth profiling (QDP) data provided through GDOES.

Major Accomplishments: In ongoing work begun during fiscal year 2001, a GDOES QDP method for SRM 2135c (Nickel-Chromium Thin Film Depth Profile Standard) is being developed. This sample consists of nine alternating layers of Ni and Cr, each approximately 55 nm thick, on a Si wafer. SRM 2135c has previously been examined in detail using Auger Electron Spectroscopy (AES) and Secondary Ion Mass Spectrometry (SIMS). Therefore, GDOES QDP results can eventually be compared to existing AES and SIMS data.

The first quantitative depth profile (i.e., mass fraction vs. depth) obtained for SRM 2135c is shown in the figure. These data were acquired at glow discharge conditions of 700 V and 20 mA using the SRM 1760 Low Alloy Steel series for calibration. One of the strengths of GDOES QDP is that calibration may be accomplished using bulk calibrants only. Referring to the figure, note that while the depth resolution decreases somewhat with increasing depth, the thicknesses of the Ni and Cr layers are approximately correct. Since these data were acquired, alternative discharge conditions that improve depth resolution significantly have been found. However, calibration has not been performed at these new conditions, and so quantitative data are not yet available. One observation that has been made during this work is that the walls of the craters formed during glow discharge sputtering may sometimes be sloped significantly, even when the crater bottoms are very flat. Upon further study, this may prove to be an important point, because common calibration algorithms assume vertical crater walls.

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

Future Plans: GDOES QDP research at NIST will likely continue for at least several more years. Specifically, during fiscal year 2002 the method for SRM 2135c will be refined and evaluated further, including comparisons of data with existing AES and SIMS data. Methods for other important sample types, such as Si wafers implanted with B and other dopants, will also be developed and evaluated. Finally, the effects of sloping crater walls on calibration will be investigated further.



M.R. Winchester (839) and D.S. Simons (837) Glow discharge optical emission spectrometry (GDOES) is being developed for the quantitative determination of the elemental composition of surfaces as a function of depth. GDOES is inherently much faster and less expensive than most competing methods of surface analysis. A major thrust of the NIST research is on evaluating and improving the quality of data. The capabilities of GDOES quantitative depth profiling are demonstrated through the development of a GDOES method for the characterization of SRM 2135c (Nickel-Chromium Thin Film Depth Profile Standard). This sample consists of nine alternating layers of Ni and Cr, each layer being approximately 55 nm thick, on a Si wafer. While the development of the GDOES method for this sample is still underway, the preliminary results are encouraging. All nine alternating layers have been resolved, and the layer thicknesses determined using GDOES are approximately correct. The NIST effort to develop GDOES as a tool for quantitative depth profiling of surfaces will likely continue for at least several more years. Once fully developed, GDOES should provide United States industry with a useful metrological tool, potentially affecting many industries that rely upon thin films, coatings, and other surface layers.

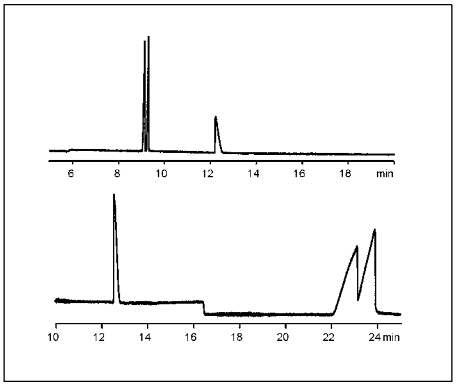
Research in Chiral Separations

CSTL Programs: Chemical Characterization of Materials, Healthcare Measurements
Author: *K.W. Phinney*

Abstract: A multi-faceted research program has been conducted to gain a better fundamental understanding of chiral separation mechanisms and to address some of the current measurement challenges. Although much of the work in this area has focused on pharmaceutical compounds (drugs), the work also has implications for other areas, including agricultural, food, and forensic sciences. This program encompasses a variety of analytical techniques including gas, liquid, and supercritical fluid chromatography, as well as capillary electrophoresis. The enantioselectivity of several sulfated cyclodextrins was investigated as part of an ongoing effort utilizing capillary electrophoresis to study chiral recognition processes. The use of well-characterized chiral selectors is necessary for reliable and reproducible chiral analysis. A liquid chromatographic method for the separation of amphetamine and methamphetamine enantiomers isolated from hair samples was developed. Determination of enantiomeric composition of these drugs is essential for distinguishing drug abuse from legitimate drug use. An SRM designed to evaluate the performance of chiral stationary phases used in liquid chromatography was also introduced during the past year. This SRM can be used to monitor changes in column performance over time and for control of column manufacturing processes.

Purpose: Enantioselective separations have grown in importance in several industries, but the greatest demand has been in the pharmaceutical industry. Chiral drugs now represent a $100 billion market, and measurements are needed to meet regulatory requirements associated with their development and production. Chiral separations also play a role in agrochemicals, foods and beverages, and forensic science. No one analytical technique can meet all the needs of these diverse fields. Therefore, our research has focused on various types of analytical methodology including gas, liquid, and supercritical fluid chromatography, as well as capillary electrophoresis. Development of rugged and accurate chiral analysis methods is facilitated by an understanding of chiral recognition processes and by the use of chiral additives or chiral stationary phases having consistent properties and compositions.

Major Accomplishments: The enantioselectivity of several commercially available sulfated cyclodextrins was investigated by utilizing them as chiral additives in capillary electrophoresis. In the forensic area, the use of liquid chromatographic methodology for the separation and quantification of amphetamine and methamphetamine enantiomers isolated from hair samples was investigated. An SRM designed to monitor the performance of chiral stationary phases for liquid chromatography was also introduced within the past year.



Separation of chlorpheniramine maleate enantiomers
with two different sulfated cyclodextrins.

Impact: Some of the methodology developed at NIST has been implemented in the pharmaceutical industry, and our research efforts have provided much needed information to guide method development efforts in a variety of fields. We also anticipate that this work will ultimately lead to the availability of improved separation technologies.

Future plans: The development of additional SRMs to validate enantiomeric composition measurements is currently being pursued, with an emphasis on chiral drugs of abuse. Efforts to characterize and improve chiral stationary phase performance will continue in conjunction with investigations of chiral recognition mechanisms.

Fourier-Transform Microwave Spectroscopy for Quantitative Measurements

CSTL Programs: Chemical Characterization of Materials, Environmental Measurements
Authors: *R.S. DaBell, and P.M. Chu; and R.D. Suenram (844)*

Abstract: There is a continuing need for improved analytical techniques to measure the concentration of trace gases for monitoring hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. In particular, a method that can conclusively identify and quantify multiple analytes in ambient air samples is critical for addressing pressing issues such as global climate change. The use of Fourier-transform microwave (FTMW) spectroscopy as a quantitative analytical technique is being investigated as its high spectral resolution and high sensitivity spectroscopy suggest that it can provide near real-time response and unambiguous identification of analytes in a gas sample. After incorporating instrument optimizations and modifications in response to initial evaluation studies, NIST measurements support the potential of FTMW spectroscopy to quantitatively measure analytes in nitrogen or blended air matrices. As work to optimize the instrument performance continues, an effort will also focus on evaluating the capability of FTMW spectroscopy to measure analytes in gas matrices that more closely match ambient air.

Purpose: To establish the feasibility of Fourier-transform microwave (FTMW) spectroscopy as a reliable technique for quantifying trace-gas analytes.

Major Accomplishments: There is a continuing need for improved analytical techniques to measure the concentration of trace gases for monitoring hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. In particular, a method that can conclusively identify and quantify multiple analytes in ambient air samples is critical for addressing pressing issues such as global climate change. Towards this end, the use of Fourier-transform microwave (FTMW) spectroscopy as a quantitative analytical technique is being investigated. The high spectral resolution and high sensitivity of FTMW spectroscopy suggest that the technique can provide near real-time response and unambiguous identification of analytes in a gas sample. The principal goal of the present program is to evaluate the technique's potential as a reliable and robust tool for quantitative measurements of trace gases in ambient air samples. To achieve these goals requires: 1) evaluation of the instrument function; 2) consideration of sample delivery issues; 3) comparison with other established analytical techniques; and 4) blind sample testing.

Initial evaluation of the instrument revealed that measured peak positions for a given analyte were reproducible to within 5 kHz providing powerful and reliable information for species identification. The signal intensities, however, varied substantially over long measurement periods (12 h), with worst-case scenarios showing complete loss of signal. To regain signal intensity, it was necessary to adjust or modify a number of instrument parameters. To improve the reliability of signal intensity, the performance of each individual component was evaluated and several instrument modifications have been incorporated. Currently, the remaining drift is ≈10 % over 12 h and the chief source of drift has been traced to fluctuations in the resonant frequency of the Fabry-Perot cavity caused by temperature induced changes of the optical cavity length. Concomitant with our effort to improve the instrument performance, we have also undertaken preliminary tests to examine the signal intensity as a function of the sample concentration and determine the detection limits using NIST standard reference samples of sulfur dioxide in a nitrogen matrix and ethanol in a blended air matrix. For sulfur dioxide, using a sample concentration range of 50 µmol/mol to1000 µmol/mol, a 0.14 µmol/mol detection limit was obtained. For ethanol, using a concentration range of 200 µmol/mol to 1400 µmol/mol, the detection limit was 1.3 µmol/mol. In both cases, the signal intensity was linear with the sample concentration over the entire ranges. These measurements support the potential of FTMW spectroscopy to quantitatively measure analytes in nitrogen or blended air matrices.

Publications: DaBell, R. S., Chu, P. M., Fraser, G. T., Suenram, R. D., "Evaluation of Fourier-transform microwave spectroscopy as a tool for quantitative analysis: Signal stability considerations", Proc. SPIE, 4574, to be published (2001).

Impact: After incorporating instrument optimizations and modifications in response to initial evaluation studies, NIST measurements support the potential of FTMW spectroscopy to quantitatively measure analytes in nitrogen or blended air matrices. Next steps will focus on evaluating its capability to measure multiple analytes in gas matrices that more closely match ambient air. Improved, validated analytical techniques are needed to measure the concentration of trace gases for monitoring hazardous air pollutants, industrial emissions, chemical warfare agent release, etc. In particular, a method that can conclusively identify and quantify multiple analytes in ambient air samples is critical for addressing pressing issues such as global climate change.

Future Plans: As work to optimize the instrument performance continues, an effort will also focus on evaluating the capability of FTMW spectroscopy to measure analytes in gas matrices that more closely match ambient air. Since this technique is based on probing rotational transitions and requires a supersonic expansion of the gas sample to concentrate the population distribution, different sample matrices will strongly impact the properties of the expansion and ultimately affect the detection limits. To date, quantitative measurements have not been the major driving force of FTMW studies and most measurements are made using an idealized balance gas of helium and neon. An effort will be focused on characterizing the signal intensities for samples with high concentrations of nitrogen, oxygen, carbon dioxide, and water, which are at high concentrations in analytically relevant samples.

Quantitative Infrared Database Developed to Support Remote Sensing Applications

CSTL Program: Environmental Measurements
Authors: *P.M. Chu, F.R. Guenther, G.C. Rhoderick, and P.A. Johnson*

Abstract: Over the last decade, growing concerns about the environment in general and air quality in particular have stimulated the development of improved, cost-effective field monitoring methods. With the recent advances in optical-based technologies, methods such as open-path Fourier transform infrared (FT-IR) spectroscopy are now becoming viable tools for field measurements. The *in-situ* and real-time nature of this approach offers several advantages over traditional point source monitoring techniques. Since molar absorptivity data in the literature widely differ, a validated quantitative database traceable to national measurement standards is a critical part of the infrastructure required for establishing emerging infrared-based monitoring technologies. NIST developed Standard Reference Database 79 (SRD 79) "Quantitative Infrared Database" to support optical-based measurements of chemical emissions and hazardous air pollutants (HAP). SRD 79 data are based on NIST primary gravimetric standards prepared with starting materials of assessed purity and procedures that minimize contamination. Currently, absorption coefficient data for 40 HAPs on a US EPA priority list is available as SRD 79, Version 1.1 with Data Release 3.0. Additional spectra will be added to the database as they are acquired and updates will be available over the internet. Plans include continued data acquisition for the compounds listed in the 1990 US EPA Clean Air Act Amendment, as well as for those compounds that are of concern in global warming and emissions trading.

Purpose: To develop the NIST Standard Reference Database 79 (SRD 79) "Quantitative Infrared Database" to support optical-based measurements of chemical emissions and hazardous air pollutants (HAP).

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

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

The primary effort of this project has focused on acquiring data at 25 ºC. To address the data needs of communities measuring industrial stack emissions by passive FT-IR and other infrared-based technologies, we have measured absorption coefficients for industrially important target gases, sulfur dioxide and methanol, at temperatures ranging from 25 ºC to 200 ºC. This project included studies to quantify sample emission contributions to the measured single-beam spectra.

Major Accomplishments: Currently, absorption coefficient data for 40 HAPs on a US EPA priority list is available as the NIST Standard Reference Database (SRD) 79, Version 1.1 with Data Release 3.0. The data are stored in the standard JCAMP-DX format to enable universal access to the data. The 0.12 cm^{-1} resolution data were processed to generate data at a number of different resolutions and apodizations to provide users with data that closely match their experimental parameters. A digital signature accompanies each data file, allowing users to ensure the integrity and source of the data file and traceability to NIST.

Data comparisons with Battelle Pacific Northwest laboratories and the higher temperature data project have helped us characterize two additional FT-IR intensity artifacts. Both of these artifacts are associated with the single limiting aperture design in most commercial FT-IRs. The first artifact is attributed to the detector viewing the warm annulus of the aperture in higher resolution measurements (0.2 cm^{-1} or higher). The off-axis rays distort the line shapes and increase the observed band intensities. The second artifact is due to the light exiting the interferometer, which is directed towards the source, can be reflected off the aperture back into the interferometer. This double modulated light produces a 2f alias throughout the spectrum. Reducing the reflectivity of the aperture wheel minimizes this artifact.

Adding an additional aperture system or a multipass cell after the interferometer minimizes the off-axis rays that reach the detector and in turn the contributions from these artifacts. For SRD 79, all of the data was acquired with a multipass cell and the potential biases due to these artifacts are within the stated uncertainties in the finger print region and no biases are expected at higher frequencies. The higher temperature absorption coefficient data required additional measurements with the infrared source off to appropriately account for sample emission issues. At 200 ºC, the sample emission correction is approximately 10 % for bands in the spectral region near 1000 cm^{-1} for the instrument and optical configuration used.

Recent Publications:
"*Intensity Artifacts in Gas Phase FTIR Spectroscopy: Focus on the Aperture*", S.W. Sharpe, R.L. Sams, T.J. Johnson, and P.M. Chu, Fourier Transform Spectroscopy, Trends in Optics and Photonics Series, Vol 51, Optical Society of America, 2001.

"*Infrared Absorptivity Temperature Dependence of Gas Phase Methanol and Sulfur Dioxide*", P.M. Chu, G.C. Rhoderick, P.A. Johnson, NISTIR 6773, National Institute of Standards and Technology, Gaithersburg, MD 20899, August 2001.

"*Removing Aperture-Induced Artifacts from FTIR Intensity Values*", T.J. Johnson, R.L. Sams, T.A. Blake, S.W. Sharpe, and P.M. Chu, submitted to Applied Optics.

Data Release 3.0 for NIST Standard Reference Database 79 CD, P.M. Chu, F.R. Guenther, G.C. Rhoderick, National Institute of Standards and Technology, Gaithersburg, MD 20899-8393, August 2001.

Impact: New infrared-based technologies coupled with the NIST Standard Reference Database 79 (SRD 79) "Quantitative Infrared Database" to support the optical-based measurements of chemical emissions and hazardous air pollutants provides both industry and EPA with a tool for assessing regulatory compliance that is both cost-effective and less invasive.

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

Establishing a system for primary realization of atmospheric ozone measurements

CSTL Programs: Environmental Measurements, International Measurement Standards
Authors: *P.M. Chu, and J.E. Norris*

Abstract: Ozone, at tropospheric levels, is a health concern and contributes to climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. These concerns have led to the establishment of air quality standards and international protocols to reduce the emissions of pollutants that either contribute to tropospheric ozone formation or deplete stratospheric ozone. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest in ozone measurements has prompted eleven national laboratories to acquire NIST SRPs.

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

The concentration of ozone in the atmosphere remains a significant issue from both scientific and political perspectives. Ozone, at tropospheric levels, is a health concern and contributes to climate change as a greenhouse gas, while stratospheric ozone protects earth from harmful UV radiation. These concerns have led to the establishment of air quality standards and international protocols to reduce the emissions of pollutants that either contribute to tropospheric ozone formation or deplete stratospheric ozone. Since 1983, NIST has provided Standard Reference Photometers (SRPs) based on UV photometry to ten US Environmental Protection Agency (EPA) facilities to provide an infrastructure for the calibration and traceability of ozone measurements within the US. More recently, the international interest in ozone measurements has prompted eleven national laboratories to acquire NIST SRPs.

Major Accomplishments: To support the growing national and international need of improved ambient ozone measurements, NIST is partnering with BIPM to develop an advanced primary ozone reference standard 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 technology with respect to current and future ozone measurement requirements. 3) Provide recommendations for potential improvements of the SRPs and survey other candidate secondary standards. 4) Develop a primary reference standard with capabilities of SI-traceability verification.

The major challenge of this project is to incorporate a secondary method that can provide a solid foundation for the primary reference standard at relevant ozone concentrations (0 nmol/mol to 100 nmol/mol). Additionally, any uncertainties due to ozone transport must be eliminated. Several approaches are being considered. Gas phase titration with nitric oxide is an attractive method, since the ozone concentration can be directly linked to primary gravimetric NO and NO_2 standards. Success of this project, independent of the verification method, demands instrumentation with high sensitivity, accuracy, precision, and rapid time response for measuring chemical species at atmospheric levels (nmol/mol and lower). In fact, minimum detection sensitivities better than 1 nmol/mol will be required to maintain uncertainties at the 1 % level for the current ground level ozone concentrations. A number of in-situ techniques are being considered, including time-gated laser-induced fluorescence detection, tunable diode laser absorption spectroscopy, and cavity ring-down spectroscopy for detection of relevant chemical species and possible contaminants in the carrier gas.

Current efforts have focussed on the construction of two new NIST SRPs which are to be transferred to BIPM shortly and an initial performance evaluation of the current SRPs. During an electronics upgrade of eleven instruments, a distinct 0.5 % slope calibration bias was noted for most of these instruments compared to SRP 2, the current principal NIST reference photometer. The calibration bias may be due to: sample memory effects in SRP 2, fluctuations in both temperature and pressure measurements for a number of instruments, variations in the zero offset dependent on the sample configuration, and significant detector drifts. An ongoing effort is dedicated to identifying and eliminating this bias.

Impact: As a collaborative project between NIST and BIPM, the advanced primary ozone photometer will provide an internationally validated ozone standard that can be used to anchor all ozone measurements through a defined traceability structure. The accuracy of the NIST SRP would be checked for consistency with the NIST SRM gas program and primary standards developed by BIPM.

Future Plans: NIST collaboration with BIPM will continue with the transfer and evaluation of the two new NIST SRPs. NIST is working to identify and eliminate the measurable calibration bias as discussed above. We are also currently surveying appropriate verification methods and state-of-the-art instrumentation that can provide a solid foundation for the new primary reference standard.

High-Accuracy Coulometric Analysis of Gas Mixtures

CSTL Programs: Environmental Measurements, Chemical Characterization of Materials
Author: *K.W. Pratt*

Abstract: Acidic gases that form strong acids when dissolved in water are of interest in global warming studies and for assigning intensities of absorption bands for calibrations in gas-phase infrared spectrophotometry. Standard gas mixtures of assessed composition are needed for calibration of instrumentation, method validation, and quality control purposes. The state of the art technique for generating standard gas mixtures is gravimetric preparation, in which the masses of the component gases introduced into a cylinder are measured – providing concentration data. However, for acidic gases, this approach often fails. The introduced active gas can react with the inside surface of the cylinder, changing its concentration in the mixture. Long-term storage of such mixtures is often impossible without change in the concentration of the acidic gas component and methods are needed to provide accurate measurements of these strong acid components in gas mixtures. Initial studies at NIST have demonstrated the feasibility of coulometric analysis to obtain accurate measurements of strong acid components in gas mixtures.

Purpose: To demonstrate the capability to obtain an accurate measurement of strong acid components in gas mixtures by coulometric analysis. Acidic gases that form strong acids when dissolved in water are of interest in global warming studies and for assigning intensities of absorption bands for calibrations in gas-phase infrared spectrophotometry.

The state of the art technique for generating standard gas mixtures is gravimetric preparation, in which the masses of the component gases introduced into a cylinder are measured. However, for acidic gases, this approach often fails. The reason is that the introduced active gas reacts with the inside surface of the cylinder, changing its concentration in the mixture. Therefore, long-term storage of such mixtures is often impossible without change in the concentration of the acidic gas component and methods are needed to provide accurate measurements of these strong acid components in gas mixtures.

Major Accomplishments: Provided that the gas of interest can be quantitatively recovered into an aqueous solution, coulometric titrimetry can easily determine the amount of strong acid present in the solution. This approach has the advantage that long-term storage in cylinders is not required. However, it is necessary to establish that the collection procedure is quantitative. To achieve traceability, one also requires detailed knowledge of the components of uncertainty for the titration of the strong acid, as well as a traceable measurement of the amount of gas that passes through the collection system.

The analytical approach evaluated and adopted uses an automated gas manifold, two impingers, and a mass flow controller (MFC). The gas mixture to be analyzed is passed for an accurately-known collection time through the impingers via the gas manifold. Solution from the first impinger is then titrated as a dilute solution of the strong acid using the NIST automated coulometry system. To establish quantitativeness, the pH of the solution in the second impinger is measured immediately following collection, taking care to exclude any interference from atmospheric carbon dioxide.

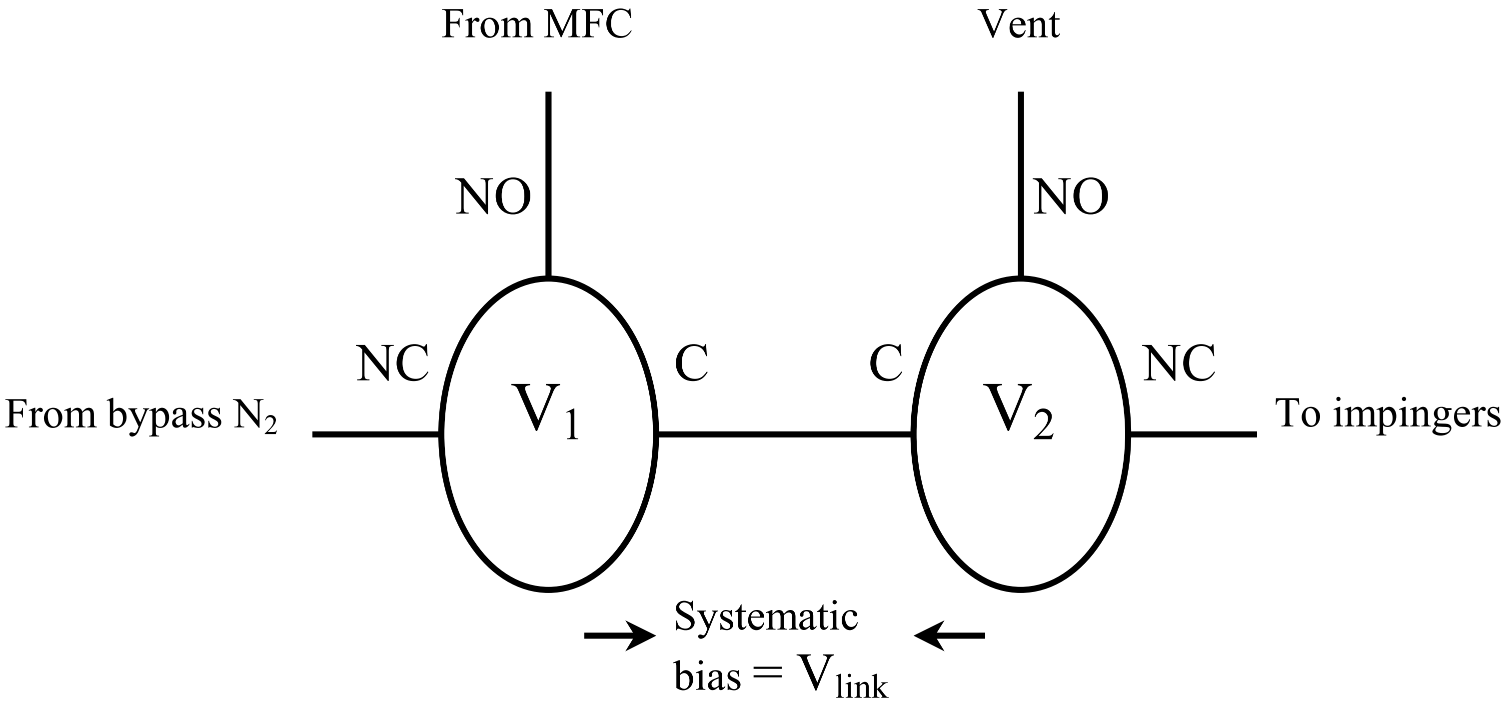
The gas manifold is shown in the attached **link**. The manifold switches between the flow of hydrogen chloride (HCl) and inert gas (nitrogen), used to purge carbon dioxide from the system and flush out the HCl lines. The manifold also supports venting of pressurized lines to the atmosphere, as required to obtain an accurate collection volume. The various states required for effecting the collection are noted in the **link**. The electronic interface that powers the solenoid valves also provides a digital timing signal used to obtain the collection time for the gas sample. The manifold is connected to the two impingers, connected in series. The concentration of hydrogen chloride in the gas mixture is obtained from the amount of acid titrated and the calibrated flow rate of the MFC. The MFC is calibrated using a traceable gas displacement system.

Initial tests demonstrated feasibility of this approach. The results of the initial tests using a hydrogen chloride in nitrogen mixture are very promising. For a 10 min collection time, a mixture with a nominal concentration of 974 µmol/mol, and a nominal flow rate of 99.1 cm^3/min (STP), the precision obtained is 0.055 % (standard deviation of the mean of 9 results). The blank (value obtained substituting nitrogen for the HCl gas mixture) is less than 0.05 % on a relative basis, using a 10 min collection time. It should be feasible to obtain similar precision levels (and overall uncertainty values) for lower concentrations with corresponding increases in the collection time.

Impact: Acidic gases that form strong acids when dissolved in water are of interest in global warming studies and for assigning intensities of absorption bands for calibrations in gas-phase infrared spectrophotometry. Often, gravimetric preparation techniques typically used for other less reactive gaseous mixtures cannot be used to provide accurate composition data for acidic gas standard mixtures due to reactions such as those between the active gas and the inside surface of the gas cylinder that change its concentration in the mixture both initially and over time. Initial studies at NIST have demonstrated the feasibility of coulometric analysis to obtain accurate measurements of these strong acid components in gas mixtures.

Future Plans: Future work will be directed toward expending this principle to other acidic gases, notably sulfur dioxide and nitrogen oxides. These gases present additional difficulties, requiring close control of the chemistry of the collection procedure to ensure quantitative recovery.

Solenoid valve manifold for HCl collection



States:
NO = 0
NC = 1

Truth Table

V_1	V_2	Output	Action
0	0	0	MFC to vent
0	1	1	MFC to impingers
1	0	0	bypass N_2 to vent
1	1	0	bypass N_2 to impingers

Potassium Bromate Assay by Redox Titrimetry Using Arsenic Trioxide

CSTL Programs: Chemical Characterization of Materials, Environmental Measurements
Author: *J. Smeller*

Abstract: A NIST bromate standard solution SRM is needed by environmental laboratories performing wastewater analyses and by NIST NVLAP accredited providers of proficiency testing for calibration, method validation, quality control and in pathways for demonstrating traceability to national standards. Redox titrimetry with arsenic trioxide was used to assay a high purity potassium bromate source material as the first step in the provision of this SRM.

Purpose: To assay potassium bromate, for use as a source material for a new NIST anion standard solution SRM. Many environmental labs have a regulatory need to be traceable to NIST and, increasingly, providers of commercial calibration solutions, "reference" solutions, and proficiency test materials also need a mechanism for demonstrating traceability to national standards. Bromate, one of the analytes of interest in drinking and wastewater analysis, is not currently certified as a NIST Standard Reference Material (SRM). No bromate primary standard is available. Environmental laboratories are limited to using ACS reagent grade bromate salts as a bromate source. Typically, the purity of these salts has been assayed by a titration using only two or three measurements. Only a small number of elemental impurities, usually listed as less than values, have been determined in these salts. No uncertainty information is given regarding the assay measurement, assay method, or impurities.

Major Accomplishments: An ACS reagent grade salt having a high stated purity was assayed by titrimetry and impurities covering the periodic table were determined semi-quantitatively by glow-discharge mass spectrometry (GDMS). The assay was performed by titrimetry because, as used for this analysis, it is considered a primary method. A primary method is defined as the following: having the highest metrological qualities; its operations can be completely described and understood; and, a complete uncertainty statement can be written down in terms of SI units. The method was validated for its appropriate use in this application. Potassium bromate ($KBrO_3$) was assayed by redox titrimetry using arsenic trioxide (As_2O_3), SRM 83d. Three aqueous $KBrO_3$ solutions were prepared from the $KBrO_3$ salt (one bottle). A nominal (0.1g) sample of As_2O_3 was dissolved in 10 mL of 5 mol/L sodium hydroxide. The solution was acidified with hydrochloric acid, and about 95 % of the $KBrO_3$ titrant was added gravimetrically. The end point was then determined by addition of dilute (1:3) $KBrO_3$ titrant using an autotitrator.

The $KBrO_3$ assay was determined to be 99.76 % ± 0.20 %, based on a bromate mass fraction of 76.40 % ± 0.15 %. The uncertainty considered the titrations of the three independently prepared $KBrO_3$ solutions. The main sources of uncertainty for each $KBrO_3$ solution were measurement replication, the mass of As_2O_3, and the volume of dilute $KBrO_3$.

Impact: A high purity potassium bromate source material was assayed as the first step in the provision of a new NIST SRM anion standard solution of bromate needed by environmental laboratories performing wastewater analyses and by NIST NVLAP accredited providers of proficiency testing for calibration, method validation, quality control and in pathways for demonstrating traceability to national standards.

Future Plans: Future plans include the development of the bromate anion SRM solution. The development steps include the following: preparing bromate standard solutions; evaluating the bromate solution concentrations for optimum concentration level; studying stability; and evaluating packaging.

Titrimetric assays of salts needed to develop additional anion SRM solutions may be performed using a similar analysis scheme.

Application of holistic gravimetry as a primary measurement of potassium in potassium dihydrogen phosphate

CSTL Program: Chemical Characterization of Materials

Authors*:* *T.W. Vetter, L.L. Yu, and J.R. Sieber; and W.F. Guthrie (898)*

Abstract: A ion-exchange gravimetric method for K in a renewal of SRM 200a, KH_2PO_4, was developed based on a previous method for K and a similar method used for Na in serum and NaCl.[1] The ion-exchange gravimetric method used here to determine K, and previously to determine Na, was applied to minimize errors and uncertainty. The critical evaluation for this application demonstrated that the holistic gravimetry (HG) approach provided excellent accuracy (as judged by results for validation materials), an expanded relative uncertainty, coverage factor of less than 0.05 %, and a very low relative measurement replication, $u_c = 0.004$ %. A similar approach could be used to separate other analytes of interest from other matrices. Though HG is best suited for high-accuracy determinations in very pure materials, because of the relative ease of complete separation, applications can be developed for more complex matrices. A holistic approach was used to consider all sources of bias and uncertainty. This critical evaluation of the gravimetric method for the determination of K in KH_2PO_4 can serve as a basis for critical evaluation of gravimetric methods, in general.

Purpose: To continue development of gravimetric methods and critically evaluate them to determine whether they can be applied by National Metrology Institutes, NMIs, to meet the requirements of a primary method. Specifically, the gravimetric determination of potassium in potassium dihydrogen phosphate, KH_2PO_4, by ion-exchange separation of K and its precipitation as potassium sulfate, K_2SO_4, is evaluated and an approach called holistic gravimetry (HG) is advocated.

NMIs are interested in determining the amount of substance using primary methods. A primary method has the highest metrological qualities, its operations can be completely described and understood, and a complete uncertainty statement can be written down in terms of SI units. Gravimetric methods are commonly considered primary; however, in order to be considered primary, any gravimetric method needs to be critically evaluated to determine if analyte is lost in the procedure and if the precipitated compound is stoichiometric and free of contaminants. Many NMIs lack the knowledge and capability to apply gravimetry as a primary method. A critical evaluation of the gravimetric method for the determination of K in KH_2PO_4 can serve as a basis for critical evaluation of gravimetric methods, in general. In addition, an overall approach that is required for highly accurate gravimetry has not been specified.

Major Accomplishments: A ion-exchange gravimetric method for K in a renewal of SRM 200a, KH_2PO_4, was developed based on a previous method for K and a similar method used for Na in serum and NaCl.[1] A holistic approach was used to consider all sources of bias and uncertainty. This approach included steps prior to the analysis such as identification of potential contaminants by semi-quantitative analysis of the KH_2PO_4 sample and designing the analysis to obtain a representative sample, reveal sample-to-sample and run-to-run differences and minimize sample loss and contamination. Each step in the determination (Figure 1) was critically evaluated and monitored for contributions to the loss of analyte or contamination of the precipitate. Measurements were made and validation samples were analyzed to access accuracy and determine the magnitude of any errors and the uncertainty associated with any errors. The magnitude of any undetected losses was critically estimated. Instrumental techniques, capable of determining trace amounts, were used to determine K in the fractions collected from the ion-exchange column, before and after the K fraction, and contaminants in the precipitate and in the crucibles used to ignite the K_2SO_4 precipitate.

This critical evaluation demonstrated that holistic gravimetry can be applied by NMIs as a primary method. Excellent accuracy (as judged by results for validation materials) and an expanded relative uncertainty, coverage factor (k) = 2, of 0.043 % were achieved. In addition, a very low relative measurement replication, $u_c = 0.004$ %, was obtained. Specifically, the ion-exchange gravimetric method used here to determine K, and previously to determine Na, can be applied to minimize errors and uncertainty. A separation by ion exchange, instead of the normal precipitation and filtration to separate the analyte from the matrix, leads to a relatively pure precipitate with a higher recovery of the analyte. A similar approach could be used to separate other analytes of interest from other matrices. The requirement for the ion-exchange procedure is that the analyte of interest can be easily separated from the matrix and that there be minimal retention of the analyte on the ion-exchange column after the elution of the analyte is completed. A similar gravimetric procedure as that used for Na and K, to produce a relatively pure precipitate (such as the sulfate) with a known stoichiometry, is also desirable. Though HG is best suited for high-accuracy determinations in very pure materials, because of the relative ease of complete separation, applications can be developed for more complex matrices.

Impact: A ion-exchange gravimetric method was critically evaluated and used for the determination of K in a renewal of SRM 200a, KH_2PO_4. A similar approach could be used to separate other analytes of interest from other matrices. A holistic approach was used to consider all sources of bias and uncertainty. This critical evaluation of the gravimetric method for the determination of K in KH_2PO_4 can serve as a basis for critical evaluation of gravimetric methods, in general.

[1] J. R. Moody and T. W. Vetter, *Journal of Research of the National Institute of Standards and Technology*, 1996, 101, 155.

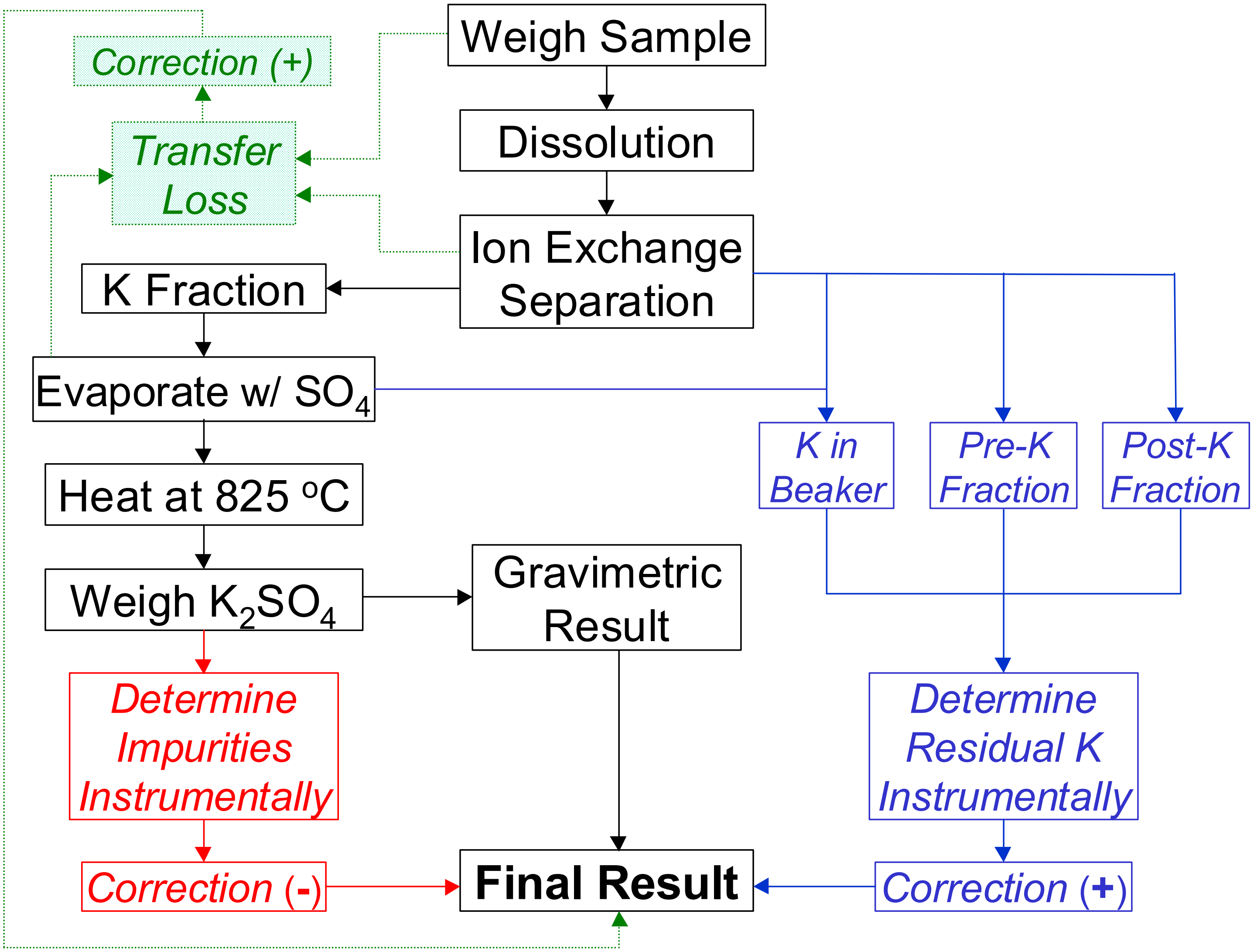


Figure 1. Schematic diagram of holistic gravimetry in the determination of w_K.

Diesel Dye Standard SRM 2037 to Assist in the Regulation and Taxation of Off-Road Diesel Fuel

CSTL Program: Chemical Characterization of Materials
Authors: *P. DeRose, and G. Kramer*

Abstract: Federal excise tax regulations require that tax-exempt diesel fuel, which is used in off-road vehicles, contain Solvent Red 164 (SR164) at a concentration that is spectrally equivalent to 11.1 mg/L or greater of dye standard Solvent Red 26 (SR26). Standard photometric test methods are used to determine the concentration of SR164 in diesel fuel. The accuracy and reproducibility of these methods are strongly dependent on the purity of the dye standard. Since the currently specified dye standard SR26 is not commercially available at high or consistent purity, the IRS requested NIST to secure and certify an alternative dye Standard Reference Material® (SRM®) for this purpose. NIST extensively evaluated two materials, one custom synthesized (SR 26) and one obtained commercially (SR24). Following the difficult purification process that utilized HPLC, each dye was analyzed for impurities using HPLC, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and LC-MS and then assessed for performance as a dye standard. Both appear to make equally effective standards for determining the concentration of SR164 in diesel fuel. SR24 was chosen for use as the reference material due to its ready availability and simpler purification requirements. SRM 2037 will be available in FY 2002 with certified values for purity and extinction coefficient in kerosene and xylene. Since solutions of all these Solvent Red dyes decolorize on exposure sunlight, it will be issued as a solid.

Purpose: To secure, characterize, and certify a dye standard that will enable the concentration of Solvent Red 164 dye in diesel fuel to be quantified reproducibly and accurately.

Federal excise tax regulations require that tax-exempt diesel fuel, which is used in off-road vehicles, contain Solvent Red 164 (SR164) at a concentration that is spectrally equivalent to 11.1 mg/L or greater of dye standard Solvent Red 26 (SR26) [Figure 1]. The U.S. Internal Revenue Service (IRS) enforces this regulation by (1) monitoring the concentration of SR164 in off-road fuel at refineries and fuel terminals, and (2) checking on-road diesel fuel at service stations and in vehicles for the presence of SR164. Standard photometric test methods are used to determine the concentration of SR164 in diesel fuel. The accuracy and reproducibility of these methods are strongly dependent on the purity of the dye standard. Since the currently specified dye standard SR26 is not commercially available at high or consistent purity, the IRS requested NIST to secure and certify an alternative dye Standard Reference Material® (SRM®) for this purpose.

Major Accomplishments: Two dye standard candidates, SR26 and Solvent Red 24 (SR24) [Figure 2], were acquired for study. The SR26 was custom synthesized, and the SR24 was obtained commercially. Both dyes required purification by preparative HPLC. The low solubility of SR26 in chromatographic solvents made its purification difficult. After purification, each dye was analyzed for impurities using HPLC, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and LC-MS and then assessed for performance as a dye standard. In solution, SR24 (or SR26) can be present as four equilbrating forms [Figure 3]. The overall structure consists of two tautomeric structures (azo-phenol and hydrazone), and each tautomer can have a *cis* and a *trans* isomer. The *trans* hydrazone is thought to be predominant form, but the less much prevalent *cis* hydrazone seems to account for an early eluting HPLC peak that was initially thought to be an impurity. The precise amounts of the different forms depend on other variables such as the type of solvent, solution temperature, etc.

Highly purified SR24 and SR26 appear to make equally effective standards for determining the concentration of SR164 in diesel fuel. SR24 was chosen as the reference material (SRM 2037) due to its ready availability and simpler purification requirements. SRM 2037 will be available in FY 2002 with certified values for purity and extinction coefficient in kerosene and xylene. It is intended to replace the impure dye SR26 standards currently being used in existing test methods. Since solutions of all these Solvent Red dyes decolorize on exposure sunlight, SRM 2037 will be issued as a solid, instead of a more convenient to use set of solution standards.

Impact: SRM 2037, a Solvent Red dye, will enable the concentration of Solvent Red 164 dye in diesel fuel to be quantified reproducibly and accurately by standard photometric test methods. This facilitates the U.S. Internal Revenue Service's enforcement of regulations requiring that a Solvent Red dye be added at minimum prescribed levels to tax-exempt diesel fuel for off-road vehicles.

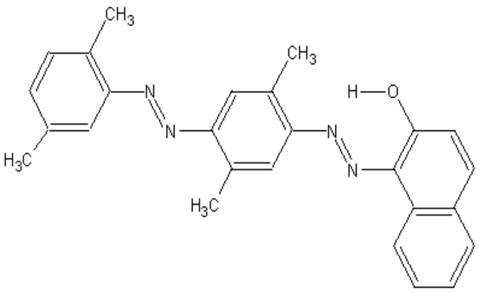


Figure 1. Solvent Red 26.

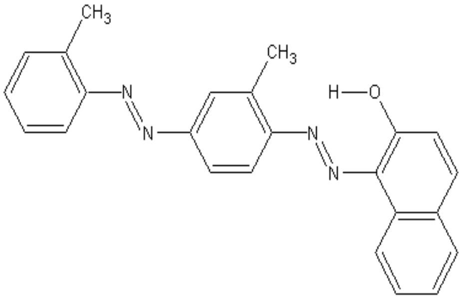


Figure 2. Solvent Red 24.

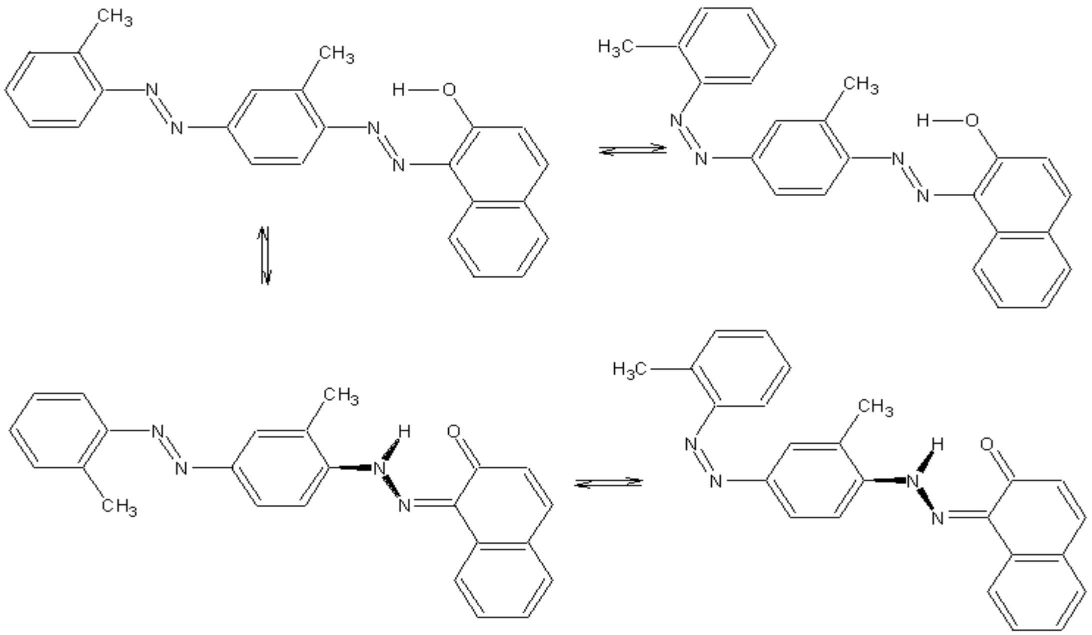


Figure 3. Equilbrating Forms of Solvent Red 24 in Solution.

Design and Applications of Plastic Microfluid Systems

CSTL Program: Nanotechnology
Authors: *L. Locascio ,E. Waddell, T. Johnson, and A. Henry; D. Ross, and M. Tarlov (836); and M. Gaitan (812)*

Abstract: In both analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in these systems must be well-controlled to provide accurate and reliable results. NIST pioneering microfabrication methods for the design and development of plastic microfluidic devices, results of its current studies on the measurement, characterization, and modulation of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods and by laser ablation, and future such studies contribute to the optimization of performance, promotion, and acceptance of such devices.

Purpose: To promote the development of plastic microfluidic devices by (1) developing methods for fabrication and characterization of plastic microchannels; (2) modulating surfaces to control microflow; (3) integrating active components into microfluidic systems.

In both analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in these systems must be well-controlled to provide accurate and reliable results.

Major Accomplishments: In recent years, our group has pioneered microfabrication methods for the design and development of plastic microfluidic devices. This year, we have focused much of our efforts on the measurement, characterization, and modulation of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods and by laser ablation.

Microflow measurement is critical to the development of microfluidic devices due to the fact that the majority of measurement errors are related to flow uncertainties. As in HPLC and CE, flow fluctuations in microfluidic devices can lead to increased dispersion, decreased resolution and ultimately a reduction in device performance and system reliability. Fluctuations in temperature can also induce dispersion and flow fluctuations in microfluidic devices. We have developed several methods for characterizing flow and temperature in plastic microfluidic devices. For flow measurement, we have developed several methods including current monitoring, caged fluorescent dye imaging, and particle imaging. For temperature measurement, we have developed methods that rely on the temperature-dependent fluorescence of Rhodamine B, a common fluorophor. Also, during the past year, we have introduced techniques for controlling microflow by direct write laser micropatterning. Methods for control and modulation of microflow can be used to induce mixing or eliminate dispersion in microfluidic devices.

The development of techniques to measure bulk fluid flow rates in microfluidic channels has enabled us to provide critical data on flow properties that are materials dependent, such as EOF mobility. Measurements of dispersion have allowed us to elucidate the causes of dispersion related to different fabrication methods and different materials. Ultimately, industrial and academic researchers can use both of these measurements to predict device performance based on the substrate material and fabrication method.

New methods for temperature measurement developed in our group using fluorescence imaging and temperature dependent fluorescence of Rhodamine B allow us to measure temperature distribution in networks of microfluidic channels in real-time with high spatial resolution (Figure 1).

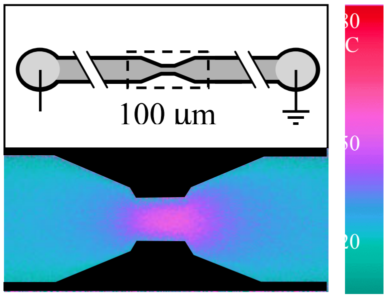


Figure 1. Temperature distribution in plastic channel.

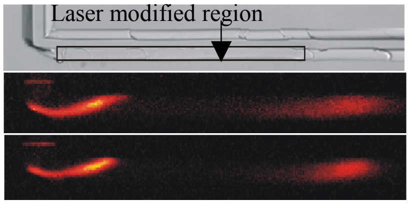


Figure 2. A. Brightfield image of microchannel with laser modified region. Fluorescence images of flow around turn in B. nonmodified channel and C.

We have also developed direct write laser micropatterning as a method to modulate flow in plastic microchannels. In this technique, portions of a preformed plastic microchannel are exposed to a UV-laser by direct write patterning thereby locally increasing the surface charge. The ability to modify the surface charge on specific surfaces within a preformed plastic microchannel allows for fine control, adjustment, and modulation of the electroosmotic flow without using wall coatings or changing the geometry of the channel to achieve the desired flow profile. We have used this approach to decrease the dispersion induced by flow around turns as shown in Figure 2.

Impact: In both analytical and biotech industries, there has been tremendous interest in recent years in the development of chip-based technologies incorporating microfluidics. It is predicted that miniaturization of chemical processes using these technologies will have a huge impact on rapid point-of-care screening as well as high throughput screening. For these devices to become commercially viable, the chemistries performed in these systems must be well-controlled to provide accurate and reliable results. NIST pioneering microfabrication methods for the design and development of plastic microfluidic devices, results of its current studies on the measurement, characterization, and modulation of flow and surface chemistry in plastic microchannels fabricated by NIST imprinting methods and by laser ablation, and future such studies contribute to the optimization of performance, promotion, and acceptance of such devices.

Future Plans: In future work, we will continue the development of micropatterning methods for application in both flow control and surface derivatization. New approaches to surface derivatization will also be used to pattern chemical selectivity into plastic microfluidic systems.

Imaging and Chemical Analysis of 100 µm Particles Through Neutron Focusing and Prompt Gamma Activation Analysis Techniques

CSTL Program: Chemical Characterization of Materials
Authors: *H.H.Chen-Mayer, G.P. Lamaze, D.F.R. Mildner, and R.Zeisler*

Abstract: Neutron radiography is a non-destructive evaluation tool for industrial materials such as aircraft components, while powerful, lacking quantitative information on elemental concentrations. Prompt gamma activation analysis can provide such information. Neutron focusing lenses can deliver a neutron beam to a spot size < 100 µm x 100 µm (FWHM), with a gain in neutron beam current density of > 40, allowing PGAA to be performed on a small region of the sample. The smaller focal spot size enables better spatial resolution, but also makes sample alignment more challenging. We have devised a method combining neutron imaging with PGAA using monolithic capillary neutron lenses. The converging-diverging beam from the lens delivers magnified images on a neutron camera, enabling alignment of the focal spot on 100-µm samples. The measurement sensitivity for a 2.6 µg Gd sample has improved by a factor of 34. In addition to rastering samples in the lateral plane, we have also explored the possibility of profiling the inhomogeneity of the sample in the direction along the beam axis. This new tool will provide a unique capability in non-destructive analysis for industrial materials. In the future, automated scanning of lens associated with gamma ray data acquisition will give a more efficient probe for possible micro-analysis.

Purpose: Conventional neutron radiography is an indispensable tool for aircraft industry in the non-destructive evaluation of components. However, it lacks quantitative information in compositions. Prompt gamma activation analysis has been used to calibrate hydrogen in metal standards for neutron radiography, but since it is a bulk technique, the probed region must be defined to enable composition mapping. It is desirable to combine these two aspects of non-destructive analysis into a tool to investigate INTERNAL small regions of interest in a metal matrix. We have devised a method combining neutron imaging with PGAA using monolithic capillary neutron lenses for this purpose. The potential beneficiaries are the failure prevention and analysis technical areas for industry and military applications.

Major Accomplishments: Glass polycapillary fibers with hundreds of hollow micrometer-wide channels have been used for guiding and changing the direction of slow neutrons. The use of a monolithic lens for PGAA has been explored earlier. In addition, the converging nature of a focused beam can provide magnified images for neutron radiography. We combine these two uses of the focused beam from a monolithic lens and implement an alignment procedure for PGAA aided by neutron imaging.

The measurements have been performed at the end position of NG0 at the NIST Center for Neutron Research. The average wavelength of the incident beam with a Be filter is about 0.6 nm. Neutrons are imaged with a neutron camera that consists of a ZnS(Ag)/^{6}LiF scintillator coupled with a CCD camera with an average pixel size of 50 µm. The gamma-ray spectrometry system consists of a p-type germanium photon detector with a virtual pulser "loss free" counting module. The two monolithic lenses used in this study are both fabricated from a bundle of capillaries that is 50 mm long and with a hexagonal cross section of 10 mm wide (flat-to-flat) at the entrance and tapered to 3 mm at exit. The distance from the exit to the focus is 8.8 mm for lens A and 9.8 mm for lens B. An optimum focal spot from lens A is recorded with the imaging detector. Line profiles across the focal spot in both the horizontal and vertical directions are taken to determine the intensity distribution and the FWHM (H: 87 µm V:100 µm) of the focus. The gain in neutron current density within the 100 µm x 100 µm area is 46 ± 2, and within a 50 µm x 50 µm area it is 71 ± 5.

The focused beam creates a neutron "point source", with neutrons departing the focus within a cone of about 13°. Any neutron-absorbing object within the path of the divergent beam will cast a magnified shadow at some further distance. We make use of this effect in order to align a small sample, a Gd foil (thickness 25 µm) of size 2 mm x 0.9 mm, at the focus for PGAA (Fig. 1). We vary the distance L_1 between the sample and the lens focus by translating the lens along the direction of the beam. The magnified image enables a better alignment of the sample with respect to the focus. From the earlier measurement of the lens focus image, we determine the location of the focus with respect to the large hexagonal shadow cast by the lens entrance. We then move the lens such that the image of the sample is now superimposed on image of the focus. This guarantees that the focal point is indeed on the sample. Once the image is centered, the lens is translated to L_1 = 0 such that the focus is on the sample, ready for prompt gamma measurements.

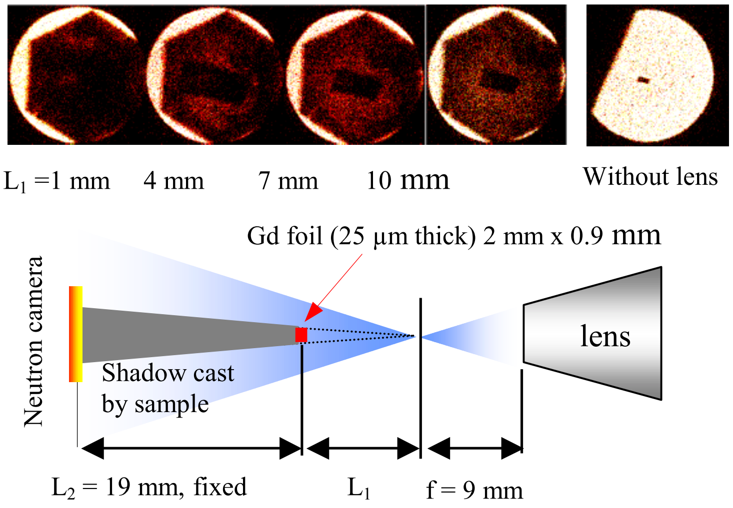


Figure 1. Magnified image of the sample in the focused beam at various distances L_1. The hexagonal shadow is cast by the lens entrance, surrounded by the background of unguided neutrons (white). The sample is the black strip in the middle of the hexagon at various magnifications. The image of the sample without the lens is also shown for comparison.

At the center of the same Gd sample described above, there is an additional indium foil of about 1 mm x 1 mm in cross section, in contact with the Gd strip. The prompt gamma measurement without the lens gives a peak intensity for Gd at 182 keV of 2.1 counts per second (cps), but the In peak at 186 keV is not visible even for a 20-min measurement (Fig. 2, right). When the lens is used in a 10-min measurement, not only has the Gd peak intensity increased, but the In peak has the Gd peak intensity increased, but the In peak has also become visible (Fig. 2, left). Since the Gd piece is much larger than the focal spot size, the gain in sensitivity for Gd is only a factor of 4. The gain for In is not estimated.

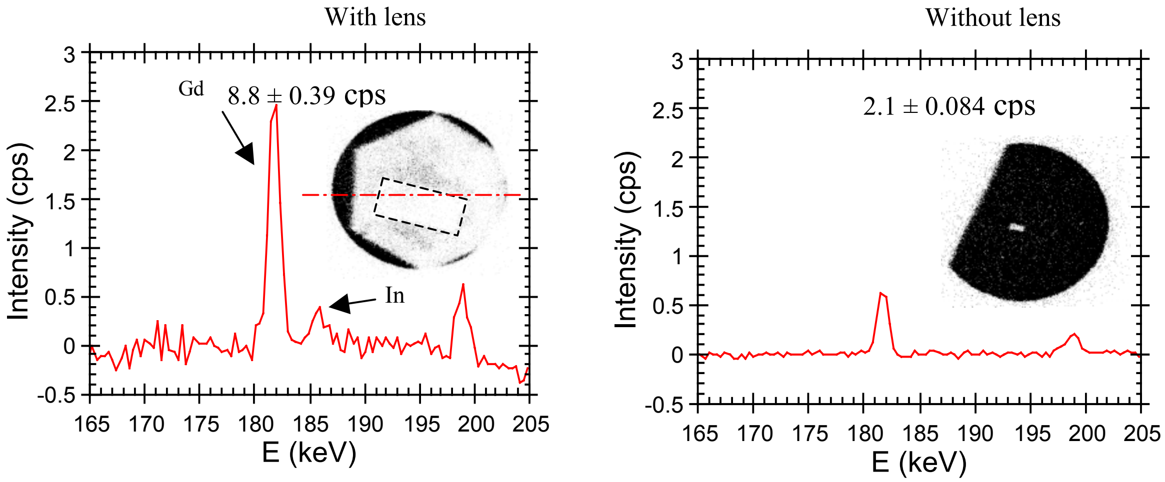


Fig. 2. Prompt gamma spectrum for Gd (182 keV) and for In (186 keV) measured with(left) and without (right) the lens, and the corresponding image of the sample. The small In signal from a small In foil attached to the Gd strip is invisible in the 20-min measurement without the lens, but becomes visible with the lens in 10 minutes.

We have also used lens B for an even smaller sample – a Gd piece of 128 µm x 103 µm x 25 µm in size and 2.59 µg in mass (effective mass ~1 µg taking into account self-absorption). Without the lens, the neutron camera cannot resolve the 100-µm particle. The gamma intensity with the lens is 17.4 cps with a 1.6% 1sigma counting statistics after 1 hour of counting, and with the direct beam 0.506 cps (3.9% 1 sigma) after 19.5 hours. The gain, given by the ratio of the two count rates, is 34 ± 1.4. A scan of the lens in the vertical direction yields a peak with a FWHM of 148 (±1.5) µm, correctly corresponding to the 100-µm sample-width and the 100-µm beam-width added in quadrature.

Impact: The effort presented here is a step toward providing quantitative elemental information to traditional neutron radiography. This new tool will provide a unique capability in non-destructive analysis for civil and defense industrial materials.

Future Plans: In the future, automated scanning of lens associated with gamma ray data acquisition will give a more efficient probe for possible micro-analysis.

Bacterial Identification by MALDI-TOF MS

CSTL Program: Chemical Characterization of Materials
Authors: *C. Nelson, B. Nelson, B. Porter, M. Welch, G. Mallard, and E. White*

Abstract: The Biological Weapons Convention is still in the process of negotiation; however, it is clear that there is a need for credible, verifiable methods for determining if a specific organism is sufficiently pathogenic to be covered by the treaty. Development of accurate fully certified techniques for the determination of biological warfare agents will require that the variations in techniques among laboratories be reconciled. Current NIST/CSTL efforts, supported by funding from the Defense Threat Reduction Agency, are focused on standardization of methods for sample preparation and data interpretation of spectra obtain using the technique known as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), both of which are critical to further database input and search algorithm development. Essentially, proteins expressed by bacteria, both intracellularly and on the bacteria surface, can produce characteristic mass fingerprints useful in distinguishing between organisms, even at the strain level. Preliminary whole cell analysis of lyophilized bacteria indicate the presence of characteristic bacterial identification markers common among 3 strains of *Escherichia coli* tested, as well as markers that may be specific to each specific strain. Distinct mass fingerprint differences were apparent between gram negative, *Escherichia coli*, and gram positive, *Bacillus subtilis*, bacteria. Standardization of bacterial sample preparation and analysis by MALDI-TOF MS, along with algorithmic development for library searches, will aid in supporting the Biological Weapons Convention and may have wider implications for bacterial identification in other areas (*e.g.* environmental, biomedical research, clinical, food industry).

Purpose: The Biological Weapons Convention is still in the process of negotiation; however, it is clear that there will be a need for credible, verifiable methods for determining if a specific organism is sufficiently pathogenic to be covered by the treaty. The requirements imposed by the need to identify conclusively what organism is involved are expected to be quite different from the requirements for early warning in combat conditions. The development of tested and certified methods of analysis, high quality databases of organisms both pathogenic and nonpathogenic, and algorithms that will adequately distinguish analytes will be essential in the development of credible verification measures. Current NIST/CSTL efforts, supported by funding from the Defense Threat Reduction Agency, are focused on standardization of methods for sample preparation and data interpretation of spectra obtain using the technique known as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), both of which are critical to further database input and search algorithm development. Variations in the methods for analysis need to be understood, especially with respect to their roll in both positive identification and the risk of false positive identification. In this project NIST/CSTL is serving its traditional role of providing independent and objective assessment of critical measurement capabilities needed by the Nation and development of standard methods, as they are needed.

Major Accomplishments: Mass fingerprint data from lyophilized whole cell preparations of gram negative, *Escherichia coli*, and gram positive, *Bacillus subtilis*, bacteria were obtained by MALDI-TOF MS. Essentially, proteins expressed by bacteria, both intracellularly and on the bacteria surface, can produce characteristic mass fingerprints useful in distinguishing between organisms, even at the strain level. Preliminary whole cell analysis of lyophilized bacteria indicate the presence of characteristic bacterial identification markers common among 3 strains of *Escherichia coli* tested, as well as markers that may be specific to each specific strain. Distinct mass fingerprint differences were apparent between gram negative and gram positive bacteria. Several variations in sample preparation were also investigated. Of the techniques attempted, choice of ultraviolet absorbing matrix appeared to have the largest impact on the quality of mass fingerprints produced. In addition to whole cell analysis, reproducible mass spectra, characteristic of proteins associated with the bacterial cell wall, were obtained using various solvent washes. These results are promising in identifying fingerprints that may distinguish bacteria even at the strain level.

Impact: Standardization of bacterial sample preparation and analysis by MALDI-TOF MS, along with algorithmic development for library searches, will aid in supporting the Biological Weapons Convention and may have wider implications for bacterial identification in other areas (*e.g.* environmental, biomedical research, clinical, food industry).

Future Plans: This work was initiated at NIST/CSTL during the past year in support of the Defense Threat Reduction Agency (DTRA), Chemical/Biological Arms Control Program (OST). Preliminary data on method protocols and acquisition of mass spectra have been obtained and the project is expected to continue for 2-3 years. NIST will continue to examine the various experimental parameters affecting the spectra of the bacteria. These will include the matrix, the intensity of the laser, the wavelength of the laser, the nature of the sample (ionic strength, growth media for bacteria, etc). Specific variability due small changes in the protocol will be examined in order to determine how exacting the specification of the standard operating procedure must be. Factors affecting reproducibility of the spectrum will be categorized as to the magnitude of the change created in the spectrum. Variation in the spectrum due to normal instrument variation will also be evaluated. A specific set of standard conditions that produce reproducible spectra under the widest set of normal instrument variations will be determined. From these experiments, standard operating procedures for the production of spectra will be developed. NIST will then create a library of MALDI-TOF MS for a wide variety of bacteria as well as bacteria that have been previously analyzed in the literature.

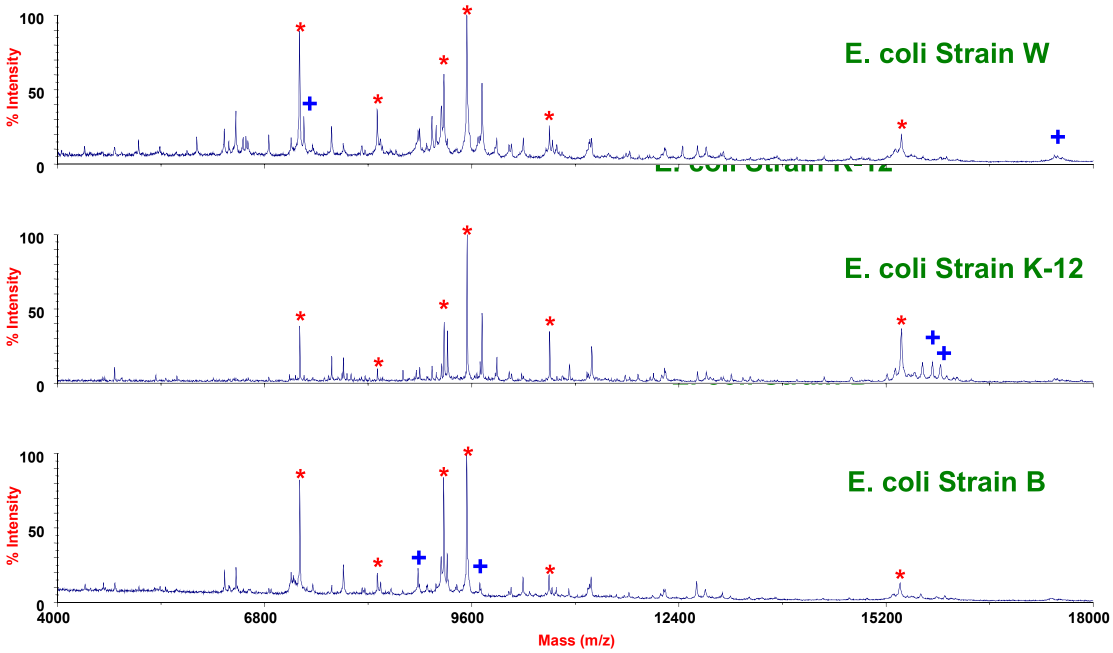


Figure 1. Whole cell MALDI-TOF mass spectral fingerprints of three strains of *Escherichia coli*. Several bacterial identification markers (BIMs) common to all three strains are indicated by “*” (red). Several BIMs that may be specific to each strain are indicated by “+” (blue).

Development of a Fluorescein Solution Fluorescence Standard to Facilitate Quantitation in Clinical Diagnostics and Biotechnology

CSTL Programs: Healthcare Measurements, Chemical and Biochemical Sensing
Authors: *P. DeRose, A. Gaigalas, L. Wang, and G. Kramer*

Abstract: Fluorescence intensity standards are in great demand in the clinical and biotechnological communities. Dramatic increases in the numbers of quantitative fluorescence assays in the past few years have generated a demand for standards to guarantee quality assurance and to satisfy regulatory agency mandates. SRM 1932, fluorescein standard, being developed and to be issued in FY2002, is a fluorescent concentration standard that will provide a viable alternative to an absolute fluorescence intensity standard that establishes a quantitative relationship between fluorescence intensity and fluorescent species concentration. Attendees of several NIST workshops on fluorescence standards identified fluorescein solution as their preferred concentration standard candidate due to its widespread use. In addition, a fluorescence standard composed of a fluorescein-labeled microbead suspension is presently being developed. These microbeads mimic biological cells labeled with fluorescein. Therefore, this microbead solution standard will serve as a fluorescence intensity calibration standard in biological assays using fluorescein.

Purpose: To prepare, characterize and certify a fluorescent standard composed of a fluorescein dye solution to facilitate the calibration of fluorescence instrumentation.

Fluorescence intensity standards are in great demand in the clinical and biotechnological communities. Since a measured fluorescence intensity is dependent on the detection system of the instrument used, as well as the incident light intensity, it is difficult to put fluorescence intensity on an absolute scale. This greatly discourages the comparison of fluorescence intensities between instruments. Dramatic increases in the numbers of quantitative fluorescence assays in the past few years have generated a demand for standards to guarantee quality assurance and to satisfy regulatory agency mandates. A viable alternative to an absolute fluorescence intensity standard is a fluorescent concentration standard that establishes a quantitative relationship between fluorescence intensity and fluorescent species concentration. These concentration standards will enable fluorescence instruments, such as flow cytometers, fluorometers, fluorescence chip readers, etc., to be calibrated for fluorescence intensity for a particular fluorophore. Attendees of several NIST workshops on fluorescence standards identified fluorescein solution as their preferred concentration standard candidate due to its widespread use.

Major Accomplishments: A commercial source of high purity fluorescein powder has been secured. Its purity has been determined using several analytical techniques, including HPLC, MS, NMR, elemental analysis, etc. We have prepared a fluorescein solution in borate buffer at pH 9.1. Its concentration has been determined gravimetrically and its extinction coefficient has also been measured.

SRM 1932 fluorescein standard will be available in FY2002 with a certified value for concentration. Fluorescein solution decomposes when exposed to light, heat or oxygen, so SRM

1932 will be composed of a set of five, single-use, amber glass ampoules that are flame sealed under argon. They should be stored in the dark at about 5 °C. The stability of SRM 1932 over time has been tested at regular intervals for more than a year and will continue to be tested for at least another year. No measurable changes in fluorescence intensity or absorbance have been observed to this point. A fluorescence standard composed of a fluorescein-labeled microbead suspension is presently being developed. These microbeads mimic biological cells labeled with fluorescein. Therefore, this microbead solution standard will serve as a fluorescence intensity calibration standard in biological assays using fluorescein.

Impact: Fluorescence intensity standards are in great demand in the clinical and biotechnological communities. Dramatic increases in the numbers of quantitative fluorescence assays in the past few years have generated a demand for standards to guarantee quality assurance and to satisfy regulatory agency mandates. SRM 1932, fluorescein standard, to be issued in FY2002, is a fluorescent concentration standard that will provide viable alternative to an absolute fluorescence intensity standard that establishes a quantitative relationship between fluorescence intensity and fluorescent species concentration. Attendees of several NIST workshops on fluorescence standards identified fluorescein solution as their preferred concentration standard candidate due to its widespread use. A fluorescence standard composed of a fluorescein-labeled microbead suspension, also being developed will serve as a fluorescence intensity calibration standard in biological assays using fluorescein as the microbeads mimic biological cells labeled with fluorescein.

Future Plans: The stability of SRM 1932 over time has been tested at regular intervals for more than a year and will continue to be tested for at least another year.

New Thermal Neutron Prompt Gamma Ray Activation Analysis Instrument

CSTL Program: Chemical Characterization of Materials
Authors: *E.A. Mackey, and R.M. Lindstrom; D.L. Anderson (US FDA); and P.J. Liposky (856)*

Abstract: A new thermal neutron prompt gamma ray activation analysis instrument became operational in FY01. The new Ge detector has a resolution of 2.0 keV (for the 1332.5 keV line from ^{60}Co) and 40% relative efficiency (relative to a 7.6-cm cube of NaI). A sapphire filter was placed in the neutron beam shutter assembly and all external components of the original system were replaced. The new beam tube is made of two sections, constructed from aluminum lined with a lithiated polymer (lithoflex). Both sections are sealed at each end, and kept under vacuum. The sample chamber is also constructed of aluminum and lined with lithoflex. The chamber may be evacuated when necessary to eliminate neutron scattering by air. Experiments were performed to measure mass fractions of Cd in SRM 2702 Inorganics in Marine Sediment, and of B, K and Cl in SRM 1575a Pine Needles. Additional experiments were performed to determine the background count rates during various operational conditions, to measure element sensitivities in this configuration, and to determine the optimum amount of neutron and gamma ray shielding that will be incorporated into the final design for the detection system. Future work will involve optimization of the detection system and shielding configuration, design and fabrication of a variable position detector support platform, and complete characterization the new system.

Purpose: This instrument has been upgraded to improve analytical sensitivities and limits of detection, and decrease the levels of radiation associated with use of the instrument. This was achieved by minimizing gamma rays created by neutron capture in the components of the instrument and support structure, and by installing an improved detection system.

Major Accomplishments: A cylindrical 5.3-cm long (4.3-cm diameter) sapphire beam filter was incorporated into the shutter assembly. This modification reduced the number of fast neutrons by a factor of five, decreased the thermal neutron flux by approximately 12%, and greatly reduced low-energy gamma-ray background. The design and fabrication of the new beam tube, beam stop, sample chamber and support structure was completed. The sample chamber and beam tube were fabricated from aluminum, and lined with lithoflex. The beam tube is kept under vacuum to reduce background gamma-rays arising from neutrons scattered by air and captured in the surrounding materials. The beam stop consists of an aluminum box welded onto the aluminum support structure, and is filled with a cube of borated polyethylene surrounded by lead. A new Ge detector with improved resolution and peak-to-Compton ratio was installed. This detector has a relative efficiency of 40% (relative to a 7.6 cm cube of NaI) and 2.0 keV (peak full-width-half-maximum) resolution for the 1332.5 keV gamma ray line from ^{60}Co. Element sensitivities obtained using this new detection system with the new instrument are approximately 1.7 times greater than those obtained with the previous instrument and detection system. Background count-rates for H decreased by approximately a factor of five, and those for N are reduced by a factor of five when the sample chamber is evacuated. Replacing the NaI Compton suppression system with a bismuth germanate suppressor eliminated Na gamma-ray background but increased the background from neutron capture in Ge. In the future, the Ge background count-rate will be reduced by increasing the amount of neutron shielding surrounding the detector.

Impact: The design changes have greatly improved the signal-to-noise ratio in the low energy region. With this improved system, we will be able to determine mass fractions of several rare earth elements that could not be measured using the previous system, which was limited because of much higher background in the low energy region. Detection limits for H have been significantly improved because the background count-rate for this element has been lowered by a factor of 10. Improvements to the detection system, overall, have provided a twofold improvement in element sensitivities. This new instrument will allow much better characterization of biological , environmental, fuel, and various other materials. During FY01, the instrument was used to determine the mass fraction of Cd in SRM 2702 Inorganics in Marine Sediment, and mass fractions of B, K, and Cl in SRM 1575a Pine Needles.

Future Plans: In FY02, the design and construction of the detector shielding will be completed. A bismuth germanate Compton suppressor will be added to the detection system to reduce background gamma radiation associated with Compton events. The design and fabrication of a variable position support structure for the detection system will be completed.

Accurate Measurement of the Half-life of ^{76}As

CSTL Program: Chemical and Biochemical Data:

Authors: *R.M. Lindstrom; M. Blaauw (Delft University of Technology, The Netherlands; and R.F. Fleming (The University of Michigan, Ann Arbor)*

Abstract: In the course of high-accuracy measurements of arsenic, we found that the most recently published and compiled half-life of ^{76}As did not fit our data as well as the earlier accepted value. To redetermine this parameter, five ^{76}As sources were measured on four Ge detector systems, and the decay data fit to an exponential function by two different nonlinear least-squares methods. We determined $t_{1/2}$ = 1.0938 d with an expanded uncertainty U = 0.0009 d. This result is 1.5% higher than the recent value, and in agreement with the older, less precise, consensus value.

Major Accomplishments: Knowledge of half-lives for radioactive decay is necessary for all applications of radioactivity. In a few cases (such as the neutron and Ti-44) accurate knowledge is needed in grand problems like the Standard Model and supernova kinetics, but in most cases moderate accuracy suffices. However, in the course of high-accuracy measurements of arsenic in silicon we found that the most recently published and compiled half-life of As-76 did not fit our data as well as the earlier accepted value. The discrepancy between old and new recommended values amounted to 5% at the end of the 4-day experiment, an unacceptable error when we were seeking an overall (1s) error of 0.3% or better. We therefore used the old 1972 value reported by Emery at ORNL, because it is the mean of seven measurements with six different kinds of detectors and because it fit our data well. The 1994 measurement was made by Ge spectrometry and published with more experimental detail than Emery had done.

Evaluation and publication of nuclear data in the USA is centered chiefly at Lawrence Berkeley and Brookhaven National Laboratories. The primary data from the literature are collected in a database called the Evaluated Nuclear Structure Data File (ENSDF). Periodically the data for a given mass number are evaluated, and recommended values are published in Nuclear Data Sheets and in the Table of Isotopes. The 1994 value was adopted because the uncertainty analysis in that paper was more complete than in older publications.

During the past year we have returned to this issue. Using several detectors and two styles of nonlinear least-squares analysis of the data, we have re-measured the half-life, with precision (and inferred accuracy) an order of magnitude better than Emery's measurement. Our result and his overlap within the 1s uncertainties, but we both disagree completely with the 1994 value.

To verify our methods, we also measured the half-life of Au-198, one of the best-studied nuclides because of its fundamental importance in the unification of the energy and wavelength in photon spectroscopy. Our number agrees with the most precise (and adopted) value to within 0.1%. The problem with the 1994 paper is that the author fitted the log A vs t rather than A vs exp(-lambda*t); the former is to lead to half-lives that are too short, just what the 1994 paper shows for the four nuclides reported (Na-24, K-42, As-76, and Au-198.

A brief account of our work is now in NIST review for publication.

Impact: An erroneous published value for a physical parameter has been corrected. Correspondence with scientists involved in data evaluation has led to a revision of the accepted value of the As-76 half-life, and has led to the establishment of a new algorithm for combining data that is superior to the one previously in use.

Future Plans: A detailed publication on the NIST-IRI methodology is in preparation. Now that the weaknesses of the published data have been revealed and the power of the NIST methodology have been established, a continuing low-level effort to redetermine a selected set of half-lives will be carried out, in collaboration with the Ionizing Radiation Division.

Mechanisms for the Delivery of Traceability and Quality Assurance for Chemical Measurements

CSTL Programs: Chemical Characterization of Materials, Healthcare Measurements, Environmental Measurements, International Measurement Standards
Authors: *Analytical Chemistry Division Staff*

Abstract: Improving accuracy in both the state of the art and the state of the practice of making chemical measurements and providing traceability and quality assurance mechanisms for chemical measurements are principal tasks of the Analytical Chemistry Division (ACD). These mechanisms include:

- Quality Assurance and Traceability with SRMs
- Quality Assurance and Traceability through Definitive/Reference Methods
- Quality Assurance and Traceability via Standard Reference Data
- Quality Assurance and Traceability through NIST Traceable Reference Materials
- Quality Assurance and Traceability using Standard Reference Instruments
- Quality Assurance via Proficiency Testing

We continue to search for new mechanisms to most appropriately meet strategic, high priority quality assurance and traceability needs of our customers.

Purpose: Improving accuracy in both the state of the art and the state of the practice of making chemical measurements and providing traceability and quality assurance mechanisms for chemical measurements are principal tasks of the Analytical Chemistry Division (ACD). While many are familiar with our most visible mechanism for these tasks—Standard Reference Materials® (SRM®s)—, ACD has long provided other, less well known approaches, has recently developed new methods, and continues to evolve new schemes to benchmark chemical determinations by leveraging NIST measurement expertise.

Major Accomplishments:

Quality Assurance and Traceability with SRMs

SRMs have traditionally been the primary tools provided by NIST for achieving chemical measurement quality assurance and traceability to national standards. Of the nearly 1300 different types of SRMs offered for sale, about 700 are certified for chemical composition. In 2001, such "chemical" SRMs accounted for ~16,000 of the 32,000 SRM units sold, and the large majority of these SRMs were certified by ACD staff. Most SRMs are certified in batches where value assignments and measurement uncertainties are determined from measurements on a statistically significant number of samples. In a few instances (e.g., gas mixtures and optical filters), SRMs are individually certified. In some of these cases, ACD provides recertification services to extend the useful life of these standards. In FY '01, 176 optical filter SRMs were recertified for 125 different customers and 104 gas cylinder SRMs were recertified for 13 companies.

Quality Assurance and Traceability through Definitive/Reference Methods

The National Committee for Laboratory Standards (NCCLS) established the National Reference System for Clinical Laboratories (NRSCL) to provide a traceability structure for clinical laboratory measurements. Part of this system was establishment of a hierarchy of methods with three levels: definitive, reference, and field. Field methods are the routine methods used in clinical laboratories. Reference methods are more complex methods that are generally within the capabilities of many laboratories and are used to directly provide traceability to the field methods. At the top of the hierarchy are the definitive methods. These methods use techniques that are considered to be primary methods by international metrological organizations and provide accurate and precise results with complete uncertainty statements. NIST's role in the NRSCL is to develop and maintain definitive methods for clinical analytes and to apply these methods to the certification of SRMs and other certified reference materials (CRMs), which are then used to test the accuracy of reference and field methods. No other U.S. laboratory develops and maintains definitive methods and no other national metrology institute has as extensive a program as does NIST for such high level methods.

In the development of the NRSCL, NIST took a leadership role in developing and evaluation of the reference methods (initially called referee methods) as well as the definitive methods applied at NIST. The first example of this was for the element calcium and the theory and practice for the successful development of a reference method was expounded in this work. The candidate reference method was an atomic absorption spectrometric method that had been published in Clinical Chemistry. The method was developed by a team of experts from clinical chemistry and evaluated with eight clinical chemistry laboratories. The conclusion of the effort was that "subject to the limitations and provisos stated, calcium in serum can be determined by the referee method to within ±2 % of the 'true or absolute' value." Reference methods were initially evaluated in conjunction with the establishment of many of the definitive methods. The results have been published in the NBS Special Publication 260 series. [NBS SP 260-36, "A Referee Method for the Determination of Calcium in Serum"; NBS SP 260-60, " A Reference Method for the Determination of Sodium in Serum"; NBS SP 260-63, "A Reference Method for the Determination of Potassium in Serum"].

For some applications, definitive methods are either not possible or not practical. In such cases, NIST focuses on development of reference methods that are critically evaluated. When it is feasible, NIST will develop two independent reference methods for a given analyte and use results from both methods for certification of SRMs. If it is not possible or practical to develop a second independent method, NIST generally organizes an interlaboratory study with laboratories that have considerable experience with measuring the analyte in a matrix similar to that of the SRM. Whenever possible, NIST incorporates existing SRMs or other CRMs into the measurement schemes to serve as controls.

The ultimate goal of any chemical measurement traceability scheme is to show a linkage to the mole. There are substances where such a scheme is not possible, e.g., proteins and other complex biomolecules with variations in structure, but it is possible for most substances for which chemical measurements are performed. The requirement is that there must be a well-characterized substance, either elemental or a compound, with a known purity, that can be weighed and used as a primary reference material to calibrate reference and definitive methods. NIST uses an arsenal of techniques to establish the purity of the substances used as primary references, many of which are SRMs. Uncertainties in the results from the best analytical techniques can be no better than the uncertainty in the purity of the primary reference material used.

Quality Assurance and Traceability via Standard Reference Data

Many analytical techniques, especially spectroscopic methods, work by measuring a parameter that can be related to chemical concentration through the use of a material dependent constant. For example, quantitative spectrophotometry relies on the Beer-Lambert-Bouguer law that states that the optical absorbance of a solution of a given material is proportional to the pathlength of observation and the concentration of the material. The material property that relates concentration to absorbance at constant path length is called the absorption coefficient. Given the ability to make high quality determinations of the physical parameters (in this case absorbance and pathlength), chemical concentration measurements then depend on having highly accurate determinations of the material property (here the absorption coefficient). NIST has long provided such data through its Standard Reference Data (SRD) program. A SRD Quantitative Infrared Database (SRM 79) was developed in ACD to provide accurate absorption coefficients for the measurement of gaseous chemical emissions and hazardous air-pollutants(HAPs) (see separate TAR). SRD 79 provides absorption coefficient data for 40 HAPs on the U.S. EPA priority list. These data were critically evaluated from measurements made on gravimetrically produced gas samples and provide a solid foundation for high quality open-path field monitoring methods.

Quality Assurance and Traceability through NIST Traceable Reference Materials

Although NIST produces SRMs, it is not a production facility. There are instances where the demand for certain SRMs overwhelms our abilities to produce them. In some cases, the use of an SRM is mandated by statute or by the sheer magnitude of the financial consequences of certain chemical measurements. Environmental gas standards were propelled to that status by the passage of the Clean Air Act that permitted emissions trading. In response to this sudden demand for traceability in gas measurements, ACD created the first NIST Traceable Reference Materials (NTRM) program. Although the details of operation differ among NTRM programs, the basic notion is that NTRMs are produced and certified by commercial vendors with NIST providing an overseeing quality assurance function. The NTRM mechanism is appropriate where there is sufficient economic incentive to justify it and sufficient measurement control to make it feasible.

The Gas Mixture NTRM Program was initiated to be real time response to the needs of the Specialty Gas Industry (SGI). The Specialty Gas Companies (SGC) constantly monitor the needs of the end user community and determine what mixtures are needed to fill the needs. The required mixtures are then prepared in batches of ten or more cylinders by the SGC, analyzed, and data submitted to NIST. NIST selects 10% of the cylinders in the batch and analyzes them so that NIST can value assign the batch. The cylinders are returned to the SGC with Certification documentation for the batch. The cylinders are under control of the SGC and can be sold to end users to provide a NIST traceable gas or the NTRM mixtures can be used by the SGC to analyze other mixtures that are then sold to provide traceability. The qualifier on the part of NIST is that NIST must have the primary standards available to perform the NIST analyses. In FY '01, 36 batches of NTRMs were certified for four different companies.

The NIST Optical Filters NTRM program provides absorbance standards for visible spectrophotometry. Currently, this program is just getting underway with three vendors. In the Optical Filters NTRM program, each vendor is certified by the National Voluntary Laboratory Accreditation Program (NVLAP) to produce visible filter standards. Traceability is provided by the use of NIST SRM optical filters to calibrate vendor spectrophotometers, and quality assurance is afforded by NIST review of all vendor calibration, certification, and recertification data, NIST measurements (one set from each vendor production batch), and blind spot checks.

Quality Assurance and Traceability using Standard Reference Instruments

Providing a reference materials for very reactive or unstable chemical entities is particularly challenging. In certain cases, traceability and quality assurance can be provided through standardization of the instrumentation used to make the measurement. Since 1983 NIST has provided Standard Reference Photometers (SRP) for determining the concentration of ozone in air (see separate TAR). Based on UV photometry, ten ozone SRPs form the basis of the U.S. EPA's infrastructure to monitor ozone. Thirteen additional SRPs are now located around the world. Each of these SRPs was built and calibrated at NIST and is returned periodically to NIST for recalibration against one of two master SRPs maintained at NIST. In addition to providing SRPs, NIST offers a service that calibrates commercial ozone photometers against one of the NIST master SRPs. As the need for ozone measurements becomes more international, it seems appropriate that the primary reference standard be international rather than national. Accordingly, NIST is now working with the BIPM to develop a primary reference standard for ozone that can anchor all ozone measurements through a defined traceability structure.

Quality Assurance via Proficiency Testing

Ensuring that the providers of chemical measurements services actually provide accurate results is an important quality assurance issue everywhere, but it is a critical issue in regulated industries, such as clinical and environmental laboratories. In general NIST does not consider the testing of service laboratories to be within its purview. However, it is a NIST role to ensure that such testing is accurately done. Accordingly, NIST becomes involved in "testing the testers." ACD is beginning to provide this service for environmental laboratory Performance Test (PT) providers. PT providers offer performance evaluation services to individual laboratories by sending samples to the laboratory for analysis and evaluating the results. NIST helps provide a level playing field by providing NVLAP accreditation for PT providers and by assuring the quality of the PT samples. Traceability is provided through the use of NIST SRMs similar to the PT samples, while quality assurance is provided by ACD measurements on samples from the PT batches sent to NIST by the PT providers.

NIST has helped improve the state of the practice in certain areas by using a combination of its quality assurance and traceability tools. One example of this is the on-going Micronutrients Measurement Quality Assurance Program. In this program, ACD conducts interlaboratory test studies on ACD-analyzed samples sent from NIST to participating laboratories. Recent studies included one for vitamin C and two on fat-soluble vitamins. After analyzing the samples, the participating laboratories send their results to NIST where they are statistically evaluated.

Results are made available individually to each participant, and the collective results are presented at a group meeting. For laboratories with results outside the acceptable range, NIST provides consultation and assistance on analytical techniques. Using this mechanism, the spread of analytical values among the participants has narrowed markedly over the years.

A New Traceability Tool

The accuracy of measurements of lead concentration in blood, or arsenic in soil, or sulfur in an aircraft alloy, are all dependent on the availability of accurate elemental solution standards for calibration. Such standards are available from NIST as the SRM 3100 Series, but the volume of demand for such standards worldwide is too great for NIST to play a significant role as a direct supplier of such standards. Most laboratories purchase their standards from a commercial source, but require that these standards be traceable to the NIST SRM 3100 Series. It is here that these SRMs play their most important role as national standards for element concentration.

The certificates that accompany commercial elemental solution standards generally include a statement on the traceability of the standard to the matching SRM from the 3100 Series. A valid claim of such traceability requires a comparison measurement between the traceable standard and the SRM, with a stated measurement uncertainty. The uncertainty of the value of a standard that is traceable to an SRM must include the uncertainty of the SRM (typically 0.3 %) together with the uncertainty of the comparison measurement. Ideally this measurement uncertainty would be much less than the SRM uncertainty, but this is beyond the current capability of most laboratories using instrumental measurements. We have developed the technique we call High-Performance ICP-OES with the capability of performing comparisons between solution standards with relative standard uncertainties of 0.03 % using unmodified commercially available ICP-OES instrumentation, and are promoting its use by standards providers who wish to establish traceability between their standards and the SRM 3100 Series. As an aid to implementing this comparison method we have created an interactive spreadsheet-based "traceability tool" to guide an analyst through the procedure. This tool has been tested by several standards providers, who have seen significant reductions in the uncertainty of their ICP-OES comparison uncertainties using their existing instrumentation. Traceable analyte mass fractions have been assigned to standards with uncertainties that are virtually identical to the uncertainties of the SRM.

Impact: Improving accuracy in both the state of the art and the state of the practice of making chemical measurements and providing traceability and quality assurance mechanisms for chemical measurements are principal tasks of the Analytical Chemistry Division (ACD). These efforts impact U.S. industry's productivity and competitiveness as well as provide the measurement and standards infrastructural support for environmental quality and human health assessments.

Future Plans: In ACD, we continue to search for new mechanisms to provide quality assurance and traceability to our customers. For example, an international intercomparison exercise conducted this year has given rise to a notion of "intrinsic " standards that combine concepts from both SRD standards and reference methods. This idea is still evolving and will require further definition and research to determine if and/or where it is practical. Given the breadth of the U.S. economy and the diversity of our customer base, NIST will always have too much to do. Only by constantly re-evaluating our methods, leveraging our measurement capabilities, and continuing to develop new ways of delivering our services can we hope to keep up and push both the state of the art and the state of the practice in chemical measurements.

A NORAMET Evaluation of Holmium Oxide Solution Reference Materials for Wavelength Calibration in Molecular Absorption Spectrometry

CSTL Programs: Healthcare Measurements, Chemical Characterization of Materials, International Standards

Authors: *J.C. Travis, D.L. Duewer, M.V. Smith, M.D. Maley, and G.W. Kramer*

Abstract: Molecular absorption spectrometers operating in the ultraviolet and visible spectral regions ("UV/vis" spectrophotometers) are widely used in clinical laboratories and the pharmaceutical and chemical industries. To provide accurate measurements, these instruments must be validated or calibrated – with regard to both their intensity (transmittance) and location (wavelength) axes. NIST supplies a suite of SRM$^{®}$s designed to help users of UV/vis spectrophotometers ensure the validity of their measurements. Recently, NIST conducted a study among five laboratories within the three NORAMET NMIs to assess the current degree of comparability of wavelength measurements among these participants under "routine" operating conditions using commercial instrumentation and between the aqueous Ho_2O_3 wavelength standard produced by NIST (SRM 2034) in the U.S. and that provided by CENAM (DMR 41) in Mexico. The study also produced Ho_2O_3 solution spectral data from a variety of spectrometers for use in a NIST study of wavelength assignment algorithms and to provide a basis for a possible reassessment of the NIST-certified wavelength values. The resulting data show a substantial level of comparability across laboratories, measurement events, CRM preparations, and peak location algorithms. The results demonstrated that the wavelength comparability of the five participating instruments actually could be improved by calibrating all of the instruments to the consensus band locations of Ho_2O_3 solution. This finding supports the value of absorption-based wavelength standards for absorption spectrophotometers.

Purpose: To demonstrate comparability of wavelength measurements among the three National Metrology Institutes (NMIs) of the North American Cooperation in Metrology (NORAMET) and between the aqueous Ho2O3 wavelength standard produced by NIST (SRM 2034) in the U.S. and that provided by CENAM (DMR 41) in Mexico. The comparability study also was designed to acquire Ho_2O_3 solution spectral data from a variety of spectrometers for use in a NIST study of wavelength assignment algorithms and to provide a basis for a possible reassessment of the NIST-certified wavelength values for SRM 2034.

Molecular absorption spectrometers operating in the ultraviolet and visible spectral regions ("UV/vis" spectrophotometers) are widely used in clinical laboratories and the pharmaceutical and chemical industries. To provide accurate measurements, these instruments must be validated or calibrated – with regard to both their intensity (transmittance) and location (wavelength) axes. The NIST Chemical Science and Technology Laboratory supplies a suite of SRM$^{®}$s designed to help users of UV/vis spectrophotometers ensure the validity of their measurements.

Holmium oxide (Ho_2O_3) in dilute acidic aqueous solution provides a safe, convenient, and effective signal for UV/vis wavelength calibration, having numerous fairly narrow and symmetric absorption bands well distributed over the wavelengths of interest [Figure 1]. NIST SRM 2034, Holmium Oxide Solution Wavelength Standard from 240 nm to 640 nm, was first produced and certified in the mid-1980s. Since spectrophotometric instrumentation and practice have become vastly more "computerized" since that time, NIST conducted this NORAMET study to reevaluate the performance of aqueous Ho_2O_3 Certified Reference Materials (CRMs) under "routine" operating conditions using commercial instrumentation. This study was not intended to intercompare national wavelength scales but to demonstrate comparability of wavelength measurements among the participants and between the NIST and CENAM CRMs.

Major Accomplishments: In the NORAMET study, aqueous Ho_2O_3 CRMs were measured by five laboratories within the three National Metrology Institutes (NMIs) of the North American Cooperation in Metrology (NORAMET) under "routine" operating conditions using commercial instrumentation. The resulting data show a substantial level of comparability across laboratories, measurement events, CRM preparations, and peak location algorithms. At the same time, it demonstrated that the wavelength comparability of the five participating instruments can actually be improved by calibrating all of the instruments to the consensus band locations of Ho_2O_3 solution [Figure 2]. This finding supports the value of absorption-based wavelength standards for absorption spectrophotometers. Coupled with the demonstrated robustness of the band position values with respect to preparation and measurement aberrations, it also supports the concept of extending the present approach to additional NMIs in order to certify a properly prepared Ho_2O_3 solution as an intrinsic wavelength standard; i.e., utilizing centralized data analysis at NIST of well-calibrated raw spectra accumulated by well qualified personnel from selected NMIs. The use of a variety of high-end spectrophotometers – in qualified hands – has permitted a much more realistic evaluation of uncertainties than is possible from any single instrument.

Impact: "UV/vis" spectrophotometers are widely used in clinical medicine and the pharmaceutical and chemical industries. To provide accurate measurements, these instruments must be validated or calibrated – with regard to both their intensity (transmittance) and location (wavelength) axes. The NORAMET evaluation demonstrated a substantial level of measurement comparability among NORAMET NMIs and of the NIST and CENAM certified reference materials used to calibrate these instruments.

SRM 2034: http://srmcatalog.nist.gov/nist/products.nsf/1117b1be0285f3bc85256a180051cc88/412c4d6e3a20c46e85256a2b0045e477?OpenDocument&Highlight=0,2034

NORAMET: http://www.nrc.ca/inms/int_coop/noramet.html

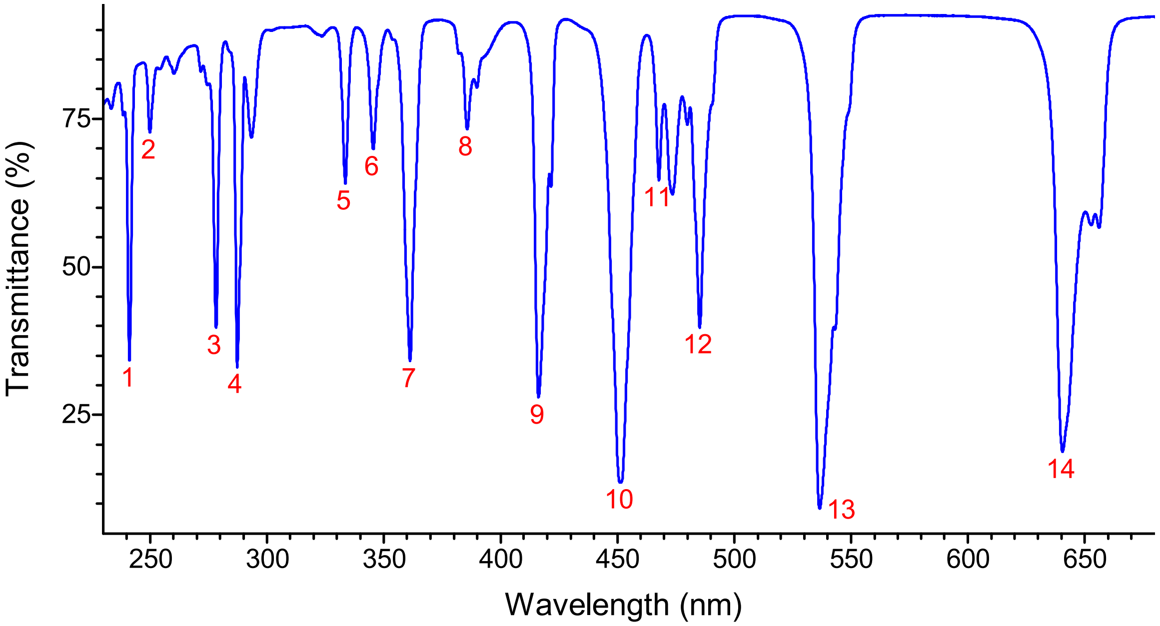


Figure 1. Spectral transmittance at 1 nm spectral bandpass of a 4 % solution of holmium oxide in 10 % perchloric acid solution. Bands having certified SRM® 2034 and DMR 41 locations are labeled "1" through "14".

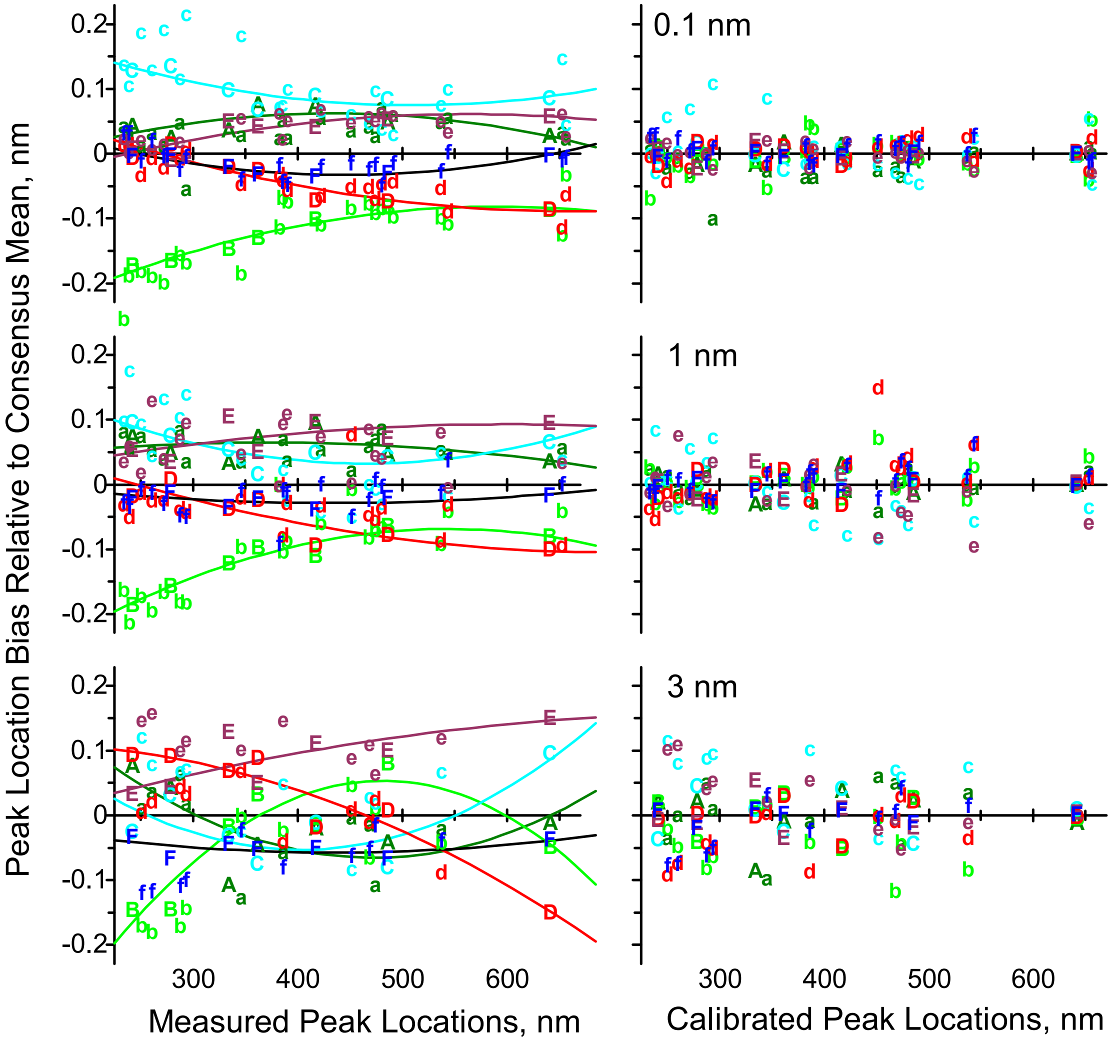


Figure 2. The three left-side segments display the among-set biases of the measured locations relative to the consensus mean for the 0.1 nm, 1 nm, and 3 nm spectral slit width (SSW) spectra. Biases are estimated as the location difference averaged over sets of eight spectra at each SSW. Quadratic polynomial calibration curves of the measured biases to the consensus locations are also displayed. The capital symbols denote the seven features used to define the calibrations. The three segments to the right display the bias data after adjustment to their calibration curve.

SpectroML—an Extensible Markup Language for Molecular Spectroscopy Data

CSTL Program: Chemical and Biochemical Data
Authors: *M.A.Rühl, M.G. Peschke, J.C. Travis, R.Schäfer, and G.W. Kramer*

Abstract: Today's analytical chemists need not only to interchange data generated within a specific technique, they, their organizations, and their colleagues need to interchange, import, export, store, and combine all their data from multiple sources at multiple sites. The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. In the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange. Over the past year, we have developed a "web-aware" mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectrometry data. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectrometry and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers among ourselves, our colleagues at other national metrology institutes, and our NIST-Traceable Reference Material (NTRM) vendors. We created SpectroML in the Extensible Markup Language (XML) and defined and documented it with both a DTD (Document Type Definition) and a schema. We have developed a few demonstration applications, applets, and stylesheets (used as templates to map document type conversions). SpectroML will evolve as the ASTM task group refines and expands it to work across the breadth of molecular spectrometry and perhaps even beyond to atomic spectrometry and chromatography.

Purpose: To develop, document, and provide demonstration applications of a "web-aware" mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange.

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 almost every commercial FT-infrared system can interchange their 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 year, we have developed a "web-aware" mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML: an extensible markup language for molecular spectrometry data. SpectroML was created initially to serve as a model implementation of a markup language for molecular spectrometry and ultimately to provide a web-based mechanism for interchanging UV/visible spectral data generated on different spectrophotometers among ourselves, our colleagues at other national metrology institutes, and our NIST-Traceable Reference Material (NTRM) vendors. Limiting our scope solely to UV/visible data, a very limited subset of molecular spectrometry data, has allowed us to keep our initial project manageable.

The vocabulary of SpectroML was gleaned from the terminology, data dictionaries, and concepts embodied in existing standards, instrument software, and data interchange formats. Our objective was to take advantage of the large body of work that has been done in the field of spectrometric data interchange rather than re-inventing it. With this concept of reuse firmly in hand, we studied terminology definitions in normative standards, spectrometer operation and software manuals, and existing native and interchange formats, extracted the most useful parts of each, and organized it into five categories: file (header information), instrument (information about the instruments used), sample (information about the samples processed), measurement (information about the measurement process), and data (the result data values and information about their structure). The information in each category consists of two main parts: the "data," which are the result data values from the experiment, and the "metadata," which are descriptive data about the "data." It is essential to keep both components together, because separately they both become useless.

We created SpectroML in the Extensible Markup Language (XML) and defined and documented it with both a DTD (Document Type Definition) and a schema. We have developed a few demonstration applications, applets, and stylesheets (used as templates to map document type conversions) and have used these demos to convince both instrument vendors and the ASTM E13 Molecular Spectrometry Committee that an XML-based approach is the way to go. At the 2001 Eastern Analytical Symposium, the ASTM E13 committee established a task group to support a markup language for molecular spectrometry based on SpectroML. Representatives from several major instrument companies have already signed on. SpectroML will evolve as the ASTM task group refines and expands it to work across the breadth of molecular spectrometry and perhaps even beyond to atomic spectrometry and chromatography.

Impact: The NIST-developed SpectroML, a "web-aware" mechanism, enables instrument-to-instrument, instrument-to-application, and application-to-application data interchange needed by todays analytical chemists. Representatives from several major instrument companies and the

ASTM E13 Molecular Spectrometry Committee support a markup language for molecular spectrometry based on SpectroML. NIST has developed demonstration applications but the impact of SpectroML will expand and evolve as an ASTM E13 task group and others refine and implement its use to applications across the breadth of molecular spectrometry and perhaps to other techniques.

Future Plans: At NIST, we are now developing the application programs to make SpectroML work with our and our NTRM vendors' instruments, so that we can begin to utilize it for UV/visible data interchange across the Internet.

UV-VIS-NIR Wavelength Standards for Transmission Measurements

CSTL Program: Chemical Characterization of Materials
Authors: *S.Choquette, D.L. Duewer, and D. Blackburn; L.M. Hanssen, and T. Early (844)*

Abstract: NIST continues to provide SRMs intended for the verification and calibration of the wavelength/wavenumber scale of ultraviolet (UV)-Visible-Near-Infrared (NIR) spectrometers operating in the transmission mode. SRM 2065 was developed to extend the operational range of existing NIST NIR wavelength SRMs, complement the existing NIST UV-Visible solution wavelength standard (SRM 2034) and provide a single glass optical standard useable from 334 nm to 1950 nm. A new SRM similar to SRM 2065 is currently being certified for wavelength verification and calibration of spectrometers operating in reflectance or quasi-reflectance (transflectance) modes.

Purpose: To provide SRMs intended for the verification and calibration of the wavelength/wavenumber scale of ultraviolet (UV)-Visible-Near-Infrared (NIR) spectrometers operating in the transmission mode.

Major Accomplishments: Standard Reference Material® 2065 (SRM®) is a certified transfer standard intended for the verification and calibration of the wavelength/wavenumber scale of ultraviolet (UV)-Visible-Near-Infrared (NIR) spectrometers operating in transmission mode. SRM 2065 is certified for the location of the center-of-gravity (COG) of twenty absorbance/transmittance bands, spanning the spectral region from 334 nm to 1946 nm. In addition, it is certified for the location of the COG of seven absorbance bands in the spectral region from 10,300 cm^{-1} to 5130 cm^{-1} at 4 cm^{-1} resolution.

The NIR spectra of organic analytes are composed of overtone and combination bands from the primary vibrational transitions found in the mid-infrared. The extinction coefficients of these bands are 10 to 10000 times lower than those of the mid-infrared bands. This allows NIR spectroscopy to be used directly with native matrix samples, mostly without sample preparation and typically in real-time. A growing number of chemical industries especially agriculture, food, and pharmaceutical manufacturing are looking to the NIR for methods to replace traditional spectroscopic and wet chemical techniques. Because NIR bands are broad and overlap, full-spectrum, "chemometric" models are used to correlate the chemical composition of the desired analyte with its NIR spectrum. A critical requirement for NIR determinations is the calibration of the wavelength axis. NIST currently offers three NIR wavelength Standard Reference Materials, SRM 1920, SRM 2517, and SRM 2035. Only SRM 2035 is suitable for the calibration of NIR spectrometers operating in transmission throughout the entire NIR spectral range. SRM 2065 extends the operational range of SRM 2035 to include 13 additional bands between 334 nm and 805 nm. Accordingly, this SRM complements the existing NIST UV-Visible solution wavelength standard (SRM 2034) and provides a single glass optical standard useable from 334 nm to 1950 nm [Figure 1].

The active element of SRM 2065 is a glass consisting of holmium, samarium ytterbium and neodymium oxides in a base glass containing oxides of lanthanum boron, silicon, and zirconium. The optical filter is 25 mm in diameter and 1.5 mm thick. This combination of the rare earth oxide concentrations and filter thickness yields absorption bands between 0.1 and 0.6 absorbance units (AU) in the NIR and 0.2 to 1.3 AU (5% to 60% transmittance) in the UV-Visible. The standard was batch certified using a number of FT and dispersive spectrometers in both the analytical chemistry division and optical technology division. The 95% confidence interval for the NIR band locations is better than 0.3 cm^{-1} for the seven certified NIR bands and less than 0.3 nm for the 13 certified UV-Visible peaks.

Impact: A growing number of chemical industries especially agriculture, food, and pharmaceutical manufacturing are looking to the NIR for methods to replace traditional spectroscopic and wet chemical techniques. Because NIR bands are broad and overlap, full-spectrum, "chemometric" models are used to correlate the chemical composition of the desired analyte with its NIR spectrum. A critical requirement for NIR determinations is the calibration of the wavelength axis. SRM 2065 extends the operational range of existing NIST NIR wavelength SRMs, complements the existing NIST UV-Visible solution wavelength standard (SRM 2034) and provides a single glass optical standard useable from 334 nm to 1950 nm.

Future Plans: We are currently certifying a new SRM similar to SRM 2065 for wavelength verification and calibration of spectrometers operating in reflectance or quasi-reflectance (transflectance) modes. SRM 2036 incorporates a glass very similar to that used in SRM 2065 into a sample holder containing a diffusely reflecting substrate (sintered polytetrafluoroethylene). This configuration will be useful for calibrating field spectrometers equipped with either integrating sphere or fiber probe sampling accessories

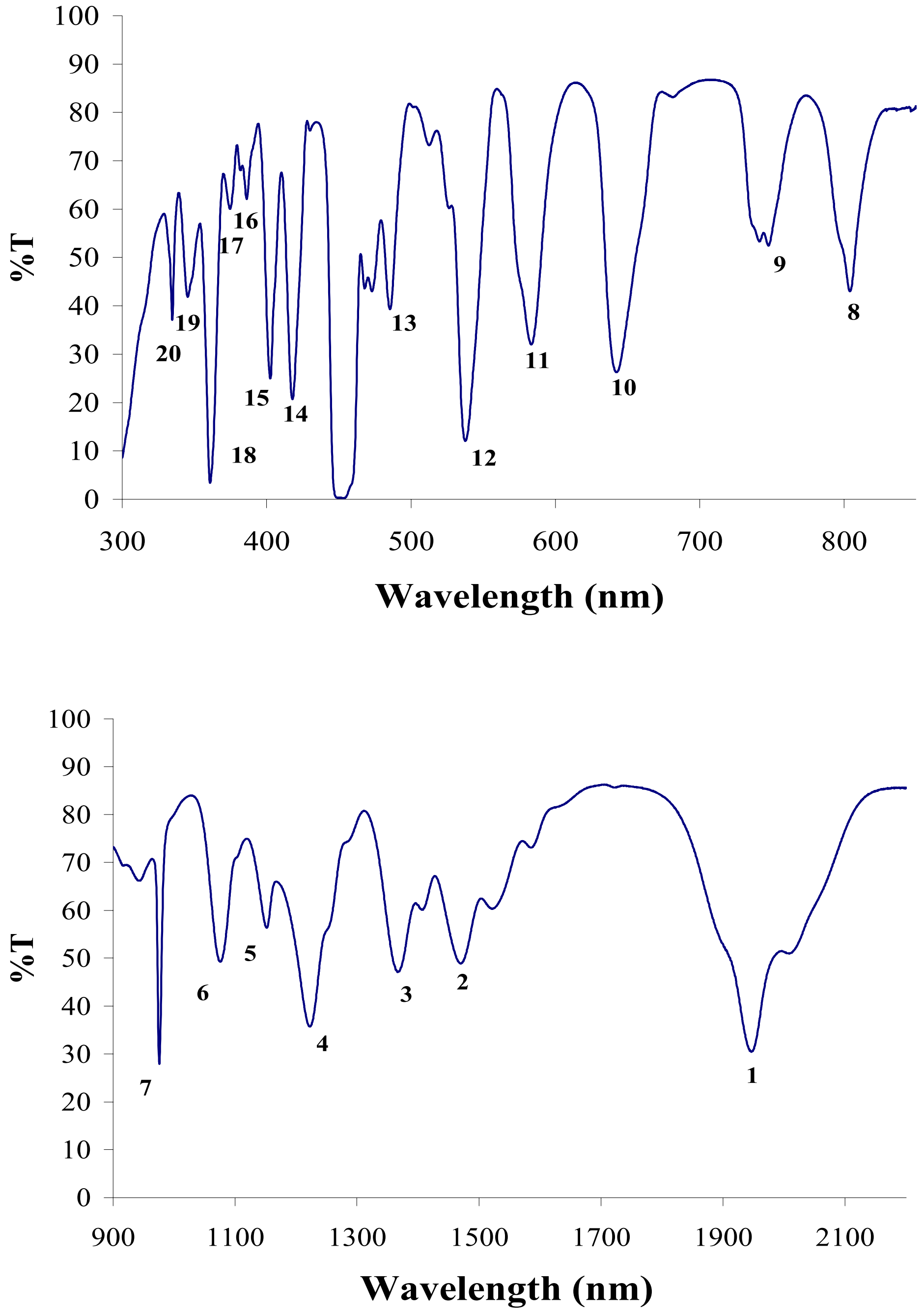


Figure 1. UV-Visible-NIR Spectra of SRM 2065

SRM 2241–A Relative Intensity Standard for Raman Spectroscopy using 785 nm Excitation

CSTL Program: Chemical Characterization of Materials
Authors: *S.J. Choquette, and D.H. Blackburn; E.S. Etz (837); and W.S. Hurst (836)*

Abstract: NIST SRMs for the calibration of the Raman intensity axis are needed to provide the analytical Raman community with the practical means to standardize Raman spectral data. This will promote the acceptance of Raman methods, facilitate the creation of standard Raman spectral libraries, and provide a means for instrument qualification as required by regulatory agencies. The first of a series of SRMs for the correction of the Raman spectral intensity is being issued this year. SRM 2241 is a certified transfer standard intended for the calibration of the spectral response function of a Raman spectrometer using 785 nm laser excitation. The active element of SRM 2241 is a fluorescent chromium-doped silicate glass that emits a broad, featureless fluorescence spectrum when excited with 785 nm laser radiation. It has been developed specifically to meet the needs of the analytical Raman community and is certified as an intensity standard for all common 785 nm excitation Raman spectroscopy systems employing macro- and micro-sampling configurations, as well as fiber-probe optical sampling. Because different materials will be needed for other laser excitation wavelengths, NIST is developing a series of Raman intensity correction SRMs for the popular excitation wavelengths. E.g., a uranium glass material is being qualified for Ar ion and doubled YAG laser excitations at 488nm/514nm and 532nm respectively (SRM 2242), a europium glass is being investigated for use with YAG lasers at 1064 nm (SRM 2243), and we are searching for a glass to use with HeNe at 633 nm (SRM 2244).

Purpose: NIST SRMs for the calibration of the Raman intensity axis are needed to provide the analytical Raman community with the practical means to standardize Raman spectral data. This will promote the acceptance of Raman methods, facilitate the creation of standard Raman spectral libraries, and provide a means for instrument qualification as required by regulatory agencies.

The first of a series of Standard Reference Materials® (SRM®s) for the correction of the Raman spectral intensity is being issued this year [Figure 1]. SRM 2241 is a certified transfer standard intended for the calibration of the spectral response function of a Raman spectrometer using 785 nm laser excitation. Using this standard allows the relative intensities of the bands observed in a Raman spectrum excited at 785 nm to be normalized to the intensity of a standard tungsten light source (white light). This, in turn, allows the direct comparison of spectra obtained on different instruments, the development of standard spectral libraries, and the verification of instrument performance. The active element of SRM 2241 is a fluorescent chromium-doped silicate glass that emits a broad, featureless fluorescence spectrum when excited with 785 nm laser radiation. It has been developed specifically to meet the needs of the analytical Raman community and is certified as an intensity standard for all common 785 nm excitation Raman spectroscopy systems employing macro- and micro-sampling configurations, as well as fiber-probe optical sampling. The fluorescence intensity of the SRM is calibrated using a certified NIST radiometric lamp standard.

Major Accomplishments: Recent advances in analytical Raman instrumentation, lasers, and detectors have revolutionized Raman spectroscopy. A variety of Raman spectroscopic techniques, employing both macro-and micro-sampling methods, are now well established in industry for process measurements and quality control. However, a general lack of accepted practices, standards, and spectral libraries hinders the acceptance of Raman spectroscopy, especially in regulated settings. While the calibration of the Raman frequency (or Raman shift, cm^{-1}) axis using pure materials and an existing ASTM standard is well accepted, techniques for calibration of the Raman intensity axis are not. Intensity calibrations of Raman spectra can be accomplished with certified white light sources, but this procedure has many drawbacks and provides numerous pitfalls to the uninitiated. These practical limitations are avoided by our approach using certified fluorescent glasses. The development of SRM 2241 and the future SRMs to be issued for other Raman laser excitation wavelengths has been closely coordinated with the Raman community made up of users from major chemical industries, instrument manufacturers, and the regulatory agencies. We have collaborated closely with the ASTM E13.08 Subcommittee on Raman Spectroscopy. For example, last year in conjunction with E13.08, we conducted an interlaboratory evaluation of the candidate glass for SRM 2241 that was critical to the determining the surface-finish characteristics and design of the holder for the glass.

The spectral characteristics and function of SRM 2241 have been characterized on three different and independent Raman systems. The detailed measurements leading to the certification of this SRM have addressed a number of experimental variables that have direct bearing on the application of this standard with different instrument designs—among these are the laser irradiance conditions employed, optical geometry and other aspects of the optical excitation and collection systems, variations of the spectral response of the luminescent glass with changes in ambient temperature, and departures from the exact (785 nm) laser wavelength. Part of the certification of this SRM entailed fitting a fourth-order polynomial to model the luminescence spectrum of the glass. The SRM user uses this polynomial equation to correct the band intensities in an acquired Raman spectrum to Raman intensities relative to white light. The mathematical uncertainties associated with these computational procedures determine the accuracy of the intensity correction. For SRM 2241, the accuracy is well within 10 percent bounds across the full Raman spectral range.

Impact: Having NIST SRMs for the calibration of the Raman intensity axis provides the analytical Raman community with the practical means to standardize Raman spectral data. This will promote the acceptance of Raman methods, facilitate the creation of standard Raman spectral libraries, and provide a means for instrument qualification as required by regulatory agencies.

Future Plans: Unfortunately, one intensity standard cannot provide intensity axis corrections for all Raman spectrometers. Different materials will be needed for other laser excitation wavelengths. We are committed to developing a series of Raman intensity correction SRMs for the popular excitation wavelengths. Accordingly, we are now in the process of qualifying a uranium glass material for Ar ion and doubled YAG laser excitations at 488nm/514nm and 532nm respectively (SRM 2242), are investigating a europium glass for use with YAG lasers at

1064 nm (SRM 2243), and are searching for a glass to use with HeNe at 633 nm and (and perhaps also diode lasers at 635 nm) (SRM 2244).

Figure 1. SRM 2241–Relative Intensity Correction Standard for Raman Spectroscopy: 785 nm Excitation.

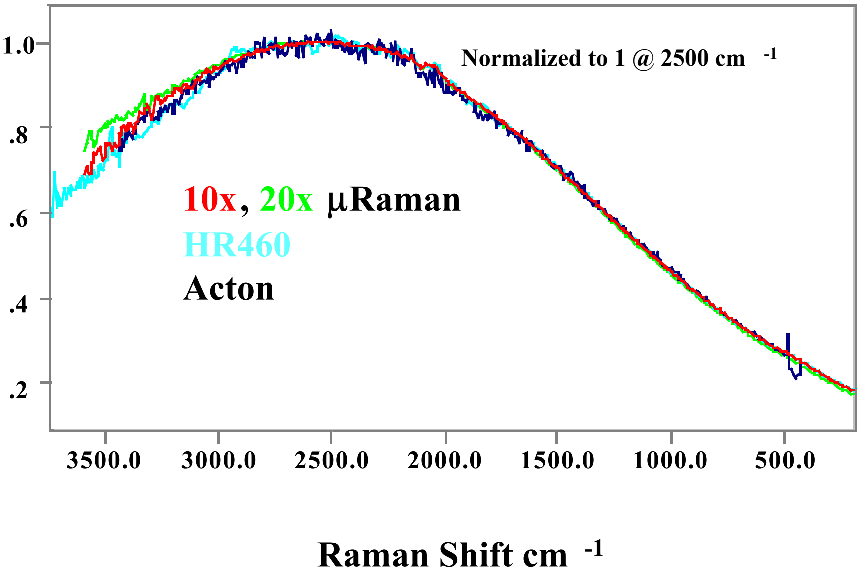


Figure 2. White light-corrected SRM 2241 on µRaman, HR460, and Acton Raman Systems

Measurement Tools and Standards for Forensic Chemistry

CSTL Program: Chemical Characterization of Materials
Authors: *Human Identity Testing Based on DNA: D.L. Duewer, and M.C. Kline*

Alcohol and Drugs of Abuse Testing: K.Phinney, M.J. Welch, L.T. Sniegoski, and S.S. Tai

Human Identification Based on Surface Components of Hair: B.A. Benner, and J.V. Goodpaster

Investigation of Handgun and Explosives Crime Scenes Based on Organic Residue Analysis: W.A. MacCrehan, and J.D. Secl

Chemical Weapons Treaty Verification: E. White, V.; W.G. Mallard (838); and E.S. Etz (837)

Abstract: Forensic chemical analyses have become important tools for solving crimes and assuring justice. Today, most forensic analysis techniques are qualitative and are used to identify or confirm the presence or absence of certain materials. However, in many cases applying quantitative analytical techniques can provide important additional information about material sources or the significance of material identifications. Over the past ten years, the NIST Analytical Chemistry Division has teamed up with the National Institute of Justice to provide measurement standards, interlaboratory comparisons, and new technology for forensic analysis.

Currently in the forensic area, the Analytical Chemistry Division develops quantitative analytical techniques for the measurement of drugs in hair samples and for the determination of gunshot residue, examines DNA measurement technologies using statistical techniques to assure the quality of such results for identification and evidentiary purposes, develops and certifies SRMs for forensic analyses that are used to validate the accuracy of methods and provide traceability for measurements made in forensic laboratories around the world and includes activities in support of chemical weapons treaty verification activities.

Purpose: To develop, evaluate critically, and apply quantitative analytical chemistry techniques for forensic analyses and to develop analytical standards for the forensic community.

Chemical measurements often play a pivotal role in criminal investigations. Making the correct identification of a rapist, determining if suspicious white powder is truly methamphetamine, or evaluating whether a kidnapped child spent time in the trunk of a suspect's car are all examples of how reliable chemical measurements can be used to help fight crime. Over the past ten years, the NIST Analytical Chemistry Division has teamed up with the National Institute of Justice to provide measurement standards, interlaboratory comparisons, and new technology for forensic analysis.

Perhaps the greatest impact of our measurement technology, standards, and quality assurance efforts has been in gaining the widespread acceptance of human identification based on DNA "fingerprints". Three NIST Standard Reference Materials (SRMs) designed for validating different fingerprinting methods have been developed and are routinely used by the forensic community. A recent NIST study reveals the importance of an accurate quantitation of DNA prior to PCR analysis.

CSTL has a long-term program to support accuracy in alcohol and drugs-of-abuse testing. Our activities include: development and maintenance of SRMs for alcohol use, urine drug testing and drug testing in hair. We have recently begun an effort to evaluate technologies used to screen substances for illicit drug content.

Research in our division has also pointed to a new idea in human identification based on surface components of the hair by evaluating the hydrocarbon profile of individual hairs. Fatty compounds such fatty acid/ester/alcohols, and cholesterol enter the hair from the sweat glands, root blood supply, shampoos and environmental exposure. By extracting a single hair into the inlet of a gas chromatography/mass spectrometry system using coupled supercritical fluid extraction, it is possible to obtain a profile for an individual. Youngsters appear to have a set of age-dependant characteristics, providing a means of identifying hair from lost or missing children.

Another problem facing forensic chemists is in the investigation of handgun and pipe bomb crime scenes. Since the 80's, we have championed the idea of quantifying the gunpowder propellant and its stabilizer additives as a means of evaluating residues from the perpetrator or crime scene and associating them with ammunition/powder samples recovered from a suspect. To aid forensic laboratories in making accurate gunpowder additive determinations, we are currently preparing a NIST Reference Material for gunpowder composition.

In addition, CSTL makes a significant contribution to the fight against international terrorism by participating in the international effort in Chemical Weapons Treaty verification.

Major Accomplishments:

Human Identity Testing Based on DNA

Working together, the Biotechnology and Analytical Chemistry Divisions of CSTL help ensure that human identify testing based on DNA measurement technologies provide unequivocal information for forensic, human remains, and paternity investigations. As an active member of the Scientific Working Group for DNA Analysis Methods (SWGDAM), we have contributed to the development of measurement technologies as well as the development of quality control and assurance tools. Starting in 1991, we have developed a series of Standard Reference Materials for evaluating and demonstrating the quality of various forms of DNA testing. We helped establish data validation criteria for the Federal Bureau of Investigation Laboratory's CODIS program. In collaboration with the Armed Forces Institute of Pathology's Department of Defense DNA Registry and others, we have evaluated the suitability of DNA archival media for long-term storage of blood stains at room temperature. Through a series of interlaboratory comparison studies now involving more than 80 DNA testing laboratories around the world, we are now evaluating the role that quantification of DNA has in the analysis of samples containing DNA from more than one source.

Current forensic human identity testing is largely based upon the identification of Short Tandem Repeat (STR) markers in DNA amplified via the Polymerase Chain Reaction (PCR). While PCR amplification has become quite routine, the amount of DNA used in the PCR reaction is widely recognized as a limiting factor. Commercially available STR typing systems are typically designed for amplification of about 1 ng of template DNA, with a stated tolerance of about a factor of two (from 0.5 to 2 ng). In a study of STR practice concluded in late 1999, we established that the expected among-laboratory standard deviation for estimating the quantity of DNA was itself about a factor of two [MSS1&MSS2]. Preliminary analysis of data from an interlaboratory study that will conclude in mid-October 2001 confirms this result [Figure 1] and suggests that it does indeed have consequences in the analysis of mixed-source DNA samples [Figure 2]. Too low an estimate of DNA quantity can lead to too much DNA being used in the amplification reaction (tending to increase the severity of amplification artifacts and thus complicates the analysis). Too high an estimate can lead to too little DNA in the reaction (tending to cause signals from minor source components to fall below established signal/noise thresholds).

DNA measurement technologies: http://www.cstl.nist.gov/biotech/DNAtech/dnatech.html

SWGDAM: http://www.for-swg.org/swgdamin.htm

Standard Reference Materials
http://srmcatalog.nist.gov/nist/products.nsf/all?SearchView&Query=dna&SearchOrder=4

CODIS program: http://www.fbi.gov/hq/lab/org/systems.htm

Department of Defense DNA Registry: http://www.afip.org/Departments/oafme/dna

STRs: http://www.cstl.nist.gov/biotech/strbase/

MSS1&MSS2: Duewer DL, Kline MC, Redman JW, Newall PJ, Reeder DJ. NIST Mixed Stain Studies #1 and #2: Interlaboratory Comparison of DNA Quantification Practice and Short Tandem Repeat Multiplex Performance with Multiple-Source Samples. In press, J Forensic Sci. (web link available soon.)

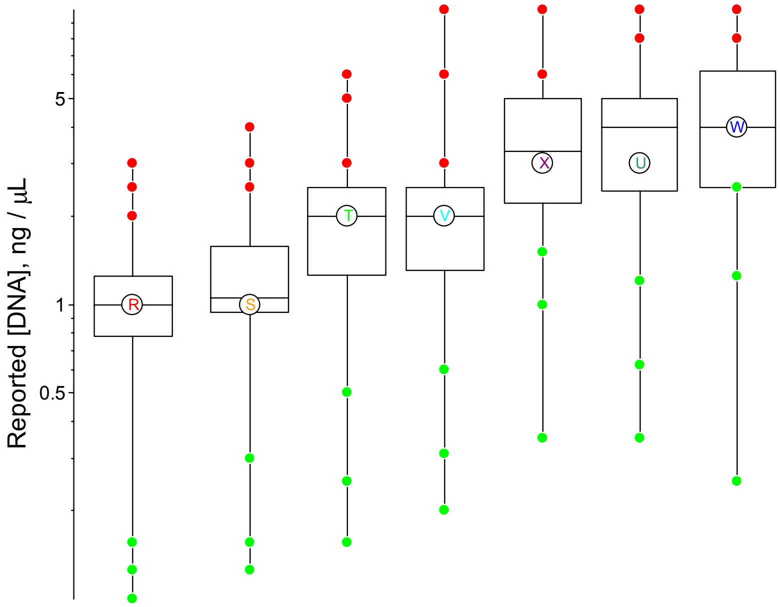


Figure 1. Figure DLD1
Distributions of Reported [DNA] Values

Box&whisker plot of reported [DNA] for seven DNA extracts distributed in the NIST-sponsored interlaboratory Mixed Stain Study #3. The "whiskers" span the lowest to the highest reported value for each extract. The "box" bounds the central 50% of the reported values; the horizontal line within the box denotes the interlaboratory median. The width of the box is proportional to the number of reported values. The open circles, labeled with the sample code, denote the designed [DNA] of each extract. The solid circles denote the same six participants, three each reporting extremely high (red) and low (green) [DNA].

For nearly all of the extracts, the interlaboratory median and the designed [DNA] are nearly identical. The cause(s) of the modest difference between the design and reported concentration for extract "U" is currently under investigation.

For all extracts, the central 50% of the participants report [DNA] that are within factors of 1.2 to 1.5 of the median. However, there are extreme values up to a factor of 8.0 from the median.

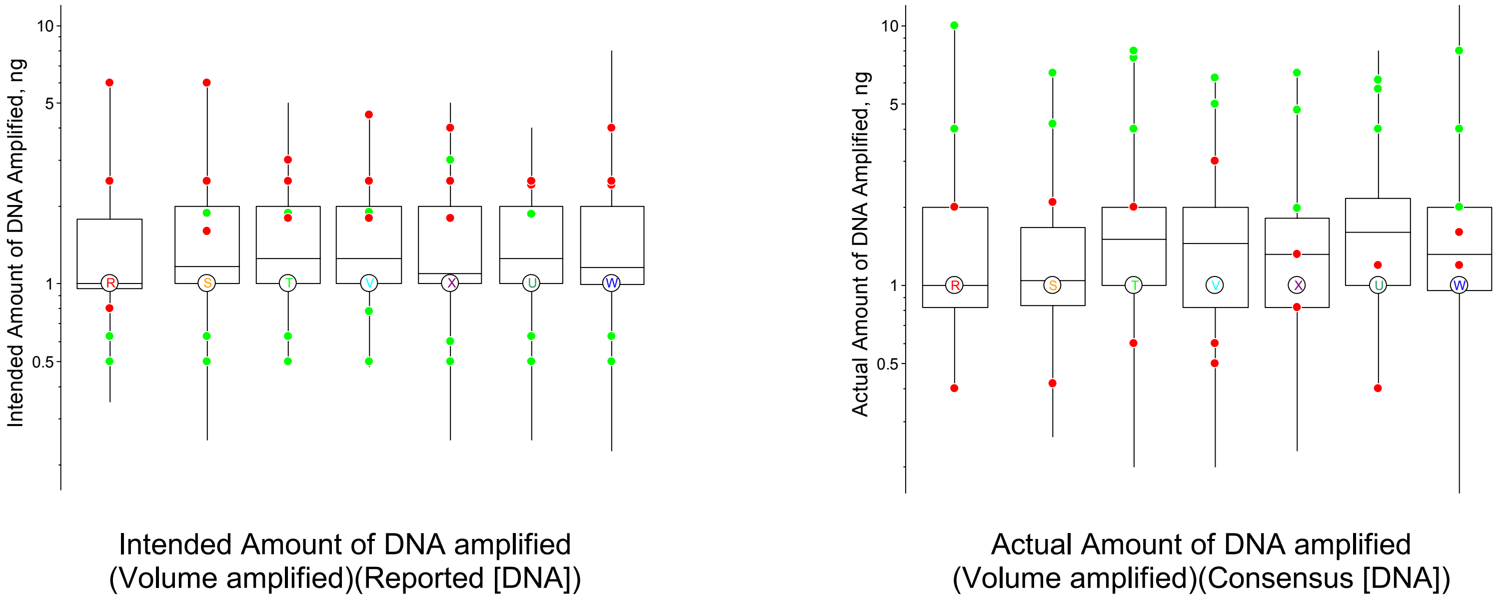


Figure 2.

The left-hand Box&Whisker shows the distributions of the amounts of DNA that the participants ***intended*** to amplify, calculated as the product of their reported [DNA] and volume of extract added to the PCR reagents. Most participants intended to amplify one to two ng of DNA, although some (such as one of those reporting very low [DNA] results) chose to target somewhat more or somewhat less based upon their past experience with their analytical system.

The right-hand Box&Whisker shows the distributions of the amounts of DNA that the participants ***actually*** amplified, using the observed median to define the "true" [DNA] for each extract. The participants reporting extremely high [DNA] (red circles) tended to amplify much less than their target amount of DNA and thus tended not to "see" alleles from the minor components of the multiple-source extracts. The participants reporting extremely low [DNA] (green circles) tended to amplify much more than their target amount of DNA. This often excessive amount of template DNA tended to increase the number of "stutter" artifacts and thus complicated their analyses.

Alcohol and Drugs of Abuse Testing

Substance abuse continues to be a major problem in the U.S. The National Institutes of Health (NIH), estimated that the economic cost of alcohol and drug abuse was $246 billion in 1992 (http://www.nih.gov/news/pr/may98/nida-13.htm), with alcohol abuse and alcoholism generated about 60 percent of the estimated costs ($148 billion), while drug abuse and dependence accounted for the remaining 40 percent ($98 billion). In Texas alone, economic costs associated with substance abuse were estimated to be $19.3B in 1997 (http://www.tcada.state.tx.us/research/economics/1997/), with productivity losses and premature death the primary contributors to the cost.

To combat the deleterious effects of substance abuse and to discourage its spread, various governmental bodies at the local, state, and federal level as well as private industry employ various drug tests. Testing may involve analysis of blood or breath for alcohol consumption, analysis of body fluids and tissues for drugs of abuse or their metabolites, or analysis of substances suspected of being illicit materials. Subjects tested may include persons suspected of driving under the influence of drugs or alcohol, suspects in other crimes, people on probation, applicants for jobs, persons holding jobs, particularly in cases where public safety is involved, and even parents in custody disputes.

There are many potential consequences of a positive drug test for an individual, including imprisonment, loss of employment, etc, therefore it is critical that tests be accurate, with very low instances of false positives. NIST has a long-term program to support accuracy in substance abuse testing. The activities include development and maintenance of SRMs for alcohol use testing; development and maintenance of Standard Reference Materials for instrument calibration and/or method validation associated with urine and hair testing as well as evaluation of products used to screen substances for illicit drug content.

SRM 1828a consists of a series of aqueous solutions with known concentrations of ethanol. For more information see (http://srmcatalog.nist.gov/). This SRM is used to calibrate breathalyzer devices used to test for the presence of alcohol in the breath of drivers suspected of being intoxicated. Levels deemed to be above the legal limit vary between states and over time. Recently NIST verified the stability of the ethanol in this SRM and plans are underway to add new levels to better address the lower levels that many states are implementing.

Over the last decade, NIST developed a series of human urine based SRMs for drugs of abuse, particularly those drugs identified as substances to be tested for in the Federal workplace (http://workplace.samhsa.gov/frames/frame_fed.htm). These SRMs include:

SRM 1507b	Marijuana metabolite in freeze-dried urine
SRM 1508a	Benzoylecgonine (cocaine metabolite) in freeze-dried urine
SRM 1511	Multidrugs of abuse in freeze-dried urine
SRM 2381	Morphine and codeine in urine
SRM 2382	Morphine glucuronide in urine

More information can be found at (http://srmcatalog.nist.gov/). NIST regularly monitors the stability of these SRMs.

In recent years, considerable attention has focused on hair testing for drugs of abuse. Hair provides a long window of detection for drug use up to several months, as opposed to urine or blood testing which only show evidence for drug use over the previous few days. There are a number of controversial issues related to using hair analysis for drug testing, but a number of commercial laboratories are now regularly performing these analyses. Applicants for jobs at many private and public organizations now must submit a hair sample for drug testing as part of the screening process and many parolees are now also being tested in this manner.

As with urine drug testing, accuracy is critical for hair testing, considering the consequences of a positive test. NIST is developing two new drugs of abuse in human hair SRMs to provide laboratories with tools for evaluating the accuracy of their methods. A total of ten analytes were soaked into the two human hair materials. The concentrations of these analytes will be certified in the two materials, using NIST measurements involving GC/MS and LC/MS. These materials will be available in FY 02.

As part of a research effort in the separation and measurement of chiral compounds, NIST is investigating the measurement of chiral drugs in hair. For example, methamphetamine, a chiral compound, is an illicit substance produced in clandestine laboratories. The illicit material is the "d" enantiomer, while the "l" enantiomer is found in over-the-counter health products. A chiral separation is necessary to separate the illicit form from the legal form, which otherwise have identical chemical and physical properties. The goal of this research is to develop methods and standards to support critical chiral separations.

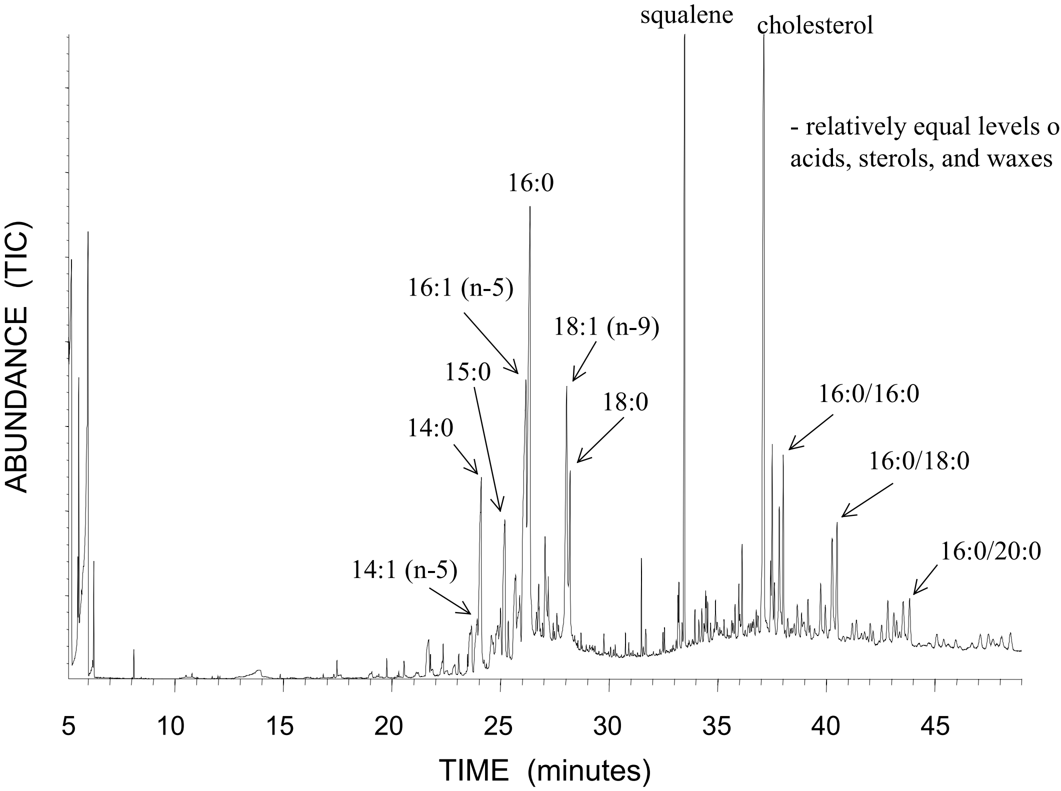
One new area for NIST involves the evaluation of spray reagents used to detect drugs of abuse. In routine police stops, substances are sometimes found that resemble drugs of abuse. The police need a quick means to determine if the substances may be drugs of abuse. Certain products are being marketed to police and perhaps, even to schools, for conducting such tests, with the warning that positive tests should be subjected to confirmatory testing. Because of concerns at the National Institute of Justice about the sensitivity and specificity of these products, NIST was asked to evaluate some of them. NIST is testing products for detecting cannibis, cocaine, and opiates for sensitivity and for reactions to non-drug materials used by people. This independent review will provide law enforcement personnel with valuable information as to the performance of such products.

Human Identification Based on Surface Components of Hair

An ongoing study in our laboratory is the characterization of surface components of small samples of hair as a trace evidence analytical technique. Results from a preliminary study of hair samples from 20 volunteers using an on-line supercritical fluid extraction-gas chromatography/mass spectrometry (SFE-GC/MS) technique suggested that the chemical profile of surface components from an individual's hair may be used to distinguish him/her from another person. This analysis might provide complementary information to other microscopic and genetic techniques currently used in forensic laboratories.

Recently, macroscopic amounts (0.2 - 1.3 g) of a composite hair sample from a single subject (collected from age 10 – 18 y) were extracted using four different extraction techniques and four different solvents. Of the techniques studied, pressurized solvent extraction was the most convenient and was amenable to sample sizes as small as 50 mg. Subsequent GC/MS analysis of the hair extracts has shown them to contain a complex mixture of lipids (see Figure below). The major components observed in the hair extracts were largely uniform across all solvents used for extraction, consisting of saturated and unsaturated free fatty acids from C_{12} to C_{18}, cholesterol, in addition to squalene and larger waxes such as fatty acid esters. Consistent with the biological origin of the material, even carbon number fatty acids and esters predominated. One interesting result was that consistently higher levels of free fatty acids and cholesterol were observed in hair from pre-pubescent versus post-pubescent individuals.

Future work will compare the chemical profiles generated from extraction and analysis of small hair samples by SFE-GC/MS and pressurized solvent extraction with GC/MS analysis. Once the most effective analytical scheme is determined, hair samples from approximately 100 individuals will be characterized and the data examined for correlations based on age, gender, and ethnicity.



Pressurized solvent extraction (dichloromethane), 0.26 g of hair, asian male, 10-18 years (composite).

Investigation of Handgun and Explosives Crime Scenes Based on Organic Residue Analysis

For over ten years, CSTL has been developing new measurement technology and investigating standards issues for the detection of handgun and explosives crime. A comprehensive listing of our publications follows this text. Much of our recent research has been sponsored by the NIST Office of Law Enforcement Standards in collaboration with the National Institute of Justice. Our work is based on determining the organic additives in smokeless gunpowder residues. When gunpowder burns, it leaves minute traces as residues that can be collected as forensic evidence. For handgun crime, unburned particles of organic gunshot residues (OGSR) may be collected from the suspect's hands, hair or clothing. At explosive's crime scenes, forensic investigators look for residue particles amongst the debris from the explosion.

In our CSTL research on handgun residues, we have investigated the measurement of compounds added to smokeless powder as markers for residue detection. By measuring additives such as propellant nitroglycerin (NG) and stabilizers including diphenylamine (DPA), DPA's primary initial nitration product N-nitrosodiphenylamine (NnDPA), and ethyl centralite (EC – N, N'-diethyl-diphenylurea) the presence of gunpowder can be confirmed. A particular emphasis of the CSTL studies has been on quantitative determination of the additive content of gunpowder and its residues. In a model study, firing known gunpowder from handguns, we have investigated the composition of powder before and after firing. Overall, we have found that the residues are basically unburned gunpowder, reflecting well the composition before firing. The amount of NnDPA is often increased relative to DPA and the variability of the composition of the residues seems higher. For NG containing powders, we have found it useful to determine the ratio (P/S ratio) of propellant NG to total stabilizer (DPA+NnDPA or EC). Good P/S value agreement was found for handgun test firings of some commercial ammunition (J Forensic Sci 2001). We are currently working on the idea of collecting gunshot residues by combing shooter's hair with a fine toothed comb.

The analysis of smokeless powder is also important to the investigation of "improvised explosives" aka pipe bomb crime. In examination of the debris form an explosion, relatively intact powder grains can frequently be collected. Investigators then can categorize the residues by making dimensional and shape observations and to determine which of the additives is present. Accurate categorization relies on correct additive identification.

Recently, CSTL/NIST OLES sponsored an interlaboratory study of smokeless powder compositional and dimensional measurements. Besides our CSTL laboratory, the nineteen participants included laboratories from state and local, national, and international forensic laboratories. We sent two smokeless handgun reloading powder samples that had been previously characterized by NIST. Overall, participants accurately identified the primary additives. Some disagreement was noted for the determination of trace additives. Four participants also submitted quantitative results.

We are currently working to develop a smokeless powder reference material. With this NIST-traceable material, forensic laboratories can validate their analytical methods. The reference material could also be used as a proficiency test sample, verify a laboratory's capability in explosives analysis. One state crime lab system used their successful participation in our interlaboratory study as partial requirements for their ASCLD-LAB accreditation for explosives determination.

Publication List

Separation and Identification of Gunshot and Explosive Constituents by Micellar Electrokinetic Capillary Electrophoresis; Northrop, D.M., MacCrehan, W.A., and Martire, D.E.; Anal. Chem. 63, 1038-1042 (1991).

*Sample Collection, Preparation and Quantitation in the Micellar Electrokinetic Capillary Electrophoresis of Gunshot Residue*s; Northrop, D. M. and MacCrehan, W. A.; J. Liq. Chromatogr. 15, 1041-1063 (1992).

Smokeless Powder Residue Analysis by Capillary Electrophoresis; Northrop, D.M. and MacCrehan, W.A.; NIJ Report 600-91 (1997).

Detection of Smokeless Powder Residues on Pipe Bombs by Micellar Electrokinetic Capillary Electrophoresis; Smith, K.D, McCord, B.R., MacCrehan, W.A., Mount, K. and Rowe, W.R.; in Proceedings of the 5th International Symposium on Analysis and Detection of Explosives, C.R. Midkiff, Ed. (1997).

Sampling Protocols for the Detection of Smokeless Powder Residues Using Capillary Electrophoresis; MacCrehan, W.A., Smith, K.D. and Rowe, W.R.; J. For. Sci. 43 (1), 119-124 (1998).

Detecting Organic Gunpowder Residues from Handgun Use; W. A. MacCrehan, K. M. Ricketts, R. A. Baltzersen, and W. F. Rowe; Proceedings of the SPIE Conference on Enabling Technologies for Law Enforcement and Security, K. Higgins, A.T. DePersia, and E.M. Carapezza, eds., SPIE Press, Bellingham, WA, (1998). pp. 116-124.

Detection of Smokeless Powder Residue on Pipe Bombs by Micellar Electrokinetic Capillary Electrophoresis; Smith, K.D., McCord, B.R., MacCrehan, W.A., Mount, K. and Rowe, W.F.; J. For. Sci. 44 (4), 789-794 (1999).

Comparing the Additive Composition of Smokeless Gunpowder and Handgun-fired Residues; Reardon, M.R., MacCrehan, W.A., and Rowe. W.F.; J. For. Sci. 45, 1197-1202 (2000).

NIST Efforts to Quality-Assure Gunpowder Measurements; MacCrehan, W.A., Reardon, M.R.; Proceedings of the JANNAF 29th Propellant Development and Characterization Meeting, CPIA Publication #697 - May 2000.

Investigating the Effect of Changing Ammunition on the Composition of Organic Additives in Gunshot Residue (OGSR); MacCrehan, W.A., Patierno, E.R., Duewer, D.L., and Reardon, M.R.; J. For. Sci. 45, 54-59 (2001).

Developing a Quantitative Extraction Technique for Determining the Organic Additives in Smokeless Handgun Powder; Reardon, M.R. and MacCrehan, W.A.; J. For. Sci. 2001; 46, 802-807.

Associating Gunpowder and Residues from Commercial Ammunition Using Compositional Analysis with the Propellant to Stabilizer Ratio (P/S); Reardon, M.R., MacCrehan, W.A., and Duewer, D.L.; J. For. Sci. 2001, *in press.*

A Preliminary Interlaboratory Study of Forensic Smokeless Powder Measurements; MacCrehan, W.A. and Reardon, M.R.; J. For. Sci. 2001; *in press.*

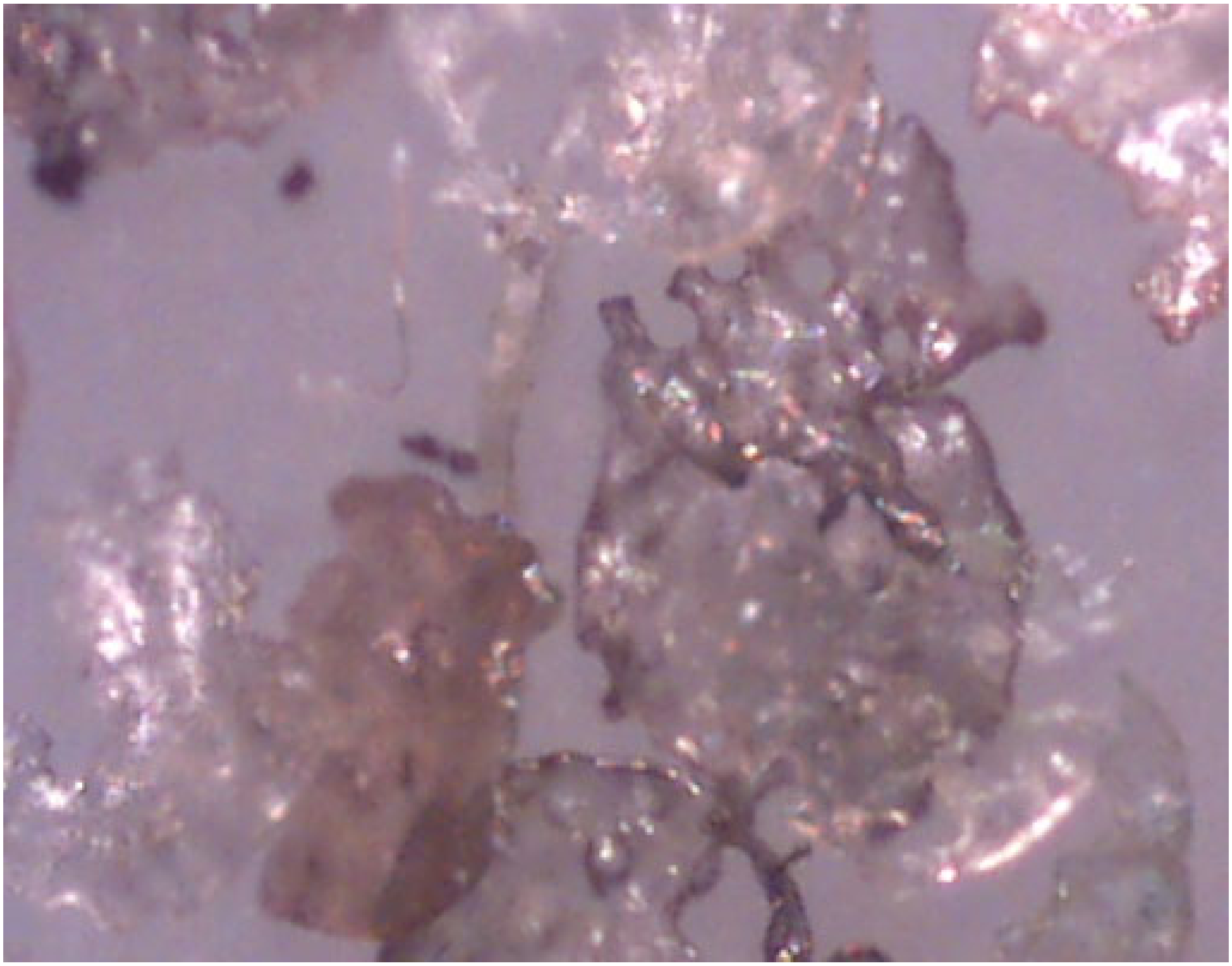
Testing a Little Gunpowder: An Interlaboratory Study of Smokeless Gunpowder Measurements; MacCrehan, W.A., Reardon, M.R., and Duewer, D.L.; *submitted to* Anal. Chim. Acta (2001)

Hot links:
Model study
http://www.astm.org/cgi-bin/SoftCart.exe/jforensicsci/PAGES/3382.htm?L+mystore+prne5938

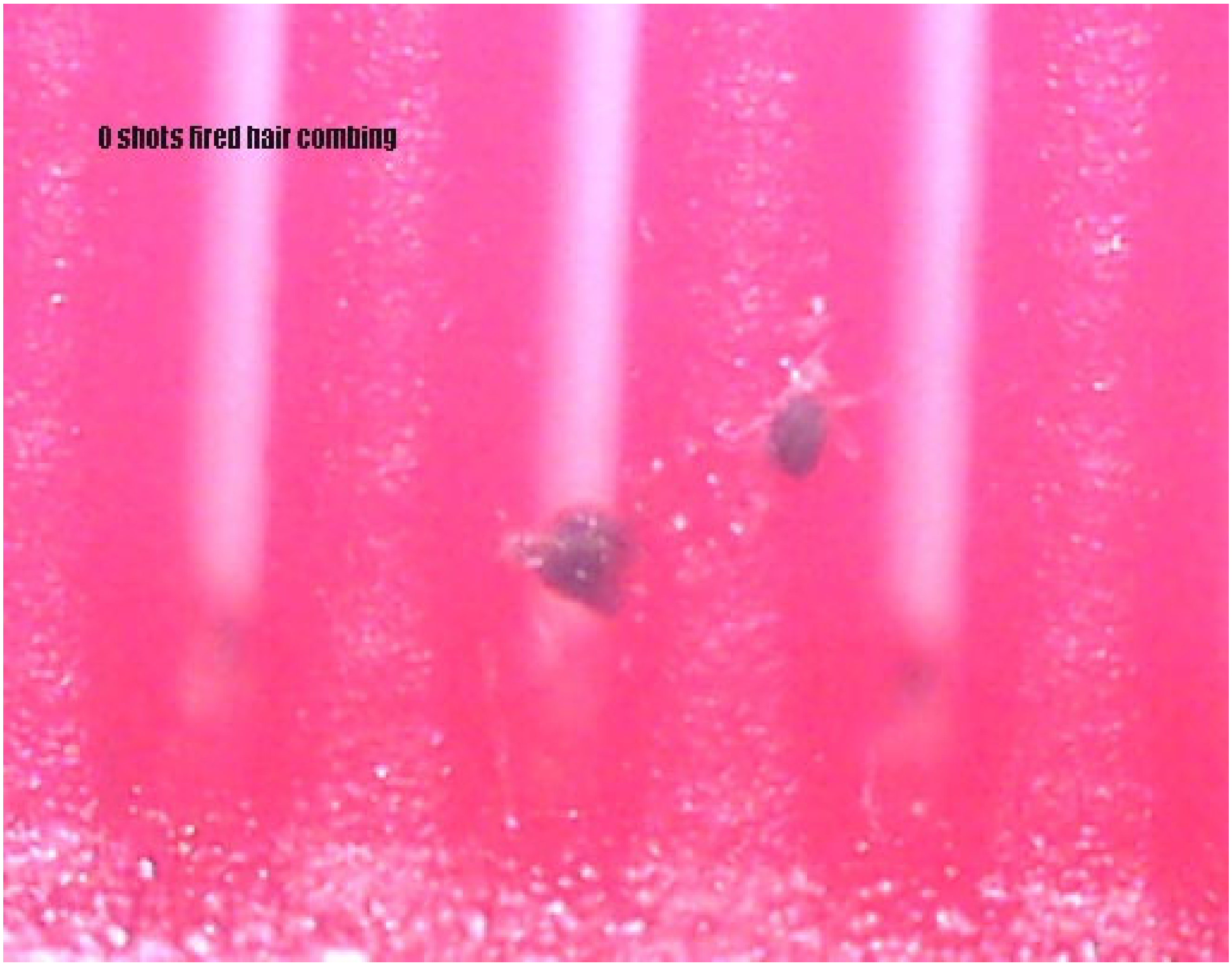
NIST Office of Law Enforcement Standards
http://www.eeel.nist.gov/oles/

ASCLD-LAB
http://www.ascld-lab.org/

Organic gunshot residues



Combing shooter’s hair



Chemical Weapons Treaty Verification

CSTL is taking a prominent role in the international effort to prevent the misuse of technology for the manufacture of chemical weapons. We are working with the Organization for the Prohibition of Chemical Weapons, whose mission is to administer the Chemical Weapons Convention (CWC). As members of the Validation Group for the Central Analytical Database, CSTL researchers from Divisions 837, 838, and 839 are responsible, along with representatives from other countries, for insuring the quality of the data added to the OPCW database. High quality data is essential to the correct identification of restricted or prohibited chemicals during inspection of chemical manufacturing facilities. There are several working groups within the Validation Group devoted to different chemical data types: infrared spectra, retention data for gas chromatography, and mass spectral data. CSTL experts support the United States Delegation to the OPCW with technical assistance on the drafting of documents relating to the taking and transfer of samples at inspection sites under the CWC. In addition, CSTL researchers provide direct onsite support for the consultations in The Hague.

The U.S. is one of the 143 signatory parties to the CWC, a global treaty bans chemical weapons. Issued in August 1994, the Convention reaffirmed principles and objectives of and obligations assumed under the Geneva Protocol of 1925, and the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction signed at London, Moscow and Washington in April 1972.

U.S. companies engaged in activities that involve certain chemicals may be required to submit reports to the Department of Commerce and may be subject to inspection by the OPCW.

Organization for the Prohibition of Chemical Weapons: http://www.opcw.nl/
Chemical Weapons Convention: http://www.opcw.nl/guide.htm

Impact: Chemical measurements often play a pivotal role in criminal investigations. Making the correct identification of a rapist, determining if suspicious white powder is truly methamphetamine, or evaluating whether a kidnapped child spent time in the trunk of a suspect's car are all examples of how reliable chemical measurements can be used to help fight crime. Over the past ten years, the NIST Analytical Chemistry Division has teamed up with the National Institute of Justice to provide measurement standards, interlaboratory comparisons, and new technology for forensic analysis.

Future Plans: The NIST Analytical Chemistry Division will continue its collaborations with the forensics community to assess needs and priorities for ACD development and critical evaluation of quantitative analytical chemistry techniques and to develop high priority analytical standards for the forensic community.