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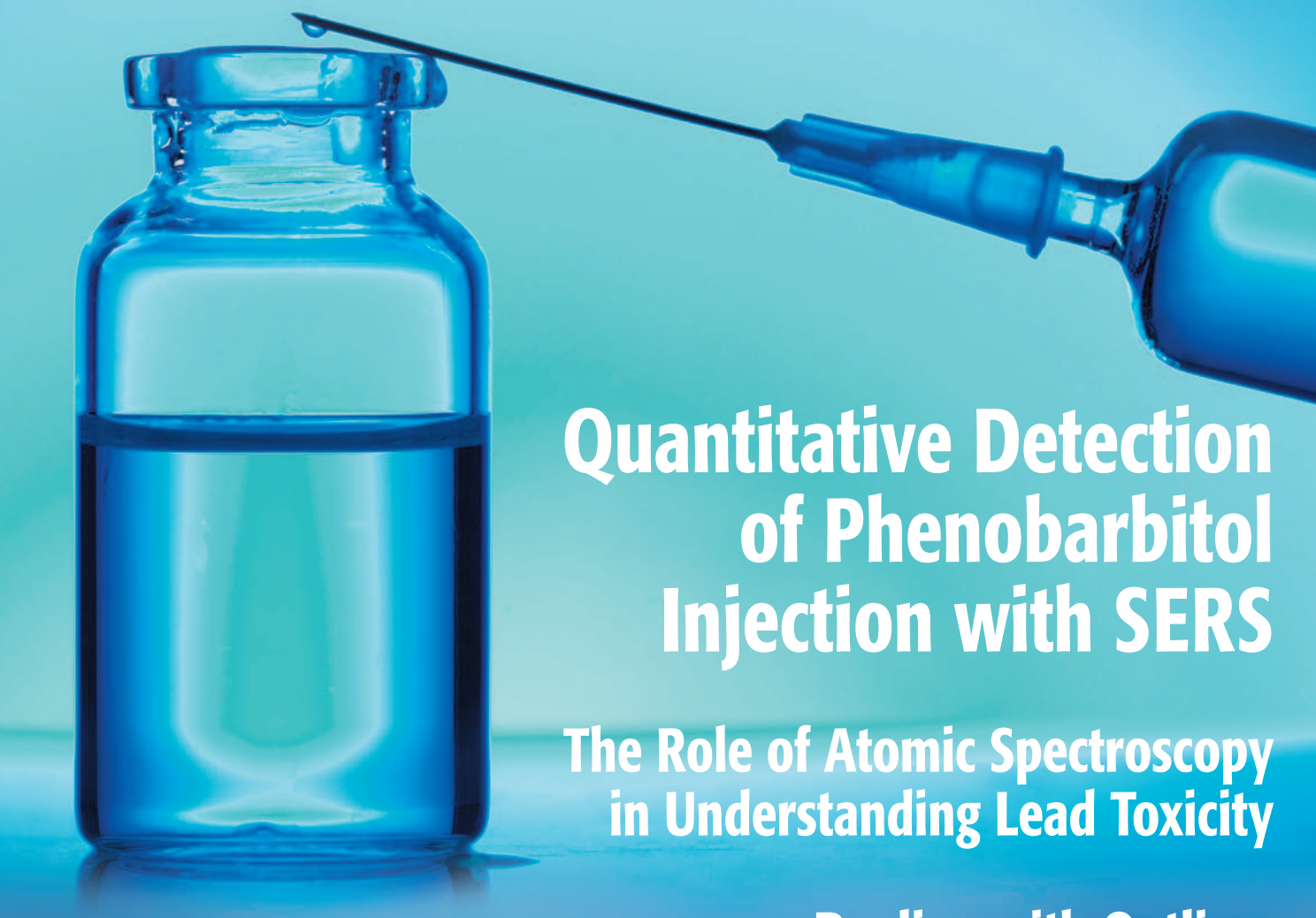

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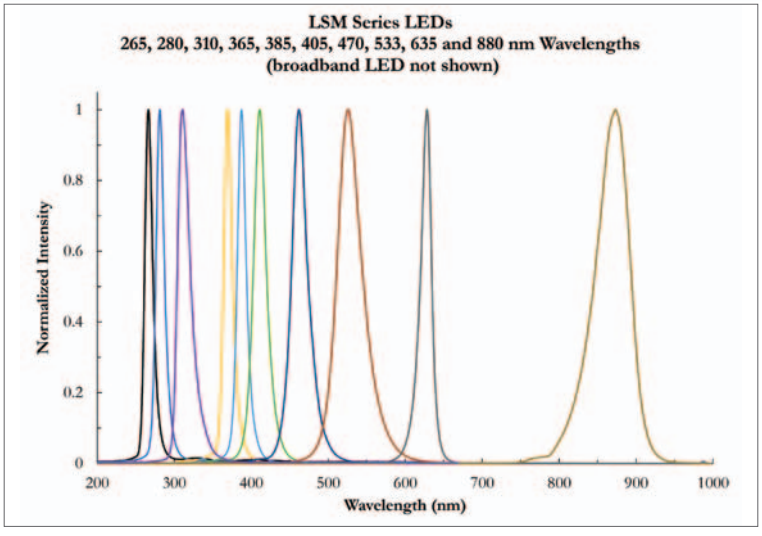

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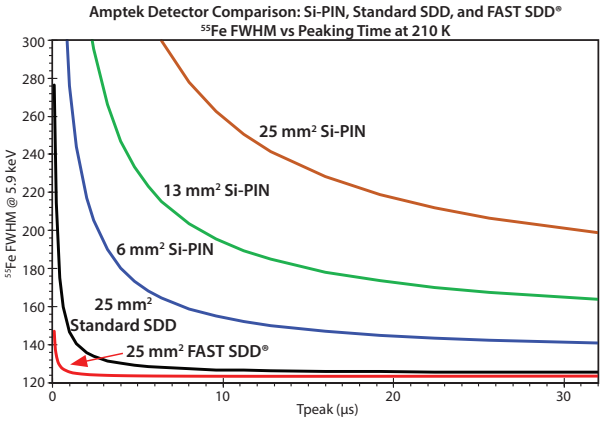

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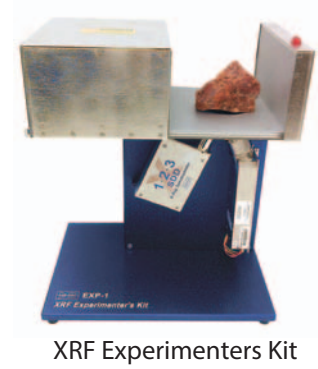

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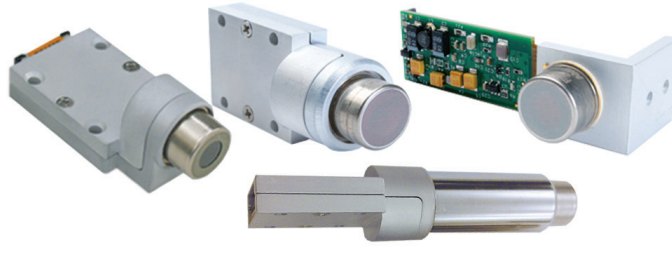
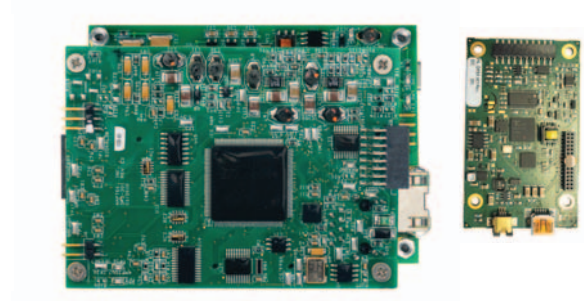


A sample of detectors with preamplifiers and heat sinks



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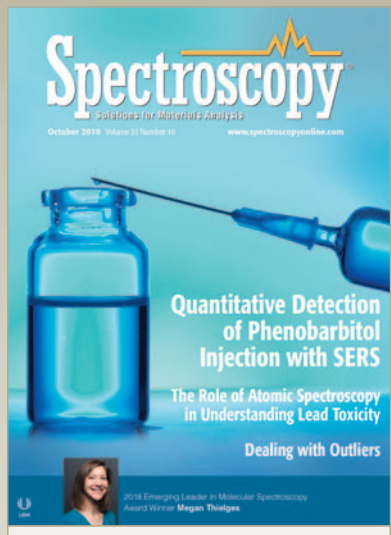

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ON THE WEB

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Chris Greatwood, MGC Advanced Polymers, and Adam J. Hopkins, PhD, Metrohm USA

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Daniel Stone, PhD, University of Leeds, and Peng Zou, PhD, Princeton Instruments

Implementing Transmission Raman for Fast Content Uniformity Testing—from Feasibility Evaluation to a Validated Release Method
Meike Römer, PhD, Grünenthal Pharma

Discover How Triple Quadrupole ICP-MS Will Help You Do More and Simplify Your Laboratory Routines
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PEER-REVIEWED ARTICLE

DEPARTMENTS

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News Spectrum

Call for Nominations: 2019 Emerging Leader in Molecular Spectroscopy

Spectroscopy magazine is seeking nominations for the 2019 Emerging Leader in Molecular Spectroscopy Award. This award recognizes the achievements and aspirations of a talented young molecular spectroscopist who has made strides early in his or her career toward the advancement of molecular spectroscopy techniques and applications. The winner must be within 10 years of receiving his or her highest academic degree in the year the award is granted. Details can be found at http://www.spectroscopyonline.com/call-nominations-2019-emerging-leader-molecular-spectroscopy

Horiba Scientific Celebrates New Facility Opening

Horiba Scientific recently celebrated the official opening of its new facility in Piscataway, New Jersey. Horiba executives, including Atsushi Horiba, the company's chairman, and leaders from industry and academia were in attendance. Also joining the celebration were local celebrities State Senator Bob Smith and Piscataway Mayor Brian Wahler.

The 132,000-square foot facility has an open office design that includes natural light, open communication spaces, informal work areas, and conference rooms for meetings and global video conferences. A tour of the facility highlighted office, engineering, manufacturing, laboratory, and cleanroom spaces, including more than 6400 square feet of applications laboratories designed to showcase the company's product line, applications training capabilities, method development, and product demonstrations.

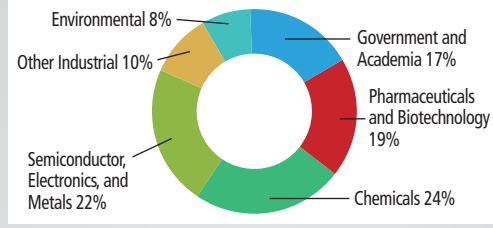
Four laboratories on the new site are designed around specific sciences: earth science, life science, materials science, and industrial science. Each laboratory reportedly specializes in the sample preparation, analysis, and data reporting appropriate for those techniques and their unique requirements; each is equipped with core instruments from various product lines related to those techniques and measurements relevant to the application space. The laboratories are used for sales demonstrations, training, customer support, engineering validation, and basic research. Training centers, adjacent to the laboratories, can be used for customer training and service training for the global network of affiliates and distributors.

The move allows for the original equipment manufacturer (OEM) facility to be located in the same building as the main office. The OEM facility has dedicated research and development (R&D) and manufacturing space, including several cleanrooms to meet production requirements.

The tour concluded with instrument and poster displays of the five Horiba segments (corporate, medical, semiconductor, automotive, process, and environmental) outside the lunchroom, followed by a buffet lunch and the cutting of a ceremonial seven-layer cake representing the color spectrum. ■

MARKET PROFILE: HANDHELD SPECTROSCOPY

The advancements of battery and computing technologies have enabled analytical instruments to have smaller form factors that take them beyond portable and into the realm of being handheld. Handheld X-ray fluorescence (XRF), near-infrared (NIR) and infrared (IR), and Raman spectroscopy are the key spectroscopy techniques available in portable and handheld formats that take analytical measurements beyond the laboratory and into the field.



Portable and handheld spectroscopy market in North America.

Like other portable and handheld devices, these spectroscopy instruments will have fewer capabilities and performance than a laboratory instrument. Handheld instruments might be used for identification or qualitative analysis, or perhaps semi-quantitative measurements. They are typically tuned for specific applications to allow for an increased level of performance. Using these handheld devices is also simpler than using laboratory instruments; they usually have point-and-shoot or push-button operation, as well as touchscreen displays. Another key characteristic of handheld spectroscopy devices, aside from portability, is that they do not require sample pretreatment, allowing for direct measurements.

These handheld spectroscopy devices are now routinely used in many industries. Handheld XRF instruments are used in the metals industry for recycling and sorting scrap materials, and can identify elements from magnesium to uranium. Portable and handheld NIR and IR devices have high utility in food quality, fungus detection, plastics recycling, pharmaceuticals, and narcotics. Handheld Raman spectrometers are used for raw materials analysis, environmental, and forensics applications.

The total market for handheld spectroscopy accounted for approximately $150 million in North America in 2017 and is forecasted to increase 8–10% annually for the next few years, with XRF and Raman spectrometers representing about two thirds of the shipments. Industrial applications in the chemicals, plastics, and semiconductor sectors are expected to drive growth for 2018. Life science applications represent a significant share of the market, particularly for Raman and NIR and IR instruments. Government and academic laboratories account for about 17% of the market. The overall market leader for handheld spectroscopy is Thermo Fisher Scientific, with its flagship Niton XRF analyzers. Other leading manufacturers include Bruker, Hitachi, Olympus, and Rigaku.

Market size and growth estimates were adopted from *TDA's Industry Data,* a database of technology market profiles and benchmarks, as well as the *2018 Instrument Industry Outlook (Midyear Update)* report from independent market research firm Top-Down Analytics (TDA). For more information, contact Glenn Cudiamat, general manager, at (888) 953-5655 or glenn.cudiamat@tdaresearch.com. Glenn is a market research expert who has been covering the analytical instrumentation industry for more than two decades.

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Atomic Perspectives

The Critical Role of Atomic Spectroscopy in Understanding the Links Between Lead Toxicity and Human Disease

The development of analytical instrumentation over the past 50 years has allowed us not only to detect trace metals at the parts per quadrillion (ppq) levels, but also to know the oxidation state, biomolecular form, elemental species, and isotopic constituents. Here, we look at how the development of atomic spectroscopy techniques has enabled a much better understanding of the links between trace metal toxicity and human disease, and, in particular, the role of lead in the health of young children.

Robert Thomas

Understanding the effects of trace metals on human health is as complex as it is fascinating. Too low or too high a concentration of essential trace elements in our diet can affect our quality of life. On the other hand, metallic contamination of the air, soil, and water can have a dramatic impact on our well-being. There are many examples that highlight both the negative and positive effects of trace metals on our lives. For instance, the effect of lead toxicity, particularly on young children, is well documented, but is it possible to pinpoint the source of the lead poisoning? The movie "Erin Brockovich" alarmed moviegoers about the dangers of hexavalent chromium (Cr VI) in drinking water, but how many in the audience realized that trivalent chromium (CRIII) metal is necessary for the metabolism of carbohydrates and fats? Dr. Oz recently alarmed his viewers about high levels of arsenic in apple juice, but what he failed to say was that it was not the highly toxic inorganic form of arsenic that had been found, but organic arsenic that had been metabolized by the apple tree to a less toxic form. Selenium, which is found in many vegetables including garlic and onions, has important antioxidant properties, but do we know why some selenium compounds are essential, while others are toxic? Clearly these are all complex questions that have to be answered to fully understand the role of trace elements in the mechanisms of human diseases. Atomic spectroscopy has an important role to play in answering these questions.

The development of analytical instrumentation over the past 50 years has allowed us not only to detect trace metals at the parts per quadrillion (ppq) levels, but also to know their oxidation state, biomolecular form, elemental species, and isotopic constituents. We take for granted all the powerful and automated analytical tools we have at our disposal to carry out trace elemental studies on clinical, toxicological, and environmental samples. However, it wasn't always that way. As recently as the 1960s, the majority of

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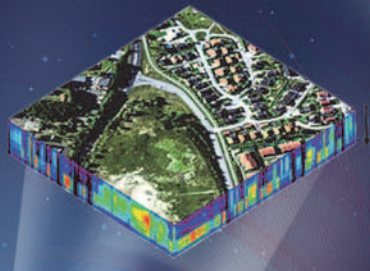

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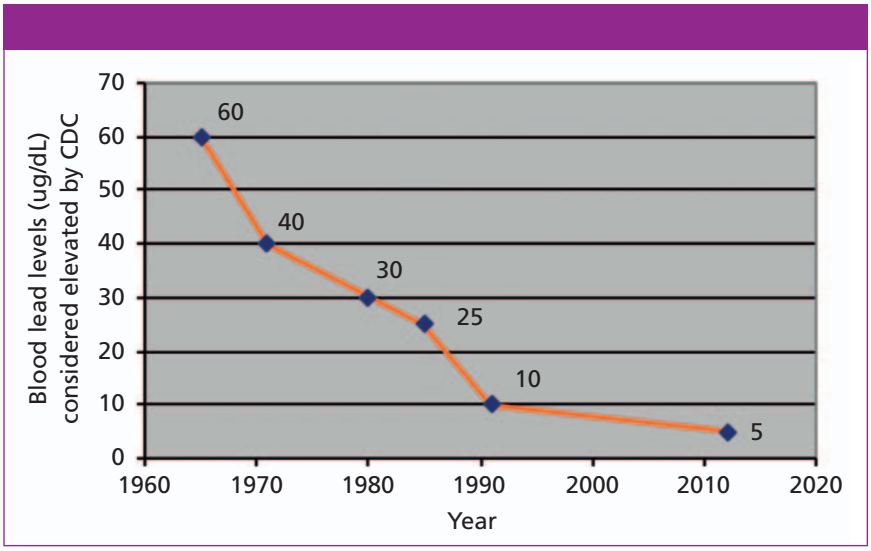

Figure 1: The trend in blood lead levels (µg/dL) in children considered elevated by the Centers for Disease Control and Prevention (CDC), since the mid-1960s.

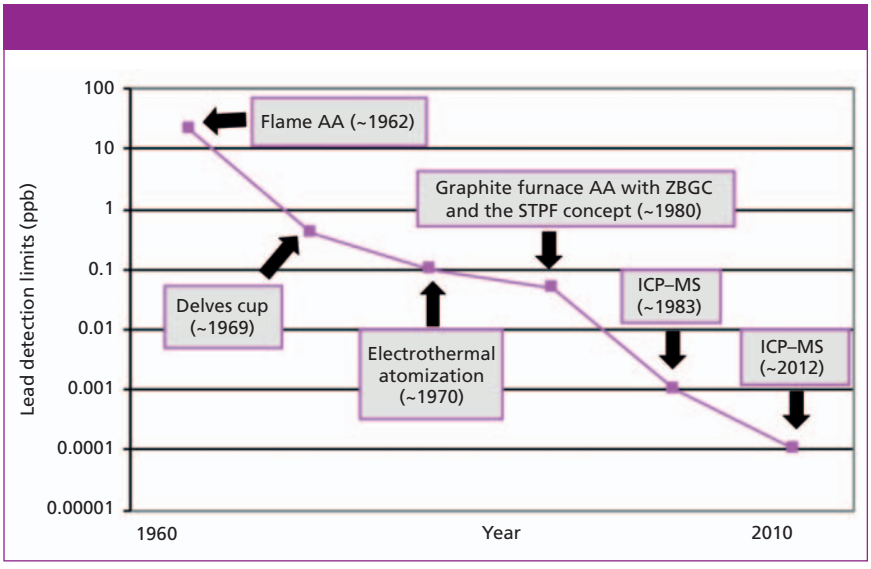

Figure 2: Comparison of detection capability (ppb) of atomic spectroscopy techniques used to monitor blood lead and the approximate year they were developed or improved.

trace elemental determinations were predominantly carried out by traditional wet chemical methods like volumetric-, gravimetric-, or colorimetric-based assays. In fact, the pharmaceutical industry has been using a sulfide precipitation colorimetric test for the measurement of lead and other heavy metals for more than 100 years; that method was only replaced in the *United States Pharmacopeia* (*USP*) in January 2018 by a plasma spectrochemical test (1).

It wasn't until the development of atomic spectroscopic techniques in the early to mid-1960s that the clinical analytical community realized they had a highly sensitive and diverse trace element technique that could be automated. Every time a major development was made in atomic spectroscopy, beginning with flame atomic emission (FAE) and flame atomic absorption (FAA) in the early 1960s, electrothermal atomization (ETA) or graphite furnace atomic absorption (GFAA) in the early 1970s, inductively coupled plasma–optical emission spectrometry (ICP-OES) in the late 1970s, and inductively coupled plasma–mass spectrometry (ICP-MS) in the early 1980s, trace element detection capability, sample throughput, and automation dramatically improved. There is no question that developments and breakthroughs in atomic spectroscopy have directly impacted our understanding of the way trace metals interact with the human body. Let us now take a look at a specific examples where atomic spectroscopy techniques have allowed us to delve deeper into understanding the impact of trace metal toxicity on our lives, focusing specifically on lead (Pb).

Lead Poisoning

Lead has no known biological or physiological purpose in the human body, but is readily absorbed into the system by ingestion, inhalation, and, to a lesser extent, by skin absorption (2). Inorganic lead in submicrometer-sized particles in particular can be almost completely absorbed through the respiratory tract, and larger particles may be swallowed. The extent and rate of absorption of lead through the gastrointestinal tract depend on characteristics of the individual and on the nature of the medium ingested. It has been shown that children can absorb 40–50% of an oral dose of water-soluble lead compared to only 3–10% for adults (3). Young children and toddlers are particularly susceptible because of their playing and eating habits, and because they typically have more hand-to-mouth activity than adults (4). Lead is absorbed more easily if there is a calcium or iron deficiency, or if the child has a high fat, inadequate mineral, or low protein diet. When absorbed, lead is distributed in the body in three main areas: bones, blood, and soft tissue. About 90% is distributed in the bones, while the majority of the rest gets absorbed into the bloodstream, where it gets taken up by porphyrin molecules (complex nitrogen-containing organic compounds providing the foundation structure for hemoglobin) in the red blood cells (5). It is, therefore, clear that the repercussions and health risks are potentially enormous if children are exposed to abnormally high levels of lead.

The Impact of Lead Toxicity on Children

The toxic effects of lead have recently been exemplified by the drinking water crisis in Flint, Michigan, where public health officials and water authority personnel failed to take remedial action when they replaced Lake Michi-

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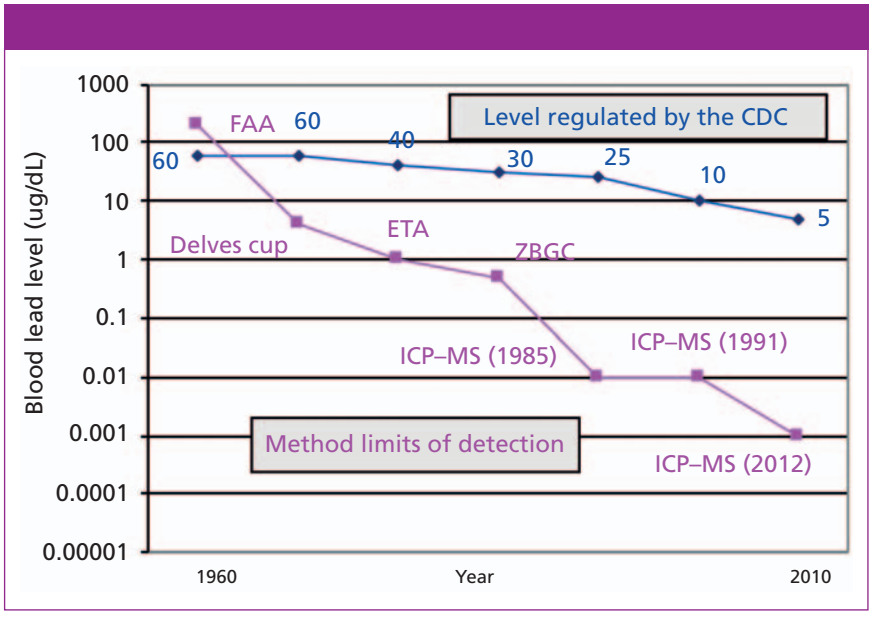

Figure 3: The improvement in real-world method detection capability (in µg/dL) offered by atomic spectroscopy techniques for blood-lead determinations compared to the trend in blood-lead levels regulated by the Centers for Disease Control and Prevention (CDC).

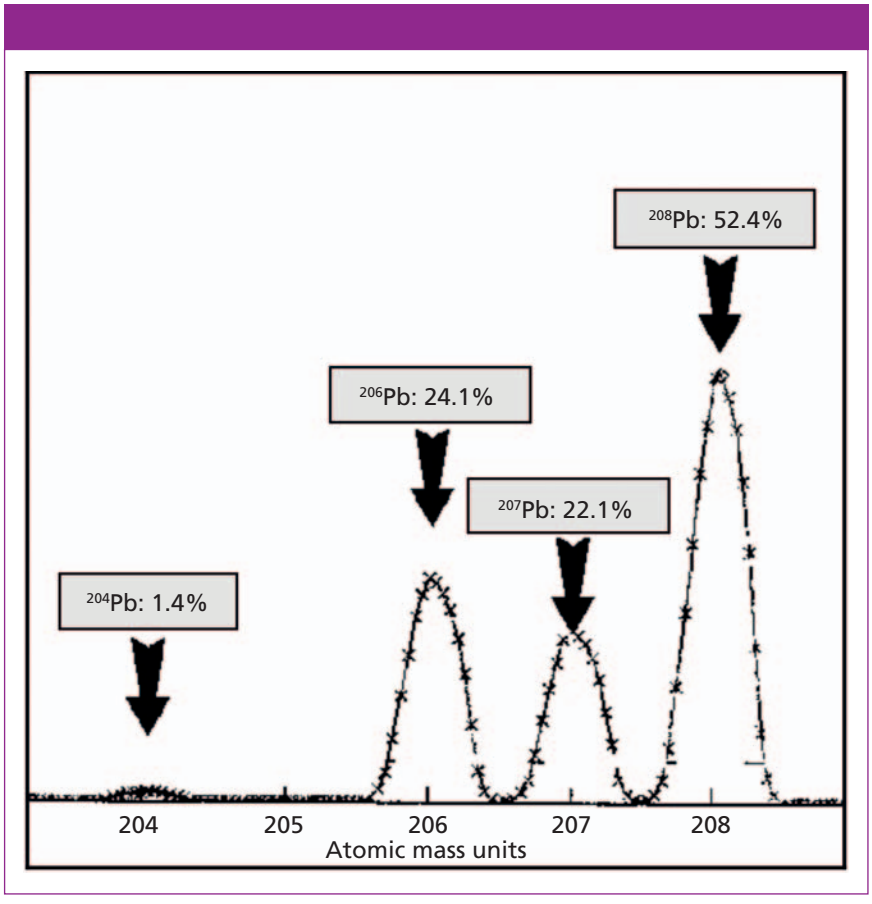

Figure 4: Mass spectrum of the four lead isotopes at 204, 206, 207, and 208 atomic mass units (amu), with their respective natural abundances.

gan with the Flint River as the source of the city's drinking water, a change that resulted in corrosion of lead pipes and high levels of lead in the drinking water supply. This particular problem is still being investigated, but the Centers for Disease Control and Prevention (CDC), recently reported that at least four million households have children living in them who are being exposed to high levels of lead from a combination of old lead paint and lead water pipes. As a result, there are approximately half a million U.S. children 1–5 years of age with blood lead levels (BLL) in excess of 5 micrograms per deciliter (µg/dL), the level at which CDC recommends remedial actions be taken (6).

Lead poisoning affects virtually every system in the body, and often occurs with no distinctive symptoms. It can damage the central nervous system, kidneys, and reproductive system and, at higher levels, can cause coma, convulsions, and even death. Even low levels of lead are harmful and are associated with lower intelligence, reduced brain development, decreased growth and impaired hearing (7). The level of lead in a person's system is confirmed by a blood-lead test, and by today's standards a blood lead level is considered elevated if it is in excess of 5 µg/dL (50 ppb) for children (8). However, the long-term effects of lead poisoning have not always been well understood. In the early and mid-1960s, remedial action would be taken if a blood lead level (or *clinical practice threshold level*, as it was known then) was in excess of 60 µg/dL. As investigators discovered more sensitive detection systems and designed better studies, the generally recognized level for lead toxicity has progressively shifted downward. In 1970, it was lowered to 40 µg/dL and, by 1978, the level had been reduced to 30 µg/dL. In 1985, the CDC published a threshold level of 25 µg/dL, which they eventually lowered to 10 µg/dL in 1991. It stayed at this level until it was reduced to 5 µg/dL in 2012. However, as our understanding of disease improves and measurement technology gets more refined, this level could be pushed even lower in the future (9). Figure 1 shows the trend in blood lead levels considered elevated by the Centers for Disease Control (CDC), since the mid-1960s.

Note that the term *blood lead reference value* (BLRV) has been used more recently

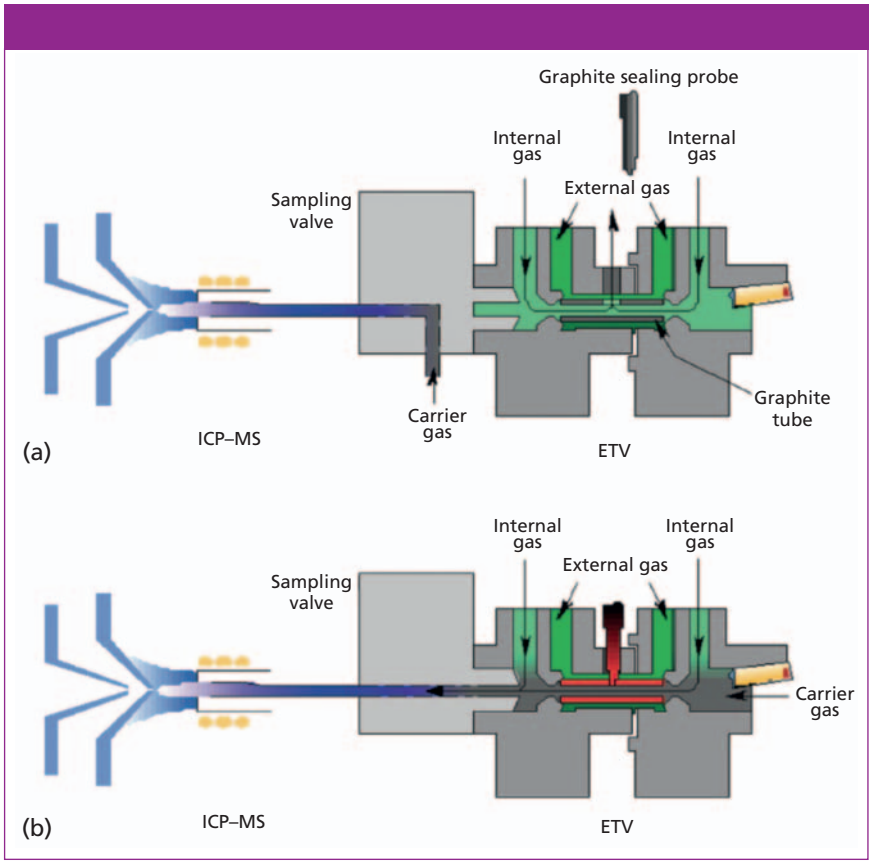

Figure 5: Schematic of ICP-MS coupled with an the electrothermal vaporization sampling accessory (ETV-ICP–MS), showing the two distinct stages: (a) prevaporization to drive off the matrix components and (b) vaporization to sweep the analyte vapor into the ICP–MS instrument for analysis. Adapted with permission from reference 22.

(since 2012), and refers specifically to the 97.5th percentile of blood lead levels for children 1–5 years old in the United States, calculated from blood lead tests performed in the National Health and Nutrition Examination Survey (NHANES). The BLRV is not a health-based toxicity threshold, nor does it define what level is considered normal. It is intended to help identify the highest risk childhood populations and geographic areas.

It is also important to point out that these thresholds were not all determined the same way. Only in 2012 (when the recognized level for lead toxicity was lowered to 5 µg/dL) was the population-based threshold called the BLRV and calculated from population statistics. Although all these levels could be said to describe thresholds of elevated blood lead levels generally, even the term *elevated blood lead level* wasn't specifically defined in CDC policy until 1978.

Currently, the major source of lead poisoning among children comes from lead-based household paints, which were used until they were banned in the United States in 1978 by the Consumer Product Safety Commission. Prior to that date, leaded gasoline was the largest pollutant, before it was completely removed from the pumps in 1995. Other potential sources include lead pipes used in drinking water systems, airborne lead from smelters, and clay pots, pottery glazes, lead batteries, and household dust. However, awareness of the problem, combined with preventative care and regular monitoring, have reduced the percentage of children aged 1–5 years with elevated blood levels (≥5 µg/dL) in the US from 26% in the early-mid 1990s to less than 2% in 2014. These data were taken from a recent National Health and Nutrition Examination Survey (NHANES) report (10).

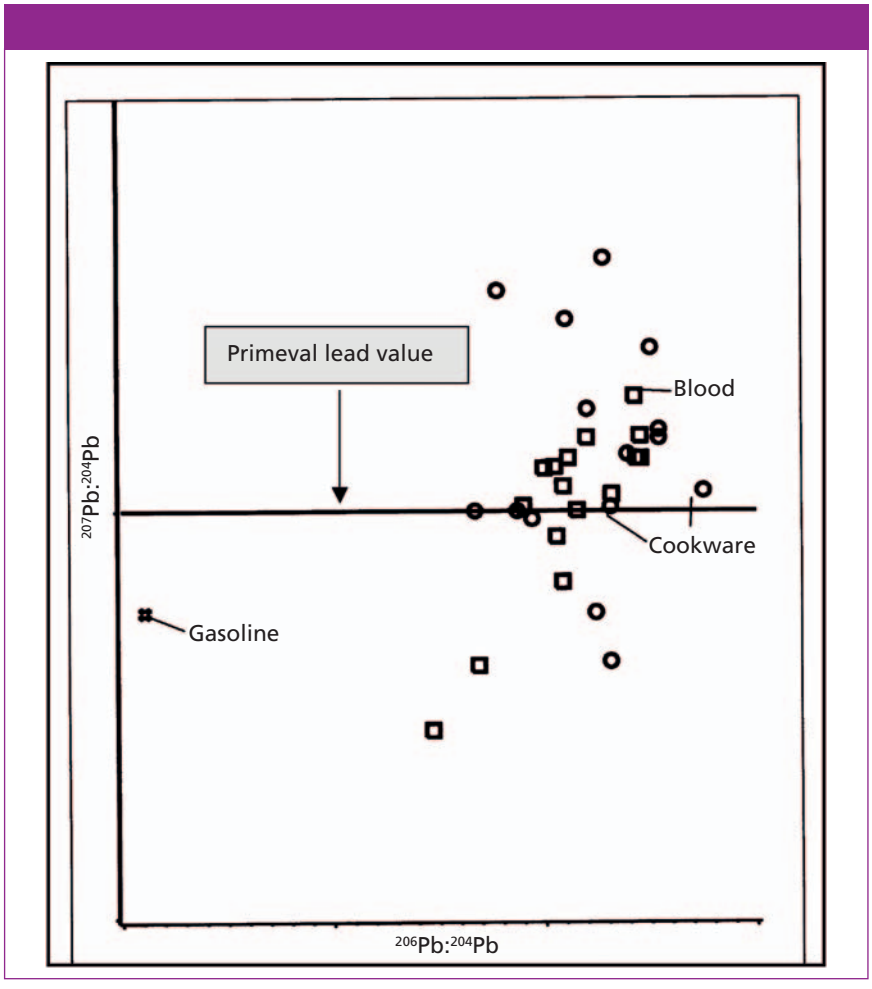

Figure 6: A plot of the ratio of $^{206}Pb:^{204}Pb$, against the ratio of $^{207}Pb:^{204}Pb$ for blood (▫), cookware (o), and gasoline (▪) samples, showing the theoretical (primeval) lead line. Adapted with permission from reference 21.

Routine Monitoring of Lead Using Atomic Spectroscopic Techniques

There is no question that the routine monitoring of children has had a huge impact in reducing the number of children with elevated blood levels. Lead assays were initially carried out using the dithizone colorimetric method, which was sensitive enough, but very slow and labor intensive. The method became a little more automated when anodic stripping voltammetry was developed (11), but blood-lead analysis was not considered a truly routine method until atomic spectroscopy techniques became available. Let's take a more detailed look at how improvements in atomic spectroscopy instrumentation detection capability have helped to lower the number of children with elevated blood lead levels, since atomic absorption was first commercialized in the early 1960s.

Flame Atomic Absorption (FAA)

When flame atomic absorption (FAA) was first developed, the elevated blood lead level was set at 60 µg/dL. Even though this level is equivalent to 600 parts per billion (ppb) of lead, which was well above the FAA detection limit of ~20 ppb at the time, FAA struggled to accurately detect lead at these levels when sample preparation was taken into consideration. The preparation of blood samples typically involved either dilution with a weak acid followed by centrifuging or filtering, or acid digestion followed by dilution and either centrifuging or filtering. More recently, dilution with a strong base like tetramethylammonium hydroxide (TMAH) and the addition of a surfactant to allow for easier aspiration has been used. When sample preparation was factored into the equation, a blood lead level of 600 ppb was reduced to 10–20 ppb, virtually the same as the FAA instrumental detection limit.

Delves Cup

To get around this limitation, an accessory called the Delves Cup was developed in the late 1960s to improve the detection limit of FAA (12). The Delves Cup approach uses a metal crucible or boat, usually made from nickel or tantalum, which was positioned over the flame. The sample, typically 0.1–1.0 mL, is pipetted into the cup, where the heated sample vapor is passed into a quartz tube, which is also heated by the flame. The ground state atoms generated from the heated vapor are concentrated in the tube, and therefore resident in the optical path for a longer period of time, resulting in much higher sensitivity and about 100x lower detection limits. The Delves Cup became the standard method for carrying out blood lead determinations for many years, because of its relative simplicity and low cost of operation.

Unfortunately, the Delves Cup approach was found to be very operator dependent, not very reproducible (because of manual pipetting), and required calibration with blood matrix standards (13). The technique became less attractive when electrothermal atomization (ETA) was commercialized in the early 1970s. This new approach offered a detection capability for lead of ~ 0.1 ppb, approximately 200x better than FAA. However, its major benefit for the analysis of blood samples was the ability to dilute and inject the sample automatically into the graphite tube with very little off-line sample preparation. In addition, because the majority of the matrix components were "driven-off" prior to atomization at ~3000 °C, interferences were generally less than the Delves Cup, which only reached the temperature of the air or acetylene flame at ~2000 °C. This breakthrough meant that blood lead determinations, even at extremely low levels, could now be carried out in an automated fashion with relative ease.

Zeeman Correction

The next major milestone in AA was the development of Zeeman background correction (ZBGC) in 1981, which com-

pensated for non-specific absorption and structured background produced by complex biological matrices, like blood and urine. ZBGC, in conjunction with the stabilized temperature platform furnace (STPF) concept, allowed for virtually interference-free graphite furnace analysis of blood samples using aqueous calibrations (14). Such was the success of the ZBGC–STPF approach, due primarily to the fact that it could be used to analyze many different kinds of samples using simple aqueous standards, that it became the recognized way of analyzing most types of complex matrices by ETA.

Even though ETA had been the accepted way of doing blood lead determinations for more than 15 years, the commercialization of quadrupole-based ICP–MS in 1983 gave analysts a tool that was not only 100x more sensitive, but suffered from less severe matrix-induced interferences than ETA. In addition, ICP–MS offered multielement capability and much higher sample throughput. These features made ICP–MS very attractive to the clinical community, such that many labs converted to ICP–MS as their main technique for trace element analysis. Then, as the technique matured, using advanced mass separation devices, performance enhancing tools, powerful interference reduction techniques, and more-flexible sampling accessories, detection limits in real-word samples improved dramatically for some elements. Figure 2 shows the improvement in detection capability (in ppb) of ICP-MS compared to ETA and the other atomic spectroscopy techniques.

It should also be emphasized that the detection limits shown in Figure 2 are instrument detection limits (IDLs), which are based on simplistic calculations of aqueous blanks carried out by manufacturers, and not realistic method detection limit (MDL) or procedural limits of detection (PLOD) that take into consideration the sample preparation procedure, dilution steps, and multiple analytical measurements. IDLs are also only intended to be used as a guideline for comparison purposes because there are so many different ways of assessing detection capability, based on variations in manufacturer, instrument design, and methodology.

Method or Procedural Limits of Detection

Figure 3 is a combination of Figures 1 and 2, and shows improvement in the blood lead method detection limit (now in µg/dL and not ppb) offered by atomic spectroscopy techniques compared to the trend in blood lead levels set by the CDC. To make the comparison more valid, a factor of 100x has been applied to the instrumental detection limits to give an approximation of the achievable "real world" method detection limit in a blood sample matrix. Both plots are shown in log scale, so they can be viewed on the same graph. The main purpose of these data is to show how the blood lead levels considered by the CDC as "elevated" over the past 50 years have dropped as method detection limits of the various atomic spectroscopy techniques have been lowered, thus giving researchers more confidence in the integrity of their data.

It should also be emphasized that a degradation factor of 50–100x is quite normal when converting an IDL to an MDL, when characterizing samples by atomic spectroscopy techniques. However, when analyzing a very complex biological matrix like blood by ICP-MS, there are many different ways of calculating LODs to encompass the entire analytical procedure. One common approach to determine the PLOD is to carry out 20 runs and plot standard deviation of the standards and spiked matrix versus concentration, extrapolating the regression line to the ordinate axis, to determine the standard deviation at zero concentration (15). In a high throughput laboratory, this approach might not be realistic, because of the additional time taken. The time involved can be somewhat shortened by taking fewer readings, but doing so will clearly negatively impact the statistical data and detection limit. Whichever approach is used, one should take into account variability in sample preparation, environmental contamination, solvents, and reagents, as well as minor sampling errors from dilution or pipetting over many runs, all of which can cause variability from day to day. Given such variability, a real-world

procedural LOD for Pb in blood is often three orders of magnitude worse than the instrument detection limit, and is typically around 0.01–0.07 µg/dL, depending on the type of ICP–MS technology and interference reduction technique used (9,16).

Identifying Sources of Lead Using Isotopic Fingerprinting

An added benefit of the ICP-MS technique is that it also offers isotopic measurement capability. This feature is very attractive to many clinical laboratories, because it gives them the ability to carry out isotope tracer (17), isotope dilution (18) and isotope ratio (19) measurements, which are beyond the realms of other atomic spectroscopy techniques. In fact, the isotopic measurement capability allows researchers to get a better understanding of the source of lead poisoning by measuring the isotope ratio of blood-lead samples and comparing them with possible sources of lead contamination. The principal behind this approach, known as *isotopic fingerprinting*, is based on the fact that lead is composed of four naturally occurring isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb, all with the same atomic number, but with different atomic masses. Thus, when naturally occurring lead is ionized in the plasma, it generates four ions, all with different atomic masses. Figure 4 shows a mass spectrum of the four lead isotopes ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb, together with their relative natural abundances of 1.4%, 24.1%, 22.1% and 52.4%, respectively.

All the lead isotopes, with the exception of ^{204}Pb, are products of radioactive decay of either uranium or thorium, the abundance of which will vary slightly depending on the rock type and geological area. This means that in all lead-based materials and systems, ^{204}Pb has essentially remained unchanged at 1.4%, since the earth was first formed (20). The ratios of the isotopic concentrations of ^{208}Pb, ^{207}Pb, and ^{206}Pb to that of ^{204}Pb will therefore vary, depending on the source of lead. This fundamental principle can be then be used to match lead isotope ratios in someone's blood to a particular environmental source of lead contamination.

However, there are known, well-understood limitations of this approach. For lead fingerprinting to be useful, potential sources of lead exposure must be limited in number and scope and the lead sources must be isotopically distinct. If more than two sources of environmental lead are likely, such as from water pipes, gasoline, smelter, paint, pottery, and glazes, then mixed or combined isotope ratios will occur and, as a result, no useful data will be obtained. In addition, if someone has chronic exposure to extremely high lead levels, the person might have brittle or broken bones, and accumulated lead in the bones would be released into the bloodstream, which could shift the lead equilibrium. This release of lead from the bones could elevate blood lead levels, independent of the source of the lead exposure or contamination.

Mexican Study

A good example of using isotope ratios to pinpoint the source of lead poisoning that worked extremely well involved a study carried out on a group of people living in a small village near Mexico City (21). A number of the residents had abnormally high levels of lead in their blood, which came from one of two likely sources: the use of leaded gasoline, which had contaminated the soil, or glazed ceramic pots, which were used for cooking and eating purposes, or both. For this experiment, the lead isotope ratios were measured using an electrothermal vaporization (ETV) sampling accessory coupled to an ICP-MS instrument. In this sample device, a heated graphite tube, similar to the type used in ETA, is used to thermally pretreat the sample. But instead of using the tube to produce ground state atoms, its main function is to drive off the bulk of the matrix before the analytes are vaporized into the plasma for ionization and measurement by the mass spectrometer. The major benefit of ETV-ICP-MS for this application is that complex matrices like blood, gasoline, and pottery or clay material can be analyzed with very little interference from matrix components (22). An additional benefit with regard to taking blood samples is that typically only a 20–50 µL aliquot is required for analysis. Figure 5 represents a schematic of how the ETV–ICP–MS system works, showing the two distinct steps: prevaporization to drive off the matrix components, and vaporization to sweep the analyte vapor into the ICP–MS for analysis.

In the Mexican study, ETV-ICP-MS was then used to determine the lead isotope ratios of ^{208}Pb, ^{207}Pb, and ^{206}Pb to that of ^{204}Pb in blood samples from a group of residents. These ratios were then compared with the two likely sources of lead contamination from the cooking pots and the gasoline samples. Figure 6 shows a subset of data taken from the study. It shows a plot of the ^{206}Pb: ^{204}Pb ratio against the ^{207}Pb:^{204}Pb ratio for the blood, cookware, and gasoline samples. It can be seen from this plot that the data for the blood and cookware are grouped very tightly together around the theoretical value of the ratios (known as the *primeval lead value*), while the gasoline data are grouped together on their own.

Based on principal component analysis of the data, this result confirms that the lead isotope ratios of the blood and cooking pots are almost identical, and are very close in composition to primeval lead, with very little addition of radiogenic lead (produced from radioactive decay). On the other hand, the alkyl lead compounds used in the production of leaded gasoline are from a different source of lead and as a result generate a very different isotopic signature. These data showed very convincing evidence that the residents of this small Mexican village were getting poisoned by the glazed clay pots they were using for cooking and eating, and not from contamination of the environment by leaded gasoline, as was first suspected.

Conclusions

There is no question that developments in atomic spectroscopy have helped us better understand the toxicity effects of lead over the past 50 years. Atomic spectroscopy advances have allowed us to lower the clinical practice threshold

level of 60 µL/dL in the mid-1960s to the current blood lead reference value (BLRV) of 5 µL/dL. More importantly, these techniques have helped to reduce elevated blood levels of children in the United States from 26% in the early to mid-1990s to less than 2% in 2014, as well as allowing us to get a much better understanding of the environmental sources of lead contamination. However, such is the power and versatility of modern atomic spectroscopy instrumentation and its accessories, that it has also dramatically improved our understanding of other trace metal-related human diseases. The toxic effects of trivalent/pentavalent arsenic and hexavalent chromium or the nutritional benefits of different selenium species would still be relatively unknown, if it weren't for the continual improvements in ICP-MS and, in particular, its use as a very sensitive detector for trace element speciation studies using chromatographic separation technology. Even though ICP-MS has been successfully applied to many application areas since it was first commercialized in 1983, its use as a biomedical, clinical, and toxicological research tool has had a direct impact on the quality of many people's lives.

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Chemometrics in Spectroscopy

Outliers, Part III: Dealing with Outliers

This column is the continuation of our previous installments dealing with the question of outliers. Here we consider what to do about an outlier, once one is detected.

Howard Mark and Jerome Workman Jr.

You have developed a set of data and have a reading that is suspected to be an outlier. You have applied one or more of the tests described in our previous column (1) and confirmed that the reading is, indeed, an outlier. Now what do you do?

There are three actions you might consider taking:

- Delete the discordant reading
- Transform the data
- Accommodate the discordant reading.

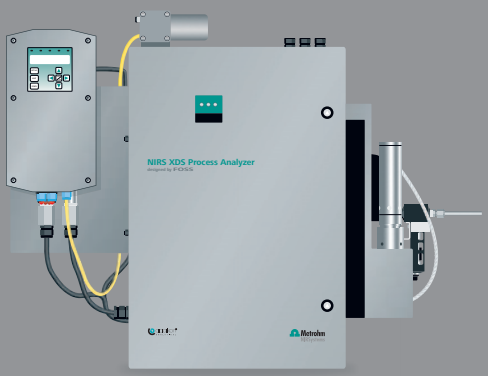
These possible actions could be considered the *statistical* approaches to dealing with the outlier. Other approaches can also be considered, and, depending on the circumstances, might be preferred. For example, if the outlier arises when calibrating a spectrometer for quantitative analysis (using chemometrics, of course, which is the default activity for all our columns), then the origin of the outlier could be either in the reference laboratory values or among the instrumental values. In either case, an alternative (nonstatistical) approach would be to identify the source of the discordant value (instrument or laboratory), and then engage in investigation of the chemistry, physics, and background of the readings to find the fundamental cause. In a pharmaceutical context, for example, this is what would be called *root cause analysis*.

A variant of this approach is to concentrate all of one's attention on just the outliers and ignore the rest of the data, with a view toward learning some new fundamental science, the rationale being that some new and unexpected effect has created the discordancy in the values. An outlier can be an indicator of a scientific accident, of the sort that has led to discoveries such as quinine, the smallpox vaccine, X-rays, insulin, penicillin, Teflon, and the cosmic microwave background (2-4) (Don't hold your breath for this, but it does happen!). After all, to a lesser degree, we saw previously (5) that the presence of a set of outliers that persisted through changes of data transformations and other manipulations of the data was indicative of a previously unsuspected systematic effect influencing the data. The discovery of this effect gave us much information about the meaning of calibration transferability, and how to achieve it.

General scientific principles come into play here. Is the effect reproducible? Can it be created or avoided at will? Does it lead to predictions of new phenomena? Is there a causal link between the discordant data and fundamental physics, chemistry, biology, or math? Can you do a controlled experiment (or more generally, what is sometimes loosely called a *statistical experimental design*)? Is there a theory (from another science, such as chemistry, physics, biology) that can explain the findings? Do other scientists get similar results, or at least have you had someone check your work? Here we will avoid departing from our mission of describing the chemometric and statistical effects on the data, and so we will not pursue those other topics. However, the reader should keep these alternate considerations in mind; after all, not everything has a statistical explanation! We will now consider the possibilities listed earlier.

COMPLETE SOLUTIONS

Handheld



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Delete the Discordant Reading

In the context of using chemometric calibration to perform chemical analysis, the most common action taken when an outlier is identified is to delete the discordant reading. As we saw in the discussion of the nature of outliers (6), however, it seems likely that samples summarily rejected this way are sometimes (perhaps even often) misidentified as outliers, when in actuality, they are simply the extreme samples of the distribution.

Alternatively, as described above, the distribution could be other than expected. If an outlier is identified based on an expectation of a normal (or at least a symmetric) distribution but the actual distribution is exponential, or χ^2, or some other asymmetric distribution, then large values are to be expected and summary deletion is obviously an incorrect action. Another incorrect action would be to deal with the data as though the assumption of normality applied.

A consequence of deleting outliers is that the data no longer represent a random sample. Given that the discordant observation is, almost by definition, extreme in one characteristic or another, deleting the outliers is a form of censorship of the data. In extreme cases, where multiple outliers are deleted from a data set, deleting the outlier is tantamount to fitting the data to the calibration model, rather than a model to the data. If more than two outliers appear to need deletion, then it is more appropriate to reconsider whether they are actually outliers of the data set, or evidence of samples from a different population than the majority of the samples, having been mixed into the data.

A careful statistician will never simply delete observations, even with good reason. Even when some observations must be deleted from a data set, a careful worker will keep a record of the deleted samples: which ones they were, their values, and the rationale for deleting them. This record will allow the purported outlier to be added back into the dataset, if it turns out that the act of deleting it was erroneous. A set of good recommendations is available in short articles by Tom Fearn (7,8), describing common sense methods of evaluating suspected outliers and making good decisions about removing them from a data set.

For example, manually entered data is notorious for being rife with data entry and data transcription errors. Modern spectroscopic instruments, even low-end ones, generally transfer data to the analysis computer electronically, so this sort of error should not occur. Reference laboratory values, on the other hand, may well be subject to being manually copied, or entered into a database, or transferred to the analysis computer by manual entry, possibly through multiple steps involving manual handling. Typos and other types of errors can occur under these conditions.

Transform the Data

Data transformations are common in spectroscopic calibration practice. This is often done in an ad hoc manner, using the shotgun method of trying every available or conceivable data transform until one of them works in some manner. While we can't completely denigrate this approach (if for no other reason than that we've used it ourselves on occasion in the past), we do wish to discourage it, and thereby introduce more science into what is currently much more of an art of calibration. Therefore when we discuss a technique, or a method, or an algorithm, we do so with the expectation (or at least the hope!) that it will be used only under the specific conditions for which it is suited, to achieve a specific predefined result.

We noted in a prior column (9) that one reason to transform data is to convert the data from having a log-normal distribution to having a normal distribution by taking the logarithm of that data. In chemometric practice, this would typically correspond the reference laboratory values. Another scenario warranting a transformation of the data is when the error of the reference laboratory measurement is not constant across the range of the analyte, but increases in proportion to the analyte value; that is, the "relative error" (instead of the absolute error) of the reference method is constant, as happens in some reference laboratory methods. In this case, also, the reference values should be replaced with their logarithms; then the errors of those reference logarithm values will be constant, and then when the calibration calculations are performed, those logarithms are used as the dependent variables, instead of the original reference values.

There is nothing wrong with transforming the reference laboratory values to their logarithms, performing the calibrations for these new (reference laboratory) values, and creating a calibration model for those new reference laboratory values. In using the model for analyzing future unknown samples, the computation will result in the transformed values being predicted by the model. The user must then remember to back-transform the result into the original domain of the data. The user must also take care to ensure that the data transform did not introduce an unwanted nonlinearity into the data.

Another transform of the data is an extension of standard good statistical practice, to collect data from multiple aliquots (at least two, although three is preferred, and may be required in some cases, as will be described). If one of the multiple readings is found to be an outlier, then it should be compared with the other readings. If the readings differ by an amount comparable to the magnitude of the outlier, then it seems clear that one of the readings is incorrect, although it may not be obvious which one is wrong. If data from three or more replicates were collected, however, then a "vote" can be taken; the two that agree are most likely correct, and the one that disagrees, especially if it is the discordant reading, is probably wrong. It may not be clear why that reading

is different, but this may be a case where a type of root cause analysis, as described above, might be warranted. If none of the three (or more) readings differs noticeably from the others, then it might be appropriate to average all the like readings together, prior to performing the calibration calculations. The data may also be "windsorized" by averaging together the highest and lowest values with their respective nearest neighbors, as a way to reduce the influence of a possible outlier. This may be ineffective if the data is multivariate, since the outlier may not correspond to any individual extreme reading.

Accommodate the Discordant Reading

Accommodation of an outlier is not necessarily a way to identify it. It is often a technique for reducing or eliminating the effect of a possible outlier on the results of the calculations, whether any outliers are present or not.

There are many methods of accommodating outliers. One that is rarely used but probably should be used more commonly, is to use what are called *robust* methods of analysis. These robust methods replace our usual methods of calculation with methods that produce essentially the same results, but are resistant to the effects of outliers. The simplest example of a robust method is the use of the median instead of the mean, as the measure of the central value of a set of data. Given that the value of the median depends almost entirely on the numbers at or near the center of the data, the values at the extremes are virtually immaterial. The largest and smallest values can be correct or off by a large amount, but that difference has no influence at all on the value of the median. Contrast that with the mean, where the influence of the extreme values increases as the square of the difference between the mean and the values themselves, so that the incorrect extreme value has undue influence on the results. There are robust equivalents to most of the common chemometric methods that are popular, including multiple linear regression (MLR) and principal component regression (PCR).

A somewhat less extreme way of accommodating the (potential) outliers is to use weighted calculations. This approach permits the use of the standard algorithms and methods of analysis. The key here is the fact that outliers are, in general, the extreme observations of some characteristic of the data. This being the case, the weighted variations of the standard algorithms are used, with the weights corresponding to the degree of extremeness of the pertinent characteristic: the more extreme the value of the characteristic, the lower the weight assigned to the sample containing that extreme value. This approach has the advantages of retaining and using all the data, while still minimizing the effect of the outlier on the results. Equations for weighted as well as unweighted calibration algorithms for implementing MLR, PCR, and partial least squares (PLS) algorithms are described in ASTM standard E1655-05 (10).

Summary

As noted at the beginning of this column, deletion of an outlier is the most common way that most spectroscopists know to deal with them. As we have seen, however, there are a number of alternate methods available to take outlier readings into account in spectroscopic analysis. Add that to the fact that in much calibration work, a great excess of enthusiasm is shown in the deletion of purported or even suspected outliers, so we hope that having alternate methods available to deal with them can reduce the overenthusiasm for deleting outliers and preserve data sets.

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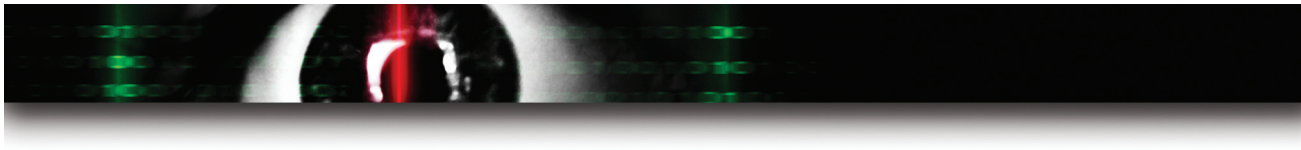

Lasers and Optics Interface

Tunable Diode Lasers for Trace Gas Detection: Methods, Development, and Future Outlook

Tunable diode laser absorption spectroscopy (TDLAS) has been a popular technique for trace gas detection for more than 20 years in academic and research circles. Commercial applications began to appear more than 10 years ago and have hit the mainstream in many markets. This article provides an overview of the methods and the development history of TDLAS, and discusses some of the future challenges for the technology.

Steven G. Buckley

Tunable diode laser absorption spectroscopy (TDLAS) has evolved along with the lasers and detectors used in the technique. Here, we will look a bit at the basics, and then focus on current capabilities and challenges.

A typical tunable distributed feedback (DFB) diode laser module tunes over a range of 2–5 nm through a combination of current and temperature tuning. These lasers have a linewidth on the order of 1–4 MHz, which at 1500 nm translates to about 20–30 femtometers, far narrower than the typical Doppler-broadened linewidth of a molecular transition. Further, the lasers can be tuned at rates up to 20 or more GHz/s, allowing rapid modulation over the absorption line of interest.

The Beer-Lambert law for small concentrations can be written with the absorbance α as a function of the absorption coefficient σ, number density N, and pathlength L:

$$\alpha = (P_0 - P)/P_0 = \sigma NL \quad [1]$$

where the absorption coefficient is measured at the line center. P_0 is the source intensity on the detector with no absorber, and P is the measured intensity on the detector in the presence of an absorber.

The perennial problem with this physics is that it is always very difficult for a detector staring at a bright source like a laser to detect a small dip in power resulting from the absorption of a trace species. In many cases in atmospheric monitoring, even when the absorption coefficient is high and the pathlength is long, direct absorption measurements are limited by the noise in the environment and the noise in the detector, and typical detection limits may be on the order of 0.1% or more.

Wavelength Modulation

The most practical solution to this problem is to superimpose a modulation on the drive current that tunes the laser in a predictable way. A common way to do this is referred to as *wavelength modulation*, in which a relatively slow ramp (10-100 Hz) sawtooth wave scans over the entire absorption line of interest, while at the same time a higher frequency (10+ kHz), small-amplitude sine wave modulates the laser wavelength.

These two drive signals are superimposed on the injection current of the laser, as illustrated in Figure 1. Each period of the slower frequency ramp contains a scan over the feature

of interest. Frequency-sensitive lock-in detection tunes to the high-frequency modulation. The amplitude of the high-frequency modulation influences the signal strength, with the maximum signal occurring when the amplitude of the modulation is 2.2 times the half-width of the absorption line.

Once the signal is detected by the lock-in amplifier, it has a characteristic shape that looks like the shape of a derivative of the absorption line shape, which gives wavelength modulation its nickname: *derivative spectroscopy*. The idealized functions are shown in Figure 2, wherein Figure 2a on the left is modeled the absorbance of a line defined by a Voigt profile with an equal Doppler and Lorentzian half-width of 0.02 cm^{-1}, with peak absorbance of 1%. In Figure 2b, on the right, the idealized first through third harmonics of the signal are illustrated, with a modulation amplitude of 0.07 cm^{-1}. Both graphs are referenced such that the line center is 0.0 cm^{-1}.

Each of the functions depicted in Figure 2b looks like the line shape that would be measured with a lock-in detector at one, two, and three times the modulation frequency as the laser is swept across the absorption line. The green dotted line corresponding to the first harmonic (marked "H1") has the shape of the first derivative, the red "H2" line looks like the second derivative, and so forth.

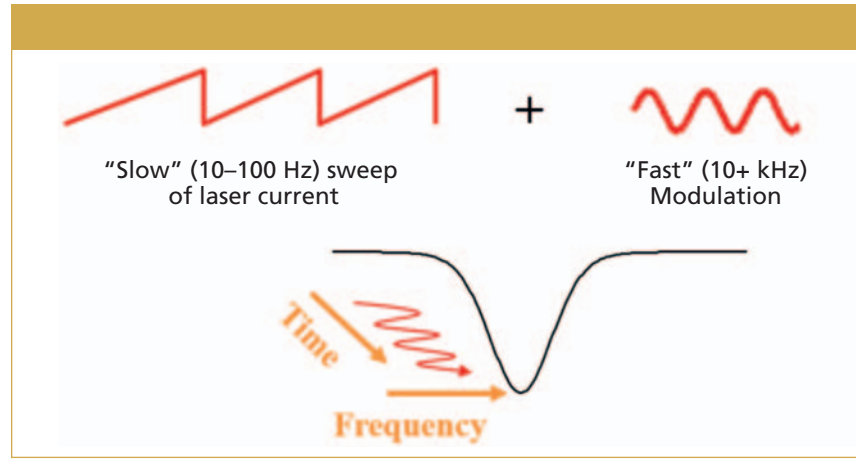

Figure 1: Wavelength modulation includes a slow sweep (left) and a faster modulation (right) of the drive current, sweeping across an absorption feature of interest (bottom).

Determination of Concentration

In practice, the first derivative signal typically has a substantial DC offset as a result of the fact that the laser power is continuously increasing during the sweep of the laser, as current is increased. For this reason, the second harmonic, which is proportional to the partial pressure (concentration) of the absorbing species, is typically used

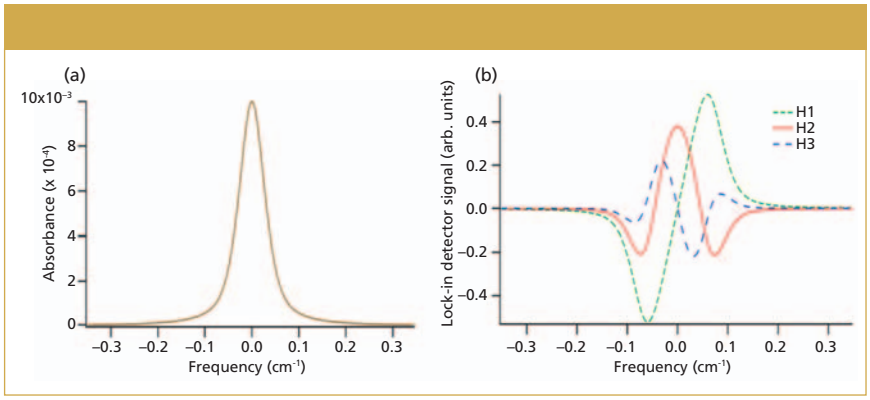

Figure 2: At left, an absorption line with 1% absorbance modeled with a Voigt profile; at right, the harmonic signals that would be measured with wavelength modulation (as described in the text) at 1, 2, and 3 times the modulation frequency.

for quantification, as it has a zero DC offset. In fact, at the line center, the intensity measurement of each of the even harmonics is proportional to the absorber concentration. The relations for the first few harmonics are:

$$I_1(\nu_0) = \bar{I}_0 \eta \quad [2]$$

$$I_2(\nu_0, \Delta\nu) = \bar{I}_0 H_2(\nu_0, \Delta\nu) P_{abs} L s_0(T) \quad [3]$$

In these equations, $I_1$and I_2 are the measured intensity at the first and second harmonic frequency, the former measured at the absorption line center frequency ν_0, and the latter also a function of the modulation amplitude $\Delta\nu$. Thus, I_1 is the DC offset of the first harmonic mentioned earlier, which is proportional to laser energy. $\bar{I}_0$ is the average laser intensity (which may be different as a result of laser or environmental conditions in the beam path). η is a proportionality constant, and is a function of the particular laser and detector pair which can be measured.

In equation 3, H_2 is the harmonic function, L is the pathlength, and $s_0(T)$ is the line strength s_0 as a function of temperature T, which is typically known (and can be measured in a laboratory if it is not known). The last parameter to be defined is P_{abs}, the partial pressure of the species to be determined.

One way to find P_{abs} is to measure I_2/I_1. With all of the parameters known, this ratio of harmonic signals allows calculation of the partial pressure of the absorbing species. This is one of the most straightforward ways to use a modulated tunable diode laser signal to determine trace gas concentration. The modulation and detection hardware are all readily available.

Other Methods for Concentration

There are other schemes for measurement of concentration as well, including higher-frequency (100 MHz–10 GHz) modulation (so-called *FM spectroscopy*) which requires a very fast (expensive) detector but pushes the intensity noise of the laser dramatically lower (1). Theoretically, this may have better performance than the wavelength modulation scheme above. A variation on this theme is the use of two FM frequencies separated by a small frequency (2). Detection at the difference (so-called *beat*) frequency allows use of a lower cost detector. Yet another method uses the ratio of two even harmonics (for example, I_4/I_2) to simultaneously determine total pressure P, through an understanding of the variation of the harmonic function with the "modulation depth," the ratio of the amplitude of modulation to the line strength (3).

Measurement of Temperature

Measurement of temperature is also straightforward, including at elevated combustion temperatures. If the variation of line strength with temperature $s_0(T)$, shown in equation 3, for two lines of the same molecule are known, then the ratio of the measured intensities of the two lines can be solved for temperature. Ideally, the variation would not only be monotonic with temperature, but relatively steeply increasing over the range of maximum interest, for best sensitivity. We did this with two adjacent lines of water that could be measured with a single laser to yield temperature over a wide range (4).

Brief History and Development of TDLAS

One of the first applications of TDLAS to atmospheric gases was published in *Science* in 1971 (5). Through the 1980s, development was limited by the laser sources, which were primarily lead salt laser diodes that needed to be cooled to cryogenic temperatures. These lasers emitted in the mid-infrared (mid-IR), thus also requiring cooled detectors. Lead selenide and mercury-cadmium-telluride (MCT) detectors were the most popular, the latter particularly so because of the possibility for fast frequency response (and thus modulation). However, even with the development of compact and reliable cryocoolers, the cost and complexity of TDLAS systems kept them mostly employed in laboratories and in very special circumstances. The lengthy chapter in Sigrist's 1994 edited book is an excellent summary of work through the early 1990s in TDLAS systems (6).

The development of near-IR (NIR) laser sources in the mid-to-late 1990s was driven primarily by the telecommunications boom, but it had major implications for TDLAS monitoring. As fast as single-mode fiber-coupled laser sources between 1.3 and 2 μm were developed for telecommunications, they were snapped up by researchers aiming to find just the right laser (remember that TDLs are tunable over only a few nm) to measure their favorite molecule of interest. These sources and detectors could be cooled by on-board TEC coolers, and were thus much more robust and easily fieldable. This more than made up for the fact that NIR bands are combination and overtone bands (rather than fundamental absorptions), which can be several orders of magnitude weaker than the fundamental bands. The modulation techniques developed to reduce laser and detector noise with the mid-IR lasers remained important to increase sensitivity with the new near-IR lasers and detectors, due to

the relative weakness of the transitions.

A stiff competition developed between very strong development groups at Unisearch (Ontario, Canada), Physical Sciences, Inc. (Andover, MA), and Southwest Sciences (Santa Fe, NM), as well as with practically oriented academic groups such as those of Prof. Ron Hanson (Stanford University), Prof. Jürgen Wolfrum (Universität Heidelberg, Germany), and (later) Prof. Volker Ebert (Technische Universität Darmstadt, Germany). The telecommunications lasers were available only in particular bands, and manufacturing techniques were under development. Therefore, there were always a few lasers in each batch that were outside of the intended wavelength range. These "rejects" were snapped up by the competing researchers, and were immediately tested for application to molecules of interest, each researcher hoping to be the first to measure, say, hydrogen sulfide or ammonia. Relationships with the TDL manufacturers were key; otherwise lead times for particular wavelength lasers could be 3–4 months or more. Each of these groups made substantial contributions to the development of TDLAS spectroscopy as it is practiced today.

Approximately a decade ago, the basic methodology, hardware, and range of gases that could be measured hit a tipping point for TDLAS technologies. Small business innovation research (SBIR) gave way to commercial application, and companies such as Siemens, Mettler-Toledo, Teledyne, General Electric, Servomex, and SpectraSensors joined Unisearch and other early companies in a now crowded field of manufacturers. A typical list of gases that can be easily measured includes O_2, HF, HCl, CO, CO_2, H_2S, H_2O, NO, N_2O, CH_4, and many others. Typical cross-stack detection limits are in the parts-per-million range (depending on the molecule) for a few meters of pathlength. Sub-parts-per-million detection can be obtained for many gases with longer paths, including with extractive sample cells.

Perhaps the most hopeful of the new entrants to the crowd of manufacturers was Zolo Technologies, which was founded in 1999, and venture-funded to the tune of $51.5 million. They developed TDLAS sensors and tomographic temperature and species mapping strategies using multiple TDLs in boilers. They were purchased by John Zink Hamworthy Combustion in March of this year. The current size of the worldwide market for TDLAS is in the hundreds of millions of dollars annually, and growing quickly.

The Future for TDLAS

There are two basic sets of challenges for TDLAS technology. The first is to continue to adapt to an increasing number of challenging measurement environments. Coal mines, refineries, cement plants, and other harsh environments bring continuing operating challenges. This requires developing fit-for-purpose sensing devices that are easy to install, which retain calibration or are easy to recalibrate under heavy wear and continuous use, and which can measure a suitable set of gases for the application. These are typical challenges for a developing technology, and will result in a spate of new implementations, as well as solutions to interesting technical challenges. Some of the most important challenges to consider for new systems are effective multiplexing, beam steering and control, and compactness.

The second set of challenges is to adapt to new laser sources. The near-IR TDLAS systems have been quite successful, with detection limits in the parts-per-million over short (~1–10m) pathlengths and relatively low costs. To improve on the technology, new room-temperature interband cascade lasers (ICLs) operating in the 3–6 μm region will allow access to more of the fundamental bands. Quantum cascade lasers (QCLs) are further out in wavelength (typically 5–12 μm), and may also be farther out in wide application because of the price associated with power, packaging, and stability requirements, but QCLs are also promising. Figuring out how to use these lasers in industrial environments has the potential to push detection limits lower by orders of magnitude as a result of the strength of transitions accessible in the mid-IR region. However, the chicken-and-egg problem of finding enough demand for the lasers to make them economical enough to implement may stand in the way, at least until the equivalent of the telecom boom of the 1990s comes to the mid-IR to drive ICL and QCL laser development. Stay tuned!

Acknowledgments

The author is grateful to have had the opportunity to learn much about TDLAS while working in partnership with Dr. Mohammadreza Gharavi, who was his graduate student from 1999–2004, and from staff for several years thereafter, at the University of California, San Diego. Later, commercial developments at Photon Machines, Inc. with Kregg Philpott continued the education. We found many of the potholes on the road to eventual success in implementation!

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SPECTROSCOPY SPOTLIGHT

LIBS FOR LIQUID SAMPLES

Although laser-induced breakdown spectroscopy (LIBS) potentially can be used for practically any kind of sample, most applications have focused on solid sample analysis. Montserrat Hidalgo, a professor in the Department of Analytical Chemistry and Food Sciences and the University Institute of Materials at the University of Alicante in Alicante, Spain, has been working with various approaches to extend the applicability of LIBS to trace-elemental analysis of liquid samples. She recently spoke to us about this research.

Your recent research has involved the use of LIBS to analyze liquid samples (1–3), as opposed to the solid samples most frequently studied by LIBS. What are the inherent difficulties associated with analyzing liquid samples with LIBS?

In fact, LIBS analysis of liquids is, in general terms, slightly more problematic than that of solids from the experimental point of view. Nevertheless, in my opinion the inherent difficulties are not associated with the LIBS technique, but with the specific experimental strategy used for LIBS analysis of the liquid sample. There are different ways by which a liquid sample can be analyzed with LIBS. For instance, excluding all those strategies involving sample preparation procedures, LIBS analysis can be performed in the bulk liquid; on the surface of the liquid; or in jets, aerosols or isolated droplets generated from the liquid sample. Bulk liquid analysis is relatively simple from the experimental or operational point of view, but sensitivity is the main limitation of this modality, because most of the plasma energy is lost in vaporization of the liquid and conversion into mechanical effects, such as the generation of a shock wave and a cavitation bubble, and only a small fraction is converted into radiative energy. Liquid surface analysis is comparatively more sensitive, because the laser-induced plasma expands in air. In this case, however, the strong mechanical effects arising from the laser–surface interaction cause experimental difficulties, such as liquid splashing on the optic components of the system, and generation of aerosol above the liquid sample or ripples on the liquid surface, which can affect both precision and sensitivity of the method. These experimental difficulties can be minimized or even eliminated using jet, aerosol, or isolated droplets strategies for LIBS analysis. However, the experimental complexity increase stemming from the need of additional devices to transform the liquid sample into the corresponding form for analysis and, in some cases, the applicability to viscous or turbid samples, can be problematic.

I think that when speaking about LIBS analysis of liquids the most critical point is not the experimental difficulty, which can be avoided by selecting the most adequate experimental strategy and experimental setup for a given application, but the analytical capability of LIBS detection for quantitative applications. Quantification limits obtained with all the aforementioned strategies are in most cases at the parts-per-million or high parts-per-billion level, which is inadequate for the quantification of most of the regulated contaminants in typical liquid samples, such as environmental or food samples. Therefore, even if these strategies can be perfectly valid when trace-elemental analysis is not a requirement, other analytical methodologies should be investigated if we want to extend LIBS applicability to a higher number of applications requiring the analysis of elements at trace levels, such as the monitoring of contaminants in liquid samples of interest. To this end, the development of analytical methodologies involving sample preparation procedures before LIBS detection seems to be a promising alternative.

What are some of the sample preparation techniques you have used to enrich liquid samples for trace-element analysis with LIBS?

Our research on liquid samples analysis with LIBS has been mainly focused on the use of microextraction methodologies as sample preparation techniques for analyte enrichment, in both liquid and solid-phase modalities. Microextraction methodologies usually lead to high enrichment factors, are more environmentally friendly than the corresponding conventional extraction modalities, and, in many cases, are also faster and more easily automatable. These two latter characteristics are very important when hyphenating sample preparation with LIBS detection if we want to use it for in situ and on-line applications. Among the existing liquid–liquid microextraction (LLME) modalities, we have tested single-drop microextraction (SDME)

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and dispersive liquid–liquid microextraction (DLLME). In the former technique, a microdrop of extraction solvent is suspended from the tip of a syringe, and introduced in the sample for a certain period of time. After extraction, the analyte-enriched microdrop is retracted into the syringe and analyzed by LIBS (2). In the latter approach, the extraction solvent is dispersed in the sample in the form of fine droplets. After extraction, the analyte-enriched organic phase is separated from the aqueous phase by centrifugation, retrieved with a syringe, and analyzed by LIBS (1,3). In both cases, the analyte-enriched organic solvent is placed on a solid substrate and heated to dryness before LIBS analysis. Even if detection limits obtained with the application of LLME procedures are quite good, at the parts-per-billion level, they involve several experimental steps and therefore are not easily automatable. This is why we have also tested the use of solid-phase microextraction (SPME) procedures. SPME is a very efficient procedure for analyte separation and enrichment, with the advantage that analytes are retained on a solid sorbent, which can be considered the best matrix for LIBS analysis. One of the SPME modalities we have tested is dispersive micro solid-phase extraction (D-μ-SPE) using graphene oxide as the sorbent, in which the extraction procedure is similar to that described for DLLME. This procedure also leads to limits of detection at the parts-per-billion level, but still the analyte-enriched sorbent needs to be separated from the sample before LIBS analysis. To avoid this step, we are currently working on a promising SPME modality called thin-film microextraction (TFME). In TFME, the sorbent is coated on the surface of a sheet-like base material, which is then immersed into the liquid sample for extraction of the analytes. After extraction, the thin film with the analyte-enriched sorbent is removed from the solution and analyzed by LIBS, therefore simplifying the sample preparation procedure, which, in this case, has good possibilities of automation.

What are the technique's advantages compared with other methods commonly used for elemental analysis of liquid samples, such as inductively coupled plasma–mass spectrometry (ICP-MS) or inductively coupled plasma–optical emission spectrometry (ICP-OES)?

It depends on the kind of measurements we are talking about. For instance, in my opinion, no advantage is gained by using LIBS for liquid analysis in routine laboratory measurements. ICP-OES and ICP-MS, among other elemental analysis techniques, are very sensitive and precise standard techniques that can be used to analyze many different kinds of liquid samples with or without the need of a previous sample preparation step, depending on the sample matrix and the

concentration of the target analytes. The main advantages offered by LIBS are not for laboratory measurements, but for in situ and on-line measurements. LIBS analysis is very fast; the instrumentation can be of small size and the setup needs no gas supply system, vacuum condition, or bulky coolant device so it can be portable, and, moreover, only optical access to the sample is needed to perform a LIBS measurement, which therefore allows stand-off analysis. All these characteristics make LIBS ideal for in situ and on-line applications such as oceanographic research and continuous environmental or industrial process monitoring, among others, which cannot be addressed with the conventional techniques commonly used for elemental analysis of liquid samples.

What are the limitations of LIBS for liquid sample analysis?

Compared with other techniques, the main limitation of LIBS is its low quantitative capability for trace-elemental analysis in liquids. It is possible to obtain very low absolute quantification limits with LIBS—for instance, at the picogram or even femtogram level when using experimental strategies such as isolated droplet analysis—due to its ability to sample extremely small amounts of liquid. However, as I have already discussed, relative quantification limits are usually at the parts-per-million or high parts-per-billion level, which makes the LIBS technique useless for applications requiring trace analysis. The use of sample preparation procedures can be a way to solve this limitation, but to improve the quantitative capabilities of LIBS analysis while maintaining its inherent advantages with respect to in situ and on-line capabilities, these sample preparation procedures should be efficient, simple, fast, and easy to automate and to hyphenate with LIBS detection. Finding a sample preparation procedure satisfying these requirements is not an easy task, and this is why it is still a challenge in LIBS research.

What are the primary samples or applications you have targeted? What makes them suitable for analysis by LIBS?

We have targeted many different applications since I started my research activity with LIBS technique more than 20 years ago. During these years, I have been studying both fundamental and applied aspects of LIBS and the targeted samples and applications have been many and varied, so it is difficult to select the primary ones. Aerosols, solids, and liquids have been the sample types on which I have focused my research.

Studies on aerosols were mainly dedicated to sulfuric acid aerosols analysis, with the goal to detect the aerosols produced during the oxidation of atmospheric dimethyl sulfide (DMS) in laboratory chemical kinetic studies on the formation of atmospheric aerosols. In this case, the suitability of LIBS arose from the ability of the technique to analyze sulfuric acid aerosols in situ and in quasi-real time, avoiding the conventional off-line procedure consisting of collection on filters and analysis by wet chemistry and ion chromatography, which is not adequate for the continuous monitoring of the reaction products of the DMS oxidation in the course of a kinetic experiment. Another great part of my research work has been dedicated to solid samples, which have been also of variate nature. Among others, soils, archaeological samples, human hair, steel, photovoltaic cells, and halloysite nanotubes have been some of the targeted samples. Usually, these solid samples were considered suitable for analysis with LIBS, because of the possibility of obtaining useful and fast analytical information by direct analysis of the sample, therefore avoiding the need for any sample preparation procedure. To conclude, in the last few years the primary samples I have targeted have been liquid samples, specifically aqueous samples. As I have already mentioned, liquids cannot be considered the most suitable samples to be analyzed with LIBS, especially if trace analysis is the pursued goal. In this case, my research activity has been precisely focused on the development of analytical methodologies able to convert these kinds of samples into appropriate forms for analysis with LIBS technique.

What are the next steps in your research?

My intention is to continue the research on hyphenation of LIBS detection with modern and efficient microextraction methodologies for liquid sample analysis. There is, nowadays, an increasing demand for portable analytical systems able to act, for instance, as early warning systems for environmental pollution control or industrial products quality control. I think that LIBS is a potential candidate to satisfy some of these demands, but, to this end, LIBS problems regarding the low quantitative capability in liquid sample analysis need to be solved, and therefore my intention is to focus the research on this critical point.

In view of the promising results we have obtained to date with the use of the thin-film microextraction technique combined with LIBS detection, the next step in the research will be to go deeper in the study of this sample preparation procedure. This research will include the study of new and efficient sorbent materials for TFME, different designs of films, evaluation of the developed methods for the analysis of different liquid matrices, and, a fundamental point, evaluation of possible ways for hyphenation and automation of both microextraction and detection processes in a single analytical system able to work in-field and to perform on-line measurements.

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SPECTROSCOPY SPOTLIGHT

MANIPULATING SOUND WITH LASERS AND OPTICS

Lasers are used for a wide range of industrial, medical, spectroscopic, and military applications. Daniel Kazal, a graduate research assistant in the Department of Chemistry and Biochemistry at the University of Maryland Baltimore County, has developed a novel technique for channeling sound using a tube-shaped laser beam that forms a thermal gradient. Based on his work with this approach, he received the 2017 FACSS Innovation Award. We recently spoke with him about this research. This interview is part of a series of interviews with the winners of awards presented at SciX last year.

Your work involves the manipulation of the direction and magnitude of sound waves traveling in air using laser light to generate photothermal barriers (1). Can you briefly describe the basic elements of the approach for both suppression and channeling of sound?

A species present in the air—in our case, ethanol vapor—absorbs the laser light, which creates a localized thermal gradient that causes the local air density to change. This air density change results in an abrupt change in compressibility at the edges of the beam, which provides an effective barrier for sound reflection. If you create a tube-shaped laser beam, you can generate an acoustic waveguide capable of channeling sound.

How is your approach an improvement over (or departure from) earlier techniques for photothermal manipulation of sound waves?

This is really the first time it has been demonstrated that it is possible to optically manipulate sound in such a manner. Before this study, the closest demonstration and observation of interacting thermally with sound waves was by John Tyndall during the 1880s, where he showed that acoustic waves can be suppressed when propagated over multiple heat barriers from a slot burner.

What were your instrumental setups or arrangements for your studies of suppression and channeling?

We employed a carbon dioxide laser, modulated with an optical chopper to create both photothermal barriers and channels inside a plexiglass chamber (used to contain the ethanol vapor). The acoustic channel was formed by using a beam expander and masking the center of the expanded laser beam with an earbud speaker, resulting in a tube-shaped channel.

What is the dependence of the suppression efficiency on wavelength and distance?

The wavelength of laser light affects the efficiency of suppression only as a function of its absorptivity by the air (or, in our case, ethanol vapor). The greater the population of excited molecules generated, the more efficient the change in compressibility will be and thus the greater the acoustic reflection will be.

How does laser power affect suppression?

Because this phenomenon relies on an initial optical absorption event, greater laser powers result in a larger number of molecules being excited and a greater thermal depletion zone. These conditions provide greater acoustic reflection efficiencies over larger distances.

What decibel range was studied? Were you able to achieve complete suppression of acoustic waves?

We studied acoustic intensities as great as 70 dB, which is about the equivalent of highway noise. This limit was due to the maximum safe amplitude that the earbud could output. By generating multiple barriers in the path of an acoustic wave, complete suppression can be achieved after just four consecutive optical barriers.

In your sound-channeling experiments, how is the doughnut-shaped laser beam formed for photothermal excitation of the air with the acoustic source located within the doughnut? What is the potential range of effectiveness for this technique?

As described before, the channel in these proof-of-principle concept studies was simply generated by blocking the center of an expanded laser beam with the earbud speaker (which doubled as the sound source). Theoretically, with enough laser power, this technique would allow sound to travel hundreds of kilometers. Even with our current setup (based on our $1/r^{0.6}$ measured acoustic decay profile), a 70 dB acoustic wave should be capable of being heard at over a kilometer away.

What are the most important applications or fields of use for this technique? Are there any potential biomedical applications?

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SPECTROSCOPY SPOTLIGHT

IMAGING MALARIA-INFECTED RED BLOOD CELLS WITH AFM-IR

In biology, the study of intracellular structures is important, and requires analytical techniques with submicrometer resolution. Atomic force microscopy-infrared (AFM-IR) spectroscopy is one technique that has the required lateral spatial resolution to observe such structures. David Perez-Guaita, PhD, a research fellow in the group of Associate Professor Bayden R. Wood at the Centre for Biospectroscopy at Monash University in Australia, is pioneering work applying AFM-IR to the study of red blood cells infected with the malaria parasite. The goal is to obtain information about the phenotype of the parasite, particularly of drug-resistant strains, which will contribute to the development of diagnostic and control measures. He recently spoke to us about this work.

You have recently used AFM-FT-IR to acquire nanoscale images of the intracellular structures of malaria-infected red blood cells (1). Can you briefly describe the steps in this approach?

The procedure can be divided in three phases. The first is sample preparation. The approach we applied is a standard procedure in parasitology, which involves the culture and synchronization of parasites and preparation of a smear. However, instead of using a glass slide, the infected red blood cells are smeared on a CaF_2 window, which is transparent to infrared light.

The second phase is generating AFM-IR multispectral images. The first stage of this second phase is to record an AFM image of a red blood cell. If the red blood cell is infected, it will exhibit topographical features caused by the presence of the parasite. The second stage is generating AFM-IR maps at different wavenumber values based on parasite bands identified in the AFM-IR spectra. Each map is a measurement of the thermal expansion produced by the laser, which is related to the absorbance of the material under the AFM tip. The wavenumber values measured are selected taking into account the molecules targeted by the analysis.

The third phase is statistical analysis, which requires coregistering the individual maps and applying multivariate data analysis to extract information about the composition of the different topographical features.

What was the role of multispectral image analysis in this work?

This critical step is to extract useful chemical information from the multispectral images. First, the different maps have to be registered to create a spectral hypercube containing a set of intensity values (one for each wavenumber) for each pixel. Then multivariate data analysis is applied using k-Means Cluster Analysis (kMCA). For example, pixels are grouped accordingly to their similarities. This approach makes it possible to identify different subcellular structures, such as a nucleolus or food vacuole. Using kMCA also provides the average values for the different classes, which can be used to study the composition of the different substructures identified.

What was the role of Raman confocal microscopy in the study?

Raman was used to confirm the presence of hemoglobin (Hb) and hemozoin (Hz) in the locations identified by AFM-IR. However, the spatial resolution of Raman spectroscopy is limited by the laser diffraction limit, and hence is much lower than the lateral resolution achieved using the AFM tip.

What challenges did you face in developing the approach?

One of the main challenges was to select the best conditions for image acquisition. Parameters such as laser power, acquisition time, and the number of coadded scans must be tuned to obtain high quality images. There is a trade-off between the signal-to-noise ratio and the acquisition time. Furthermore, cells can be damaged if the laser power is too high, or the exposure time is too long. In fact, we burned several samples before we were able to find suitable conditions for the measurements.

What were you able to see with this technique that was not possible to see with other techniques?

At the Monash Center for Biospectroscopy, we have been trying to develop IR- and Raman-based technologies to study malaria parasites for several years. The use of AFM-IR has two main advantages compared with other molecular imaging approaches.

TOM FULLUM/GETTY IMAGES

First, the lateral resolution of techniques such as Raman and IR spectroscopy is diffraction limited. In the case of Raman, Hz and Hb are strong Raman scatterers, and hence it is difficult to investigate other molecules in their vicinity. FT-IR spectroscopy, on the other hand, is able to detect changes in lipid and DNA composition, but the wavelength applied is in the size range of the parasites, making it impossible to identify subcellular structures. Our study shows that AFM-IR was able to distinguish and chemically characterize subcellular structures with submicrometer resolution. Secondly, AFM-IR not only offers information about the chemical composition of the subcellular structures, but also provides topographical information from the AFM maps. Unfortunately, the approach has also some disadvantages. The main disadvantage is that the penetration depth of the laser is not known with certainty, and hence it is hard to know to what extent we are measuring the whole cell or just the surface.

How can these images help in malaria research or other biology research? What are your next steps in this work?

This paper described is a proof of concept study that demonstrates the capabilities of AFM-IR in the study of the *Plasmodium sp.* parasites. We are currently trying to exploit these capabilities in the study of strains of the *Plasmodium sp.* parasites resistant to artemisinin. The use of artemisinin derivatives is threatened by the rapid spread of drug resistance in Southeast Asia. The World Health Organization (WHO) has warned that if the resistance reaches Africa or India, there is a limited window of opportunity to avert a regional public health disaster, which could have severe global consequences. It is known that artemisinin and artemisinin derivatives are activated by Fe-containing compounds present in the *Plasmodium* parasite to form carbon-centered free radicals, which damage the proteins of the parasite. Some strains have developed resistance to this mechanism, and we want to know why. So, our next steps in this work will include experiments comparing the chemical composition of resistant and susceptible strains using AFM-IR, and inoculation studies. By investigating the spectral differences between untreated and treated samples, one can identify the phenotypic changes induced by a drug. The study of the IR spectra of a treated susceptible strain of *P. falciparum* will potentially increase our knowledge of the mode of action of artemisinin. It will indicate the compositional changes induced by the presence of the reactive oxygen species from the activated artemisinin. The discovery of spectral markers associated with resistance can assist in the development of new drugs and increase the effectiveness of treatments.

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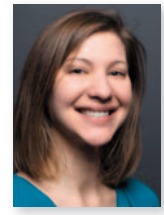
The 2018 Emerging Leader in Molecular Spectroscopy Award

This year's award recipient, Megan Thielges, is a pioneer in the use of vibrational probes, in conjunction with two-dimensional infrared spectroscopy, to investigate the structures and dynamics of proteins, with the aim of understanding biological systems.

Nicole Olson

Megan Thielges, an associate professor of chemistry at Indiana University, has won the 2018 Emerging Leader in Molecular Spectroscopy Award, which is presented by *Spectroscopy* magazine. This annual award recognizes the achievements and aspirations of a talented young molecular spectroscopist, selected by an independent scientific committee. The award will be presented to Thielges at the SciX 2018 conference in October, where she will give a plenary lecture, and be honored in an award symposium.



Megan Thielges

Thielges earned her B.S. in biochemistry at Arizona State University. She went on to earn her PhD in biophysics from The Scripps Research Institute in La Jolla, California, in 2009. After three years as a postdoctoral fellow at Stanford University, she obtained her current position as an associate professor of Chemistry at Indiana University. She has published 11 papers as a principle investigator, and 22 as a postdoctoral, graduate, or undergraduate researcher. She has given 45 presentations and received six previous awards, as well as nominations for more. In addition to her research, Thielges has served as a reviewer for numerous scientific journals and on panels for many major national departments, including the National Institutes of Health, the National Science Foundation, and the Department of Energy.

The Challenges of Studying Protein Dynamics

The focus of Thielges's work is the study of protein structure and dynamics. Specifically, she is a pioneer in the use of multisite vibrational probes in conjunction with two-dimensional (2D) infrared (IR) spectroscopy to investigate the structures and dynamics of proteins.

The study of protein structure and function is a field of great interest. Proteins are the mediators of biological functions, and the structure of proteins is complex. The simplest level of protein structure is the primary structure, which consists of a sequence of amino acids joined to form a polypeptide chain. The order of the amino acids is what affects the structure and function of the protein; a single substitution can have life-threatening consequences. The secondary structure consists of a regular pattern of substructures that are formed because of the bonds between the various amino acids that compose the polypeptide chain. Proteins are not necessarily static in their conformations, however. In the field of protein dynamics, scientists look at the transitions a protein can make between different shapes that can occur on various scales of length or time, or both. These conformational changes have been shown to affect cellular functions, such as enzymatic activity and cellular signaling. These changes can happen on extremely fast timescales, making vibrational spectroscopy an excellent technique to observe them.

Given the dense nature of protein structure and the large amounts of molecules present, however, measured signals are congested, with large amounts of spectral overlap, making it is difficult to resolve the signals. A strategy for alleviating this signal congestion is to use a vibrational probe, which involves replacing one or more molecules in the structure with another whose vibrational signature operates in the so-called *silent region* of proteins, from about 1800 to 2500 cm^{-1}, a region free from native protein signals (1). But developing effective vibrational probes is challenging. For a vibrational probe to be useful at a specific protein site, it must be strongly responsive to the physical property being investigated, be sensitive only to its immediate environment, cause little to no change to the original structure of interest, and be able to be incorporated into the system (2). This is where Thielges comes in.

Harnessing Vibrational Labels and 2D IR to Study Proteins

A major aspect of Thielges's work is the development of new vibrational labeling strategies. This is the crucial element of her contributions, according to Andrei Tokmakoff, a pro-

fessor at the University of Chicago. "Her work has resulted in a toolbox that is opening up new ways of using IR spectroscopy in protein chemistry and biophysics," he said. "By combining these new labels with ultrafast 2D IR, Megan has [developed] a new way of studying protein chemistry, where fast dynamical processes involved in catalysis or binding are directly observed rather than inferred."

Studying Enzyme Catalysis

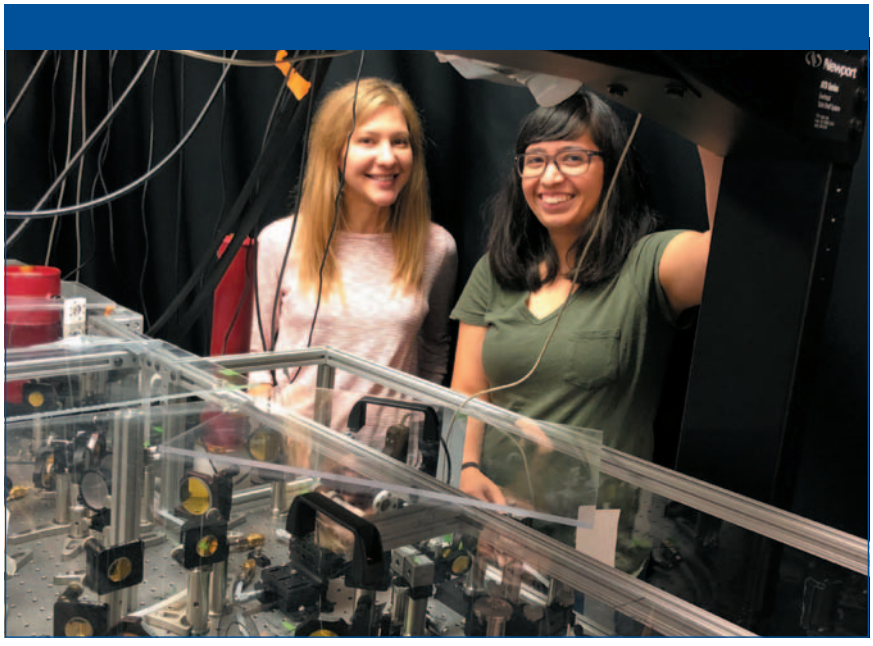
One example of this work was Thielges's use of vibrational labels to study cytochrome P450 enzymes, which play a role in the biological functions of molecular synthesis and metabolic processes. They are the major enzymes involved in the metabolism of medications, making their structure and function of particular interest to medical researchers. Thielges's work combining a new labeling techniques she developed (heme-bound CO, in this case) with IR and 2D IR spectroscopy has revealed different substrate binding configurations at the active site in these enzymes and enabled characterization of the fluctuations and exchange of these configurations. Her studies on P450 enzymes have illustrated the importance and power of 2D IR spectroscopy to measure protein dynamics occurring on very small timescales, as well as the technique's ability to gather information about the role of dynamics in biological activity (3). The National Institute of Health has awarded Thielges research grants for this work. By resolving different substrate binding configurations and the fast dynamics of conformational interchange, these experiments may one day enable understanding of the drug-degradation process undertaken by these enzymes.

Cellular Signaling

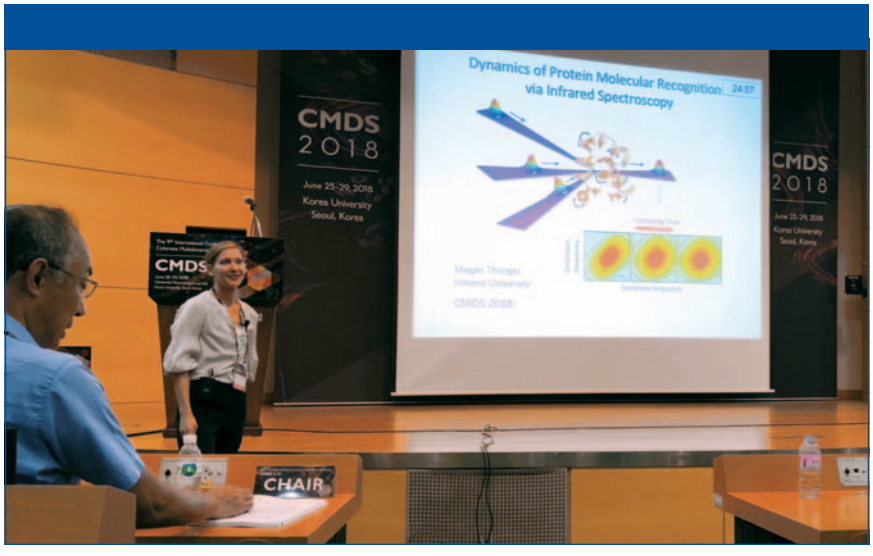
Another important application of Thielges's work is in the study of cellular signaling. The SH3 region in proteins is responsible for controlling certain signal transduction pathways, or the process through which a physical or a chemical signal is carried through a cell (cellular signaling). By introducing carbon-deuterium probes into the SH3 domain in yeast cells, Thielges was able to observe variations in the binding conformations of a proline-rich peptide (4). This study illustrated the power of IR spectroscopy to uncover rapidly interconverting states in a protein–peptide complex for the study of cellular signaling.

Enabling the Study of Proteins at the Nanosecond Timescale

With 2D IR spectroscopy, it is possible to detect the signals from vibrational probes. However, the experimentally accessible timescale has typically been limited to the picosecond regime. This limitation has led to the need to develop 2D IR probes with longer vibrational lifetimes to extend the experimental timescale. Thielges's group has introduced and studied a novel IR probe, p-cyano-seleno-phenylalanine (CNSeF), for the study of protein dynamics by 2D IR spectroscopy, that is able to extend signal lifetime into the nanosecond range. The group demonstrates that this new probe could be detected on timescales 20–50 fold lon-



Thielges with one of her students, Sashary Ramos, in the laser laboratory at Indiana University.



Thielges giving a talk at the International Conference on Coherent Multidimensional Spectroscopy (CDMS 2018) in Seoul, Korea, in June 2018.

ger than could be achieved with the popular probe cyanophenylalanine (5).

Recognition by Others

Tokmakoff wrote enthusiastically in support of Thielges's nomination for this award. He described her as an "ambitious and creative interdisciplinary scientist" who conducts "fearless research." What makes her stand out from her colleagues, he said, is not only that she is highly trained in two different scientific fields that rarely cross—protein and enzyme biochemistry and biophysics, and ultrafast laser spectroscopy—but that she is able to overcome the challenges of combining them successfully. As Tokmakoff said, leading spectroscopists often work with model systems (rather than meaningful biological problems), and chemical and molecular biologists are often unwilling to work with the long development process of new physical methods. "Megan is really the first of her kind—one who can do it all," he said. "She is truly a first class experimental scientist in both areas."

Michael Fayer, a professor of chemistry at Stanford University, where Thielges did postdoctoral research, agrees with Tokmakoff's appreciation of Thielges's combination of skills. "Megan is an amazing blend of a real biologist and a hard-core chemical physicist," he said. "This is her great strength that allows her to make important contributions to biology and biophysics."

Minhaeng Cho, the director of the IBS Center for Molecular Spectroscopy and Dynamics and a professor of chemistry at Korea University, first met Thielges at a conference three years ago, and the two have since collaborated on multiple occasions. According to Cho, Thielges's contributions to molecular spectroscopy started while she was still in graduate school. While at the Scripps Institute, he said, Thielges "introduced an extremely small and non-perturbative vibrational probe into proteins in a site-specific manner." This was important because of the spectral congestion that made using the plentiful C-H bonds to study the shapes and motions of proteins impossible. By replacing the hydrogen with deuterium at specifically targeted sites, Thielges demonstrated that the C-D stretch band, which falls into proteins' silent IR region, has "line shapes [that] are highly sensitive to protein conformations and secondary structures." Cho believes Thielges's research will lead her to address deep questions in biology, such as how protein–protein interactions and recognition are related to fast local fluctuation dynamics at the interface between proteins.

Floyd Romesberg, who was Thielges's graduate advisor at Scripps, says Thielges is a remarkable experimentalist. "She totally revolutionized our experimental approach to data collection, and her methods are still used by my group today," he said. "She also organized a study group for the students to teach themselves the theory underlying time-resolved non-linear spectroscopy."

Carlos Baiz, an assistant professor of chemistry at the University of Texas at Austin, describes Thielges as "creative, resourceful, [and] charismatic." He believes she will continue to "push the envelope" when developing more complex probes and methods to further evaluate protein structure and dynamics. He be-

lieves her new techniques will be more widely adopted by the ultrafast community in the coming years.

Peter Hamm at the University of Zurich conducts research that is very closely related to that of Thielges. But even though he characterizes their relationship as "a friendly competition," he nominated her for an award last year. Hamm describes Thielges's research as "an absolute prerequisite" for 2D IR spectroscopy to become a more versatile tool to study the structure and dynamics of proteins. "Her work is courageous," he says. "It does not seek the low-hanging fruit."

Summary

Megan Thielges's colleagues all believe she has a very bright and promising future ahead of her. Romesberg summarized this outlook well. "Megan will continue to combine sophisticated biology and spectroscopy," he said, "not only to explore protein dynamics, but to begin to elucidate how [they have] been tailored by evolution for biological function."

Megan Thielges is a pioneer at the forefront of combining biophysics with spectroscopy. She has been "fearless" and has overcome many practical obstacles to advance these two fields. She is a top-notch experimentalist who takes on big challenges. From organizing study groups to teach herself the theory behind time-resolved non-linear spectroscopy to developing methods as a graduate student that her advisor and his current group still use today, it seems there is nothing Megan Thielges can't do if she puts her mind to it.

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Thielges receiving an award for her work as a session chair at the International Conference on Coherent Multidimensional Spectroscopy in Seoul, Korea, in June 2018.

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Quantitative Detection of Phenobarbital in an Injectable Solution Based on Surface-Enhanced Raman Spectroscopy

A simple method for phenobarbital detection was established. Based on the density functional theory (DFT), the geometry optimization and spectra calculation were performed using Gaussian software on a Becke, 3-parameter, Lee-Yang-Parr (B3LYP) level for phenobarbital. The results from theoretical analysis were compared with experimental results, and the Raman frequencies and their vibrational modes were assigned. A silver colloid surface-enhanced Raman scattering (SERS) substrate, with uniform size and good monodispersity, was prepared, and samples were detected without any pretreatment. The detection conditions were optimized. A linear relationship was obtained between concentration and Raman intensity over the 12–100 µg/mL concentration range. The limit of detection (LOD) was 12 µg/mL, and the recovery rate was between 94.2% and 111.2%. This method shows great practical potential for the identification of phenobarbital.

Lin Bao, Siqingaowa Han, Yaoye Xu, Hang Zhao, Dianyang Lin, and Wuliji Hasi

Phenobarbital is a representative long-acting barbiturate. It has sedative, hypnotic, and anticonvulsant effects. If used improperly (including excessive and long-term use), it may threaten human health. Large doses can inhibit the cardiovascular and respiratory systems, and even can lead to paralysis of the respiratory center of the medulla oblongata. Long-term use can lead to dependence. Psychotropic drugs make the body dependent and can harm human health. Therefore, the use of such drugs should be strictly monitored and controlled.

Raman spectroscopy offers fast, simple, repeatable, qualitative, and quantitative analysis in a noninvasive way. It does not require complex sample preparation, or data analysis (1–3). However, the signal is weak. Thus, surface-enhanced Raman spectroscopy (SERS) is used to overcome problems related to low sensitivity (4,5). It is widely used in pharmaceutical analysis (6,7). Yokoyama and Yamada (8,9) describe a flexible substrate based on SERS for biochemical analysis. However, there is no optimization of the experimental parameters, quantitative analysis, and assignment of the characteristic peaks.

We examined the Raman spectra of phenobarbital using the droplet detection mode (10). Some of Raman characteristic peaks of phenobarbital were assigned, and the Raman peak at 666 cm^{-1} was used as the basis for stability analysis and quantitative analysis. The experimental conditions were then optimized. The linear quantitative range was 12–100 µg/mL. The main devices and procedures are shown in Figure 1.

Experimental

Reagents

Silver nitrate ($AgNO_3$) and sodium citrate was purchased

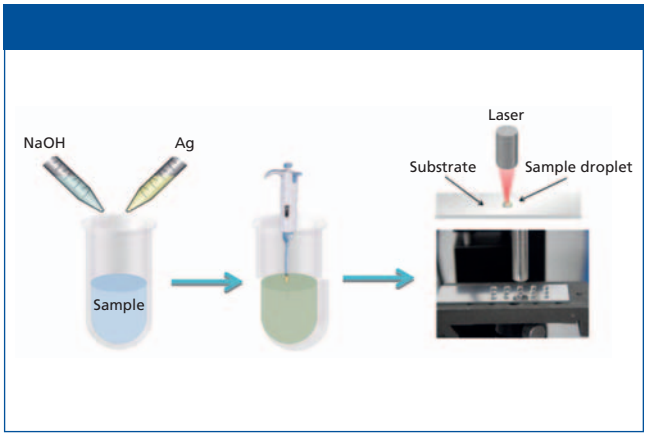

Figure 1: Schematic diagram of the method proposed for the phenobarbital detection process.

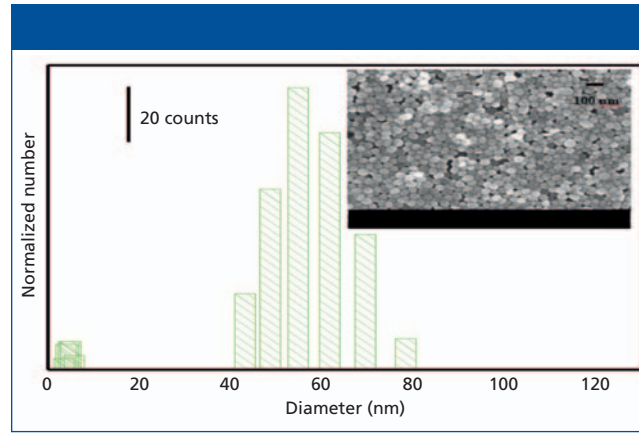

Figure 2: Size distribution of silver nanoparticles (inset: SEM picture of silver nanoparticles).

from Sinopharm Chemical Reagent Co., Ltd. Sodium hydroxide (NaOH) and potassium iodide (KI) were purchased from Xilong Chemical Co., Ltd. An injectable phenobarbital solution (0.1 mg/mL) and phenobarbital powder were purchased from Tianjin Jin Yao Pharmaceutical Co., Ltd. Hydrochloric acid (HCl) was purchased from Tianjin Kermel Chemical Reagent Co., Ltd.

Apparatus

We used a model BWS415-785H portable Raman spectrometer (B&W Tek, Inc.). The laser wavelength was 785 nm. The probe distance was 5.9 mm, and the spectral resolution was less than 3 cm^{-1}. The laser power was 120 mW, and the spectral integration time was 5 s. The data were analyzed by B&W Tek, Inc. embedded software for smoothing and background correction.

Sample Treatment and Preparation of Silver Colloid

The 0.1-mg/mL of phenobarbital injectable solution served as the stock solution. This solution was diluted with deionized water at 12, 20, 30, 50, 70, and 100 µg/mL. The silver nitrate powder was dissolved in deionized water (11) and heated. When the solution boiled, the sodium citrate solution with a mass fraction of 1% was immediately added, and then boiled for 1 h. This solution was then cooled from room temperature to 4 °C, and protected from light.

Results and Discussion

Based on the electromagnetic (EM) enhancement theory, the SERS intensity is dependent on the resonance frequency of the noble metal substrate. The plasmon resonance frequency depends on the size of the nanoparticles, especially for gold and silver nanoparticles (12–15). The morphology and size of the silver nanoparticles were characterized by scanning electron microscopy (SEM), as shown in Figure 2. As can be seen from the map, the silver nanoparticles are spherical and have uniform morphology. The average size of silver nanoparticles was about 50 nm, and its main particle size distribution ranged from 50 to 80 nm by dynamic light scattering analysis.

Raman spectra were performed with the B3LYP hybrid functional with the 6-31g (d, p) basis set. The structure of the optimized phenobarbital molecule is shown in Figure 3. To attribute the Raman characteristic peaks of phenobarbital, we collected the Raman spectra of powder and the SERS spectra of phenobarbital, and compared their Raman spectra with DFT calculation, as shown

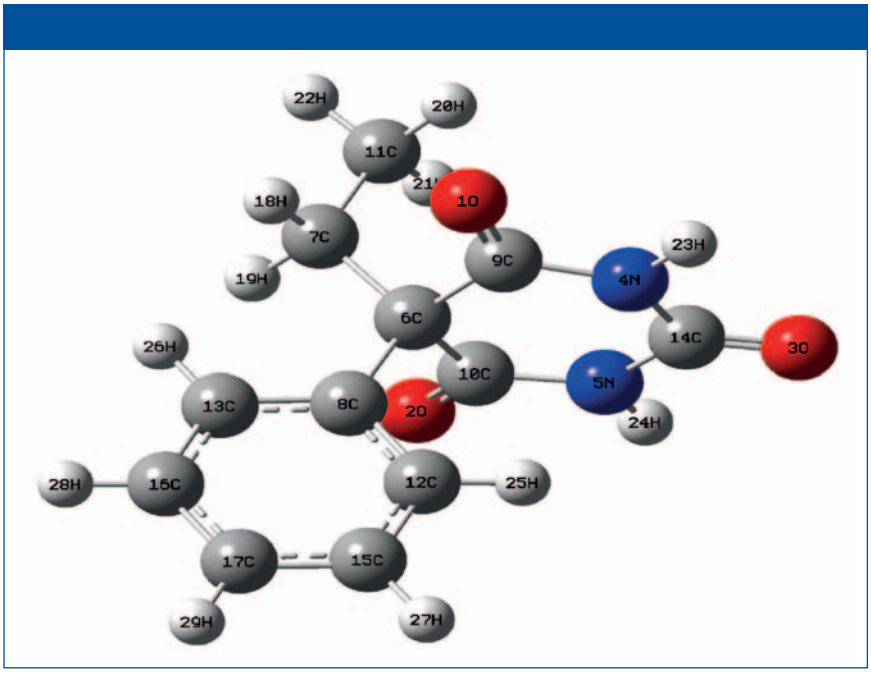

Figure 3: Optimized structure of phenobarbital.

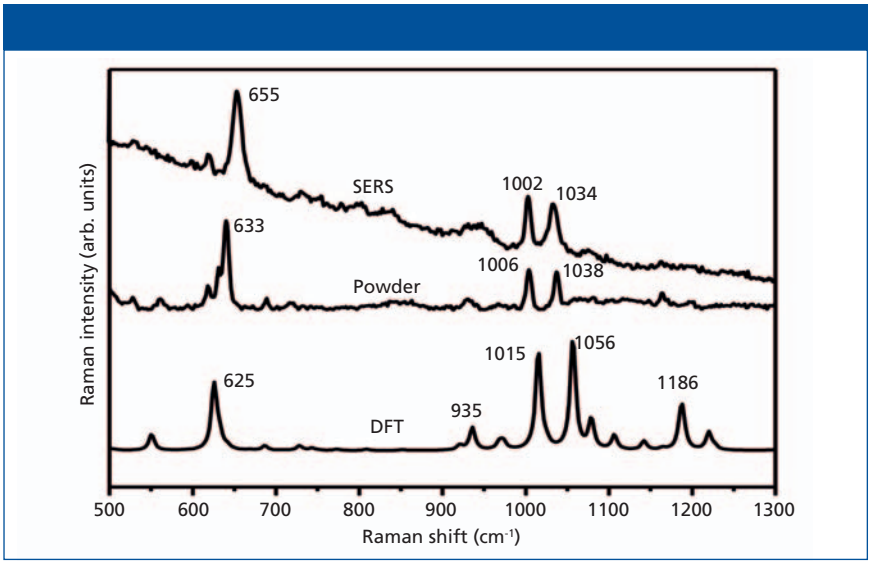

Figure 4: Raman analysis of phenobarbital: theory-calculated, powder, and SERS spectra of an aqueous solution (0.1 mg/mL).

Table I: Theoretical and experimental vibration frequency of phenobarbital

DFT (cm^{-1})	Solid powder (cm^{-1})	SERS (cm^{-1})	Ascription
625	633	655	δ(N-heterocycle)+$\delta(C_7H_{18,19})$
935	—	—	$\delta(C_{11}H_{20,21,22})+\nu(C_6\text{-}C_8)+\delta(C_7H_{18,19})$
1015	1006	1002	δ(Benzene ring)+$\nu(C_6\text{-}C_8)$
1056	1038	1034	$\delta(C_{13}H_{26})+\delta(C_{12}H_{25})+\delta(C_{15}H_{27})+\delta(C_{16}H_{28})+\nu(C_{16}= C_{17}=C_{15})$
1189	—	—	$\delta(C_{16}H_{28})+\delta(C_{17}H_{29})+\delta(C_{12}H_{25})+\delta(C_{15}H_{27})+\nu(C_6\text{-}C_8)$

in Figure 4. It can be seen from the graph that the main Raman peaks in phenobarbital are 625 cm^{-1}, 935 cm^{-1}, 1015cm^{-1}, 1056 cm^{-1}, and 1189 cm^{-1}. These peaks are basically matched to the Raman peaks of the solid powder and the Raman peaks of the SERS in the aqueous solution. The deviation of displacement is caused by different external conditions. The theoretical model is the case of a single phenobarbital in the vacuum, and the solid and SERS spectra are the results of the solid and liquid phase. The matching and attribution of the specific Raman characteristic peaks of phenobarbital are shown in Table I.

Adding coagulant can change the dispersion state of the nanoparticle sol. This addition breaks the balance potential of the sol system so that the sol particles aggregate (16). It results in more SERS hotspots with good enhancement effects. Thus, we selected 0.1 M (NaOH), acid (HCl) 0.1 M, and neutral (KI) 0.1 M coagulants. The results were collected under the same experimental conditions and show that the Raman spectra of hydrochloric acid coagulant do not induce characteristic peaks (Figure 5). The spectrum with KI coagulation has only subtle peaks at 1002 cm^{-1} and 1034 cm^{-1}. These phenomena are probably due to the theory of Hofmeister; some ions will destroy the structure of macromolecules and the denaturation strength of chloride ions in the sequence is greater than that of iodine ions, which is consistent with the experimental results. Although a certain degree of migration occurred in the 655 cm^{-1} position that shifted to 666 cm^{-1}, the spectrum with sodium hydroxide has all strong peaks in phenobarbital. Thus, all subsequent experiments used 0.1 M NaOH as the coagulant. The coagulant, sample, and silver colloid volume ratios were 1:1:1.

The SERS spectra of different concentrations of phenobarbital were obtained (Figure 6). The laser power was 120 mW, and the integration time was 5 s. All measurements were performed over five replicates and averaged. Above 12 µg/mL, the Raman

signal of the sample could still be detected at 666 cm^{-1}. The detection limit of phenobarbital in aqueous solution was 12 µg/mL. In the past, the judgment of the detection limit for the substance is more concentrated on one of the most discernible characteristic peaks. The experiment can also have 666 cm^{-1} peaks at lower concentration, and 666 cm^{-1} peaks at lower concentration could be detected. But for the sake of being precise, this article uses 666 cm^{-1} as the quantitative analysis peak, and the combination of 1002 cm^{-1} and 1034 cm^{-1} appears simultaneously as the criterion for determining the LOD.

A stable and repeatable SERS signal is important when using silver aggregates. The 666 cm^{-1} peak was selected for stability analysis. We selected three different concentrations of phenobarbital solution under the same conditions. These were repeated over 15 experiments in 5 min. The standard deviations at 80 µg/mL and 50 µg/mL were both less than 5% and

Table II: Analytical results for the samples

Spiked (µg/mL)	Sample Found (µg/mL)		Recovery (%)	RSD (%)
16	1	15.070	94.2	3.78
	2	16.256	101.6	
	3	15.728	98.3	
	Average	15.685	98.033	
60	1	65.460	109.1	4.89
	2	61.620	102.7	
	3	54.960	99.1	
	Average	60.680	103.63	
90	1	87.750	97.5	6.59
	2	93.330	103.7	
	3	100.080	111.2	
	Average	93.720	104.13	

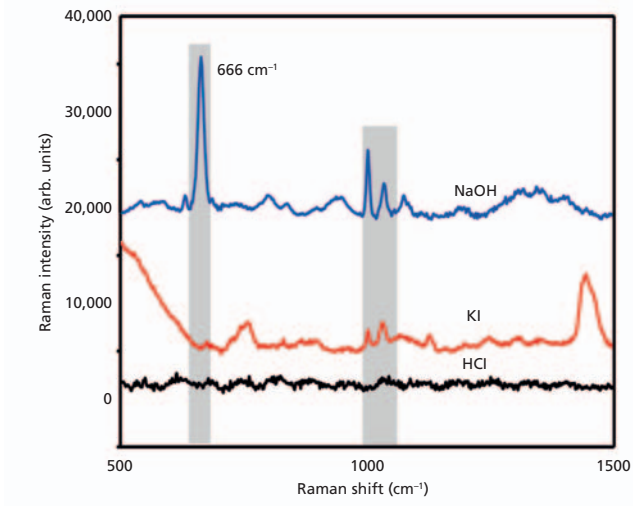

Figure 5: SERS spectra of phenobarbital sodium using different aggregating agents.

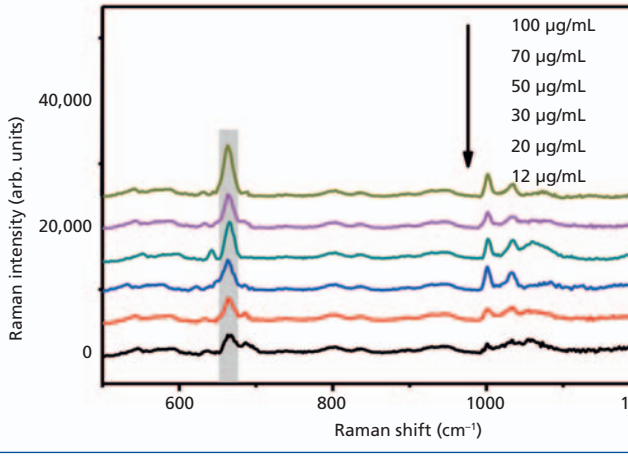

Figure 6: SERS spectra of different concentrations of aqueous phenobarbital.

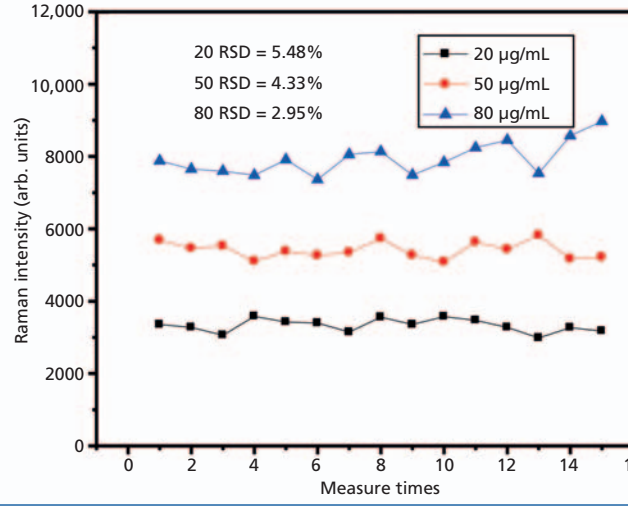

Figure 7: Reproducibility of NaOH-induced silver aggregates in solution.

approximately 5% at 20 µg/mL (Figure 7). Figure 7 shows that the silver aggregates have good repeatability.

Figure 8 shows the linear relationship between the concentration of phenobarbital and Raman intensity at 666 cm^{-1} from 12 to 100 µg/mL. The fitting equation is $y = 1582.6 + 84.896x$, where y is the intensity of the Raman peak at 666 cm^{-1} in the SERS spectrum of the phenobarbital sodium and x is the concentration of phenobarbital sodium. To ensure the accuracy of the data, all spectra were collected five times and averaged. The correlation coefficient is 0.9955, suggesting a good fit.

To test the accuracy and reliability of this method, we detected three different concentrations (16 µg/mL, 60 µg/mL, and 90 µg/mL) of the spiked samples. The SERS spectra were collected using a portable Raman spectrometer, and each sample was measured three times. We selected the 666 cm^{-1} characteristic peak intensity values to fit the formula above. We then calculated the corresponding concentration value, and calculated the corresponding recovery rate and relative standard deviation (Table II). The data indicate that SERS can accurately detect phenobarbital sodium. The recovery rate was 94.2%–111.2%, and the average relative standard deviation is about 5%. SERS detection of phenobarbital is accurate, simple, and efficient. This technology offers real time and rapid detection of phenobarbital.

Conclusions

We used SERS to detect phenobarbital in an injectable solution. First, the hydrogen phenobarbital Raman peaks were assigned to determine the characteristic peak. We then induced aggregation with sodium hydroxide. The aqueous solution of sodium hydroxide increased the SERS signal and lowered the detection limits. We used linear fitting for semiquantitative analysis, and then calculated the recovery rate. The detection limit of phenobarbital is based on the signal at 666 cm^{-1} in the range of 12–100 µg/mL. The best fit curve is $y = 1582.6 +$

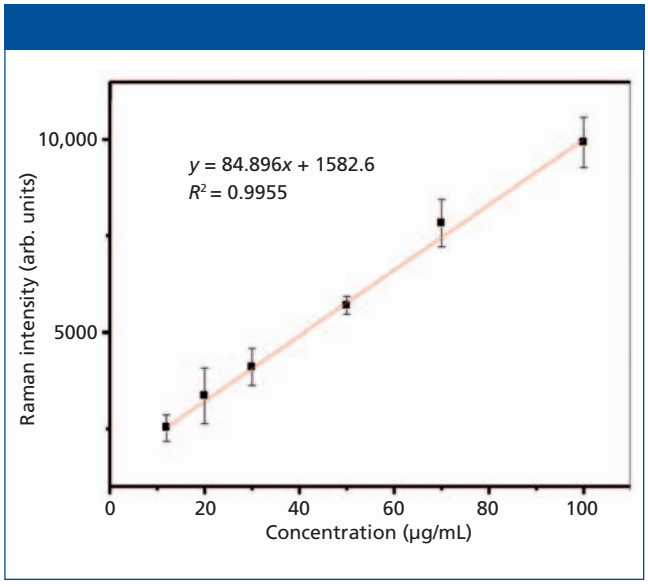

Figure 8: Calibration curve for phenobarbital.

84.896x (R^2 = 0.9955). The recovery rate was 94.2–111.2%. This method is rapid, accurate, nondestructive, and easy. It is useful for the detection of phenobarbital in an injectable solution.

Acknowledgments

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EAS 2018: Analytical Solutions to the World's Problems

November 11–14, 2018
Crowne Plaza Princeton Conference Center
Plainsboro, NJ

Mary Ellen McNally
President of the 2018 Eastern Analytical Symposium & Exposition

The theme of the 2018 Eastern Analytical Symposium and Exposition is "Analytical Solutions to the World's Problems." The EAS has always been about problem solving. This year, we focus on problems that affect the world. These problems affect our families, our health, our homes, our environment, and our planet, no matter where we live or work. Sometimes, by our best efforts, we solve one problem, only to create another. The analytical community typically is asked to respond to such difficult problems. The community has always been innovative and creative in addressing the needs of our employers, our businesses, our communities, and the world. We discover new entities, create novel instruments, define scientific processes, and provide critical analytical services to tackle even the most complicated problems and challenges.

The EAS program this year offers a multitude of analytical solutions to problems, whether societally created, technologically or industrially generated, or just the results of human curiosity and activity. Presentations on analysis in pharmaceutical, environmental, clinical, conservation, food, forensic, regulatory, proteomic, and health science contexts are planned, with experts in each of these fields offering state-of-the-art solutions.

Sustainability

We have started to make EAS a sustainable green conference with some specific green initiatives this year. With electronic posters and a reduction of pages in the final program (both of which save trees), we have begun to change our carbon footprint. Our keynote speaker, John Warner, the founder of the Warner Babcock Institute for Green Chemistry, will present on green chemistry technologies, and lead our efforts at sustainability.

Plenary Lecture

Our plenary lecture will be given by Linda P. McGown, the William Weightman Walker Chair of Chemistry and Chemical Biology at Rensselaer Polytechnic Institute, and the winner of the 2018 EAS Award for Outstanding Achievements in the Field of Analytical Chemistry. The EAS award this year is given in recognition of McGown's solutions to problems in the fields of separation science, the analysis of DNA sequences, and her development of aptamers as potential pharmaceutical substances. We also offer an early morning breakfast lecture by Mark Schure, of the Theoretical Separation Science Laboratory on the current state 2D liquid chromatography.

Overall Conference Program

In addition to these focused lectures, the program committee has finalized a wide variety of invited and contributed sessions that span the gamut of analytical chemistry. Whether it's forensics, pharmaceutical analysis, environment, food science, or cultural heritage, there are session with talks by international leaders in analytical chemistry. Of particular interest to *Spectroscopy* readers, there are 12 sessions on spectroscopy. These include an award session organized by the New York–New Jersey division of the Society for Applied Spectroscopy (SAS) and two EAS award sessions (details in the next section), and a special session celebrating the 60th anniversary of SAS. Other spectroscopy sessions cover topics such as the elemental analysis of solids, chemometrics for Raman spectroscopy, clinical applications, nano-infrared spectroscopy, materials identification, spectroscopy in food quality and safety, and mobile applications. For more information, check out the program at the EAS website: www.eas.org.

Awards

EAS has long recognized the efforts of analysts, and this year is no exception. In 2018, the Eastern Analytical Symposium & Exposition is pleased to honor the following recipients of the 2018 EAS Award for Outstanding Achievements in:

- **The Fields of Analytical Chemistry:** Linda P. McGown of Rensselaer Polytechnic Institute
- **Magnetic Resonance:** Clare Grey of the University of Cambridge
- **Mass Spectrometry:** Yinsheng Wang of the University of California, Riverside
- **Vibrational Spectroscopy:** Stephen P. Cramer of the University of California, Davis.

In addition to these field-based awards, we are pleased to present the **EAS Young Investigator Award** to Kerri Pratt of the University of Michigan.

Also, the EAS is pleased to provide a forum for these society-based awards:

- **NY–NJ SAS Gold Medal:** Igor Lednev of the University at Albany
- **New York Microscopical Society's Ernst Abbe Award:** Peter R. De Forest of John Jay College
- **American Microchemical Society's Benedetti-Pichler Award:** Ryan C. Bailey of the University of Michigan.

Posters in New Electronic Format

As mentioned earlier, this year EAS is instituting a new format for poster presentations. Rather than being presented on paper, all posters will be displayed electronically. This approach will allow for more interactive flow of information, as well provide a green alternative to the usual paper posters seen at most meetings.

Short Courses

For those who want to hone their expertise in various areas, the EAS has offered short courses designed to help the practicing analyst to develop new skills and enhance knowledge. They are specifically designed to help analysts keep current with best practices and new techniques. This year there are 36 courses that range from instructions on separations to general courses on troubleshooting and process analysis. You are sure to find topics that will provide essential knowledge and enhance your career in analysis. The instructors are experts in their specialties, and they communicate the important, and sometimes esoteric, nature of techniques and problems encountered in everyday laboratory work. The complete list of EAS short courses, descriptions, and schedule is available on our website at www.eas.org.

Exposition

Of course, EAS is well-known for the exposition, and this year we continue the tradition of providing attendees with the latest innovations in analytical technology. More than 80 exhibitors have already signed on to be a part of the action. So, whether your need is specific to a particular problem, or you want to learn what the latest innovations are, you will find it at the exposition.

Join us at the Crowne Plaza Princeton Conference Center in Plainsboro, New Jersey, November 11–14, 2018 to see everything that EAS has to offer.

Continued from page 33

We are really excited about this because there are many potential applications for this phenomenon, including standoff chemical sensing, secure communications, acoustic stealth, and dynamic acoustic manipulation. There are also potential biomedical applications, like increased tissue penetration for photoacoustic sensing, and imaging by optically guiding acoustic, or ultrasonic signals to or from specific locations within tissues.

What are the next steps in your research?

There are many. Currently, we are focusing on applying this phenomenon to the numerous ambient air applications, including acoustic suppression and standoff sensing. In addition, we are also focused on further characterizing the properties of this phenomenon, including incident acoustic angle on reflection–propagation efficiency, the effect of localized thermal gradients, and many more.

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PRODUCTS & RESOURCES

LED light sources

Ocean Optics' LSM series LED light sources are designed for fluorescence excitation and other measurements requiring narrow-band illumination. The LEDs are controlled by an LDC-1 compact single-channel driver and controller with a touchscreen. When using the internal modulation mode, the user can select sine, triangle or square waveforms.
Ocean Optics,
Largo, FL;
www.oceanoptics.com

Transmission Raman accessory

Ondax's transmission Raman accessory is designed so that measurements in both transmission and backscatter modes can be made without moving the sample. The sample chamber comes with a set of holders that accommodate pills or tablets of varying sizes, and can also be used with standard HPLC vials.
Ondax,
Monrovia, CA;
www.ondax.com

Programmable hydraulic press

The PressPRO microprocessor-controlled automated hydraulic press from PIKE is designed to produce consistent KBr pellets to enhance the uniformity and quality of transmission samples. Up to four pressure hold time and ramps may be programmed and performed within a run, and five profiles may be stored on the device. According to the company, the press is capable of a variable force from 1 to 15 US tons and can hold any force value indefinitely.
PIKE Technologies,
Madison, WI;
www.piketech.com/PressPRO-Hydraulic-Press.html

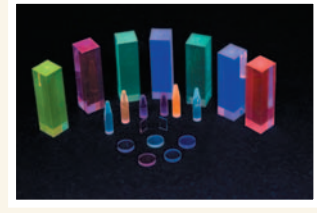
Microplastics analysis application note

An application note from Renishaw describes how it is possible to analyze microplastics with the company's InVia confocal microscope. According to the company, the analyses can locate fragments, identify the polymers present, and output statistics on particle size and composition.
Renishaw, Inc.,
West Dundee, IL;
www.renishaw.com/raman

Fluorescence instruments & accessories

PerkinElmer's FL 6500 and FL 8500 fluorescent spectrometers are designed for sample testing. According to the company, the instruments use interchangeable, plug-and-play accessories, intuitive software, and support services.
PerkinElmer, Inc.
Waltham, MA
www.perkinelmer.com

Polymer fluorescent references



Starna's second-generation polymer fluorescent references are designed for wavelength calibration and the monitoring of instrument performance in fluorescent applications. According to the company, the new references are based on new proprietary dyes and provide increased stability and resistance to photobleaching, allowing for their use as relative photometric intensity references. **Starna Cells, Inc.,** Atascadero, CA;
www.starnacells.com

UV–vis spectrophotometers

Genesys UV–vis spectrophotometers from Thermo Fisher Scientific are designed with a variety of options. According to the company, the Genesys 50 spectrometer has a single cell configuration; the Genesys 150 spectrometer provides automation for high-throughput options and room-light resistance; the Genesys 180 spectrometer includes an eight-cell changer for higher throughput environments; and the Genesys 160 spectrometer adds preprogrammed methods. **Thermo Fisher Scientific,** Madison, WI;
www.thermofisher.com/genesys

Handheld LIBS analyzer

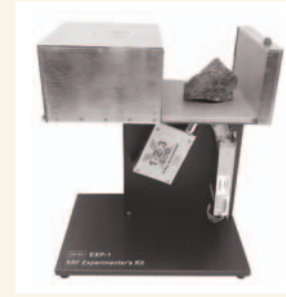
The Chemlite Plus handheld laser-induced breakdown (LIBS) spectroscopy analyzer from TSI is designed to identify base metals, including iron, copper, and nickel, in as little as 1 s. According to the company, the analyzer comes with spectrum logging and downloading capability.
TSI Inc.,
Shoreview, MN.
www.tsi.com

FT-NIR laboratory analyzer for biodiesel

ABB's MB3600-CH30 FT-NIR laboratory analyzer for biodiesel is designed for determining biodiesel properties in final product and mid-batch biodiesel samples from vegetable oil transesterification reactors. According to the company, the analyzer uses a transmission sampling method with disposable glass vials.

ABB Measurement & Analytics,
Quebec, Qc, Canada;
www.abb.com/analytical

XRF kit



Amptek's XRF kit is designed to help users quickly begin doing elemental analysis via X-ray fluorescence. According to the company, the kit includes the company's X-123 complete spectrometer with a FAST SSD or SSD detector, a Mini-X USB Controlled X-ray tube, XRF-FP QA software, a sample enclosure, and test sample.

Amptek, Inc.,
Bedford, MA;
www.amptek.com

Spectrometer

BaySpec's SuperGamutUVSWIR benchtop spectrometer is designed to cover the full spectral range of 200–2500 nm. According to the company, the spectrometer is compact, has no moving parts, and provides long-term stability and high light throughput, with low stray light.

BaySpec, Inc.,
San Jose, CA;
www.bayspec.com

Automated electric fusion fluxer

The Katanax X-300 fluxer from Spex SamplePrep is designed to produce fused beads for X-ray fluorescence or inductively coupled plasma solutions for analysis. According to the company, the benefits of electric fluxers versus gas fluxers include safety, temperature control, low power consumption, and simple installation.

Spex SamplePrep,
Metuchen, NJ
www.spexsampleprep.com/x300-fluxer

Microwave digestion system

CEM's MARS 6 sample preparation system now includes the contactless in situ iWave temperature sensor. According to the company, using light-emitting technology, the sensor renders the vessel transparent, enabling the determination of the temperature of the actual sample in real time. The sensor reportedly can measure the sample temperature of each vessel without the need for a control vessel, fiber-optic probes, or wires.

CEM Corporation,
Matthews, NC;
www.cem.com

Electron backscatter diffraction camera

The Velocity EBSD camera from EDAX is designed for highspeed electron backscatter diffraction mapping. According to the company, the camera combines indexing speeds greater than 3000 indexed points per second with indexing success rates of 99% or better, and image resolution that provides orientation precision values of less than 0.1°.

EDAX, Inc.,
Mahwah, NJ;
www.edax.com

Direct mercury analyzer

Milestone's DMA-80 direct mercury analyzer is designed to provide results that are comparable to traditional techniques in as little as 5 min per sample. According to the company, the analyzer has the ability to analyze virtually any matrix.

Milestone, Inc.
Shelton, CT;
milestonesci.com/direct-mercury-analyzer

NIR scanner

The NIRONE scanner from Spectral Engines is designed to pair wireless and Bluetooth connectivity with the company's NIRONE NIR sensor and light sources. According to the company, the battery-powered scanner is ready for measurements out of the box, and includes cloud-based analytics and a free demonstration and feasibility testing service.

Flash Photonics, a distributor for Spectral Engines,
Redmond, WA;
www.flash-photonics.com

Calendar of Events

October

21–26 October SciX 2018
Atlanta, GA
www.scixconference.org

31 October–1 November 2018 8th International Conference on Current Trends in Mass Spectrometry and Chromatography
Columbus, OH
https://massspectra.com

31 October–1 November 2018 21st International Conference on Pharmaceutical & Bio-Inorganic Chemistry
San Francisco, CA
https://pharmaceuticalchemistry.pharmaceuticalconferences.com

31 October–2 November 2018 Analytical China 2018
Shanghai, China
www.analyticachina.com/index.html

November

2–6 November 34th Asilomar Conference on Mass Spectrometry
Pacific Grove, CA
www.asms.org/conferences/asilomar-conference/asilomar-conference-homepage

4–7 November American Association of Pharmaceutical Scientists (AAPS) Annual Meeting and Exposition
Washington D.C.
www.aaps.org/aaps/annual-meeting-and-conferences/annual-meeting

12–14 November Eastern Analytical Symposium and Exposition
Princeton, NJ
eas.org

25–30 November 2018 Materials Research Society Fall Meeting and Exhibit
Boston, MA
www.mrs.org/fall2018

December

5–6 December 8th International Conference on Chemistry & Drug Designing 2018
Vancouver, British Columbia, Canada
https://chemistrydrugdiscovery.pharmaceuticalconferences.com

January 2019

30–31 January 2nd World Congress on Petrochemistry Conference
Bangkok, Thailand
https://petrochemistry.global-summit.com

February 2019

2–7 February SPIE Photonics West
San Francisco, CA
https://spie.org/conferences-and-exhibitions/photonics-west

3–8 February European Winter Conference on Plasma Spectrochemistry
Pau, France
https://winterplasma19.sciencesconf.org

16–21 February SPIE Medical Imaging
San Diego, CA
https://spie.org/conferences-and-exhibitions/medical-imaging

March 2019

17–21 March Pittcon Conference & Expo 2019
Philadelphia, PA
https://pittcon.org

April 2019

2–6 April Materials Research Society Spring Meeting & Exhibit
Phoenix, AZ
www.mrs.org/spring2018

14–18 April SPIE Defense + Commercial Sensing
Baltimore, MD
https://spie.org/conferences-and-exhibitions/defense--commercial-sensing

May 2019

28–30 May North American Workshop on Laser Ablation 2019
Austin, TX
www.jsg.utexas.edu/nawla2019/

June 2019

23–27 June SPIE European Conferences on Biomedical Optics
Munich, Germany
https://spie.org/conferences-and-exhibitions/european-conferences-on-biomedical-optics

August 2019

6–10 August Denver X-ray Conference 2019
Lombard, IL
www.dxcicdd.com

11–15 August SPIE Optics + Photonics
San Diego, CA
https://spie.org/conferences-and-exhibitions/optics-and-photonics

Short Courses

November

4 November Strategies for Effective Science Communication
Sacramento, CA
sacramento.setac.org/scientific-program/abstract-submission/training-courses

4 November Using Multiple Lines of Evidence for Sediment Quality Assessment in Regulatory Programs
Sacramento, CA
sacramento.setac.org/scientific-program/abstract-submission/training-courses

4 November mRNAseq Data Workshop: De Novo Assembly and Differential Expression Analysis
Sacramento, CA
sacramento.setac.org/scientific-program/abstract-submission/training-courses

4 November An Introduction to Data Science with R
Sacramento, CA
sacramento.setac.org/scientific-program/abstract-submission/training-courses

5–8 November European Short Course on Principles and Applications of Time-Resolved Fluorescence Spectroscopy
Berlin, Germany
www.picoquant.com/events/details/fluorescence-course

7–9 November Functional Near-Infrared Spectroscopy (fNIRS) Course
Boston, MA
www.bu.edu/neurophotonics/2018/01/23/fnirs-course_nov2018

11 November Intact and Top-Down Protein Characterization and Quantitation by Mass Spectrometry: Approaches for Pharmaceutical Drug Discovery, Development, and Bioanalysis
Princeton, NJ
easinc.org/wordpress/?page_id=5171

11 November Process Analytical Technology: Out of the Lab and into the Line
Princeton, NJ
easinc.org/wordpress/?page_id=5158

11 November Taking Advantage of the Power in Excel
Princeton, NJ
easinc.org/wordpress/?page_id=5201

11 November Interpretation of Mass Spectra with Practical Solutions to Problems
Princeton, NJ
easinc.org/wordpress/?page_id=1063

11 November Lifecycle Approach to Analytical Methods for Drug Products, Incorporating QbD Concepts
Princeton, NJ
easinc.org/wordpress/?page_id=4013

11 November Introduction to Vibrational Spectroscopy for Real Time Analysis
Princeton, NJ
easinc.org/wordpress/?page_id=1075

11–12 November Chemometrics Without Equations Parts 1 & 2
Princeton, NJ
easinc.org/wordpress/?page_id=1002

13 November Modern Portable Analytical Spectroscopy
Princeton, NJ
easinc.org/wordpress/?page_id=5165

13 November Setting Data Quality Objectives and Determining, Reporting, and Interpreting Data Quality Indicators to Meet Scholarly Publication Requirements
Princeton, NJ
easinc.org/wordpress/?page_id=5169

14 November Practical NMR Spectroscopy
Princeton, NJ
easinc.org/wordpress/?page_id=5154

14 November Analytical Instrument Qualification from a Chemical Metrology Perspective
Princeton, NJ
easinc.org/wordpress/?page_id=5258

14 November Keeping Your Analytical Procedures in Compliance with the FDA: Validation, Documentation, and Change Management
Princeton, NJ
easinc.org/wordpress/?page_id=2515

29–30 November Metabolomics Informatics
San Francisco, CA
www.asms.org/conferences/fall-workshop/fall-workshop-homepage

December

4-6 December General Multivariate Analysis Principles and Spectroscopy
Chicago, IL
www.camo.com/training/courses/general-mva-spectroscopy.html?id=819

Call for Papers

Spectroscopy invites researchers to submit their work for publication.

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References. Number the literature citations in the text consecutively in order of appearance and indicate them by Arabic numerals in parentheses. Number each reference separately. Group the references at the end of the manuscript in the order of their appearance in the text, not alphabetically.

Use Chemical Abstracts Service Source Index for journal abbreviations. Use the following format for references:

(1) R. Salzer and H.W. Siesler, *Infrared and Raman Spectroscopic Imaging* (Wiley-VCH, Weinheim, 2009), pp. 90–103.

(2) P. Matousek, *Appl. Spectrosc.* **60**, 1341 (2006).

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A number of supplemental issues are planned for 2019, for which we also invite contributions. Manuscripts for the supplements on Raman technology, IR spectroscopy, and ICP-OES & ICP-MS should be 2500–3000 words long, with up to six figures and tables combined, and should include an abstract of approximately 150–200 words. We are accepting submissions for the following issues:

Raman Technology supplement, June 2019

Submission deadlines:
Abstracts: January 11, 2019
Manuscripts: March 15, 2019

IR Spectroscopy supplement, August 2019

Submission deadlines:
Abstracts: February 1, 2019
Manuscripts: April 5, 2019

ICP–OES & ICP–MS supplement, September 2019

Submission deadlines:
Abstracts: March 1, 2019
Manuscripts: May 8, 2019

Current Trends in Mass Spectrometry supplement series

Our supplement series on mass spectrometry appears four times a year: in March, May, July, and October. Manuscripts for this series should be approximately 3500–4500 words long, including an abstract of approximately 150–200 words, plus up to eight figures and tables. We are still accepting submissions for the following issues:

Submission Deadlines:

March 2019 issue
Abstracts: November 8, 2018
Manuscripts: January 11, 2019

May 2019 issue
Abstracts: January 11, 2019
Manuscripts: March 1, 2019

July 2019 issue
Abstracts: February 8, 2019
Manuscripts: April 12, 2019

October 2019 issue
Abstracts: April 1, 2019
Manuscripts: June 7, 2019

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For more information about contributing to *Spectroscopy*, please contact

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Ad Index

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