JANUARY 2016 # 36

the Analytical Scientist

Diving into the Deep

Oceanographer Rob Munier explores the mysteries of our blue planet – and the role of analytical science.

24 – 30

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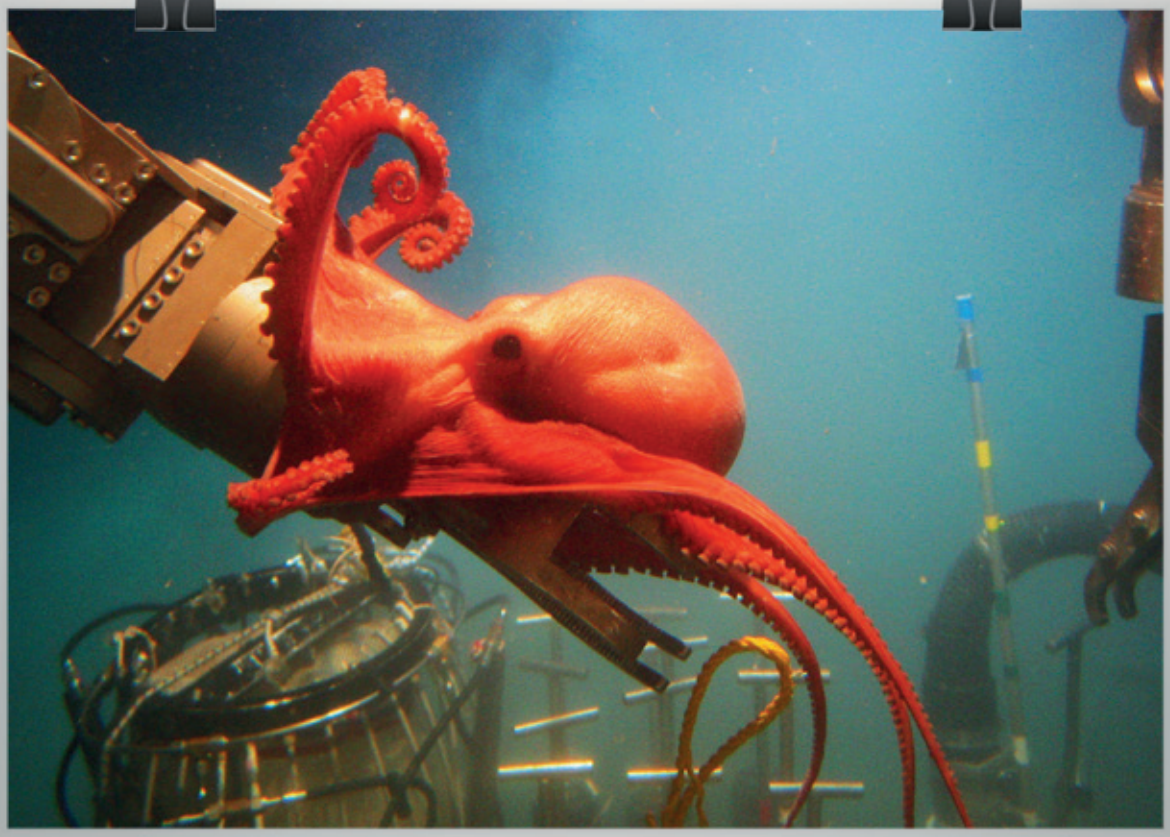
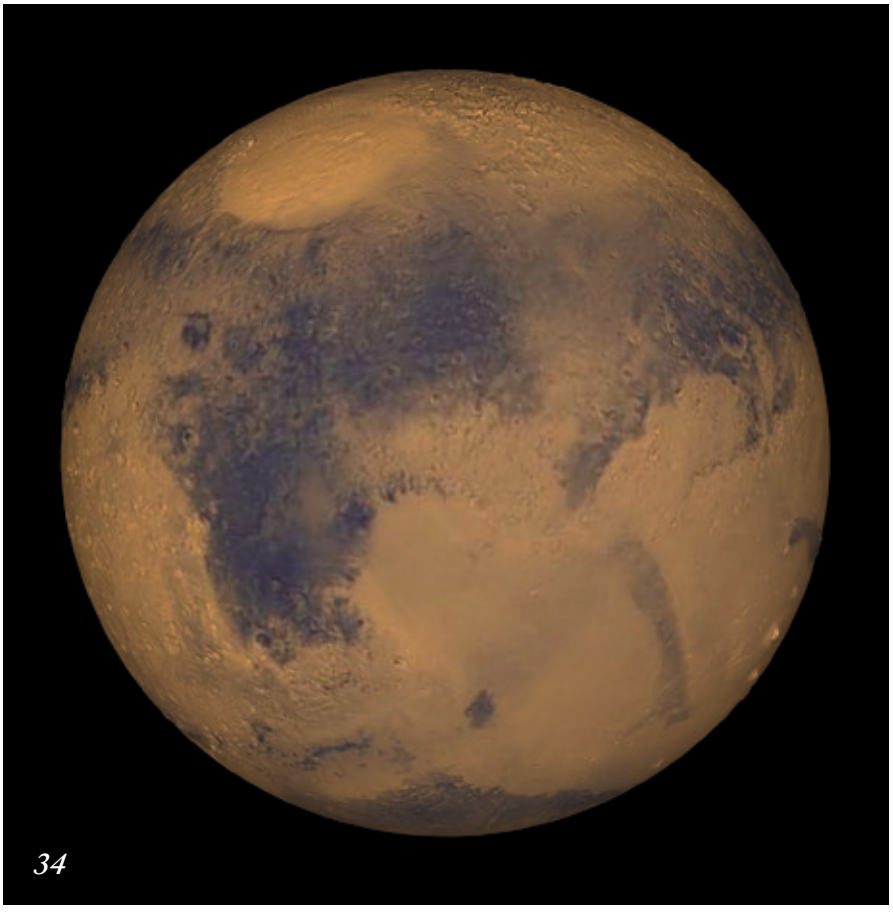
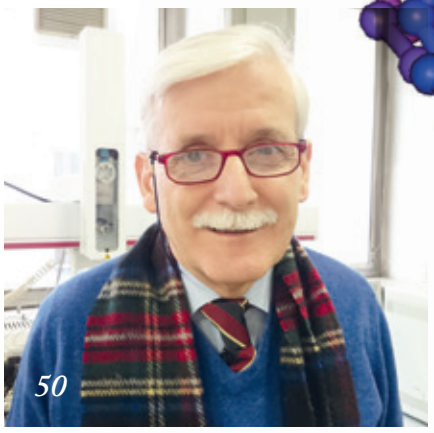


Benthoctopus sp gets overly familiar with Alvin's robotic manipulator arm at 2,300 meters down in the Gulf of Mexico.
Photo by Bruce Strickrott (Alvin pilot) from Expedition to the Deep Slope/NOAA/OER (NOAA Photo Library).

Would you like your photo featured in Image of the Month?
Send it to rich.whitworth@texerepublishing.com

Contents



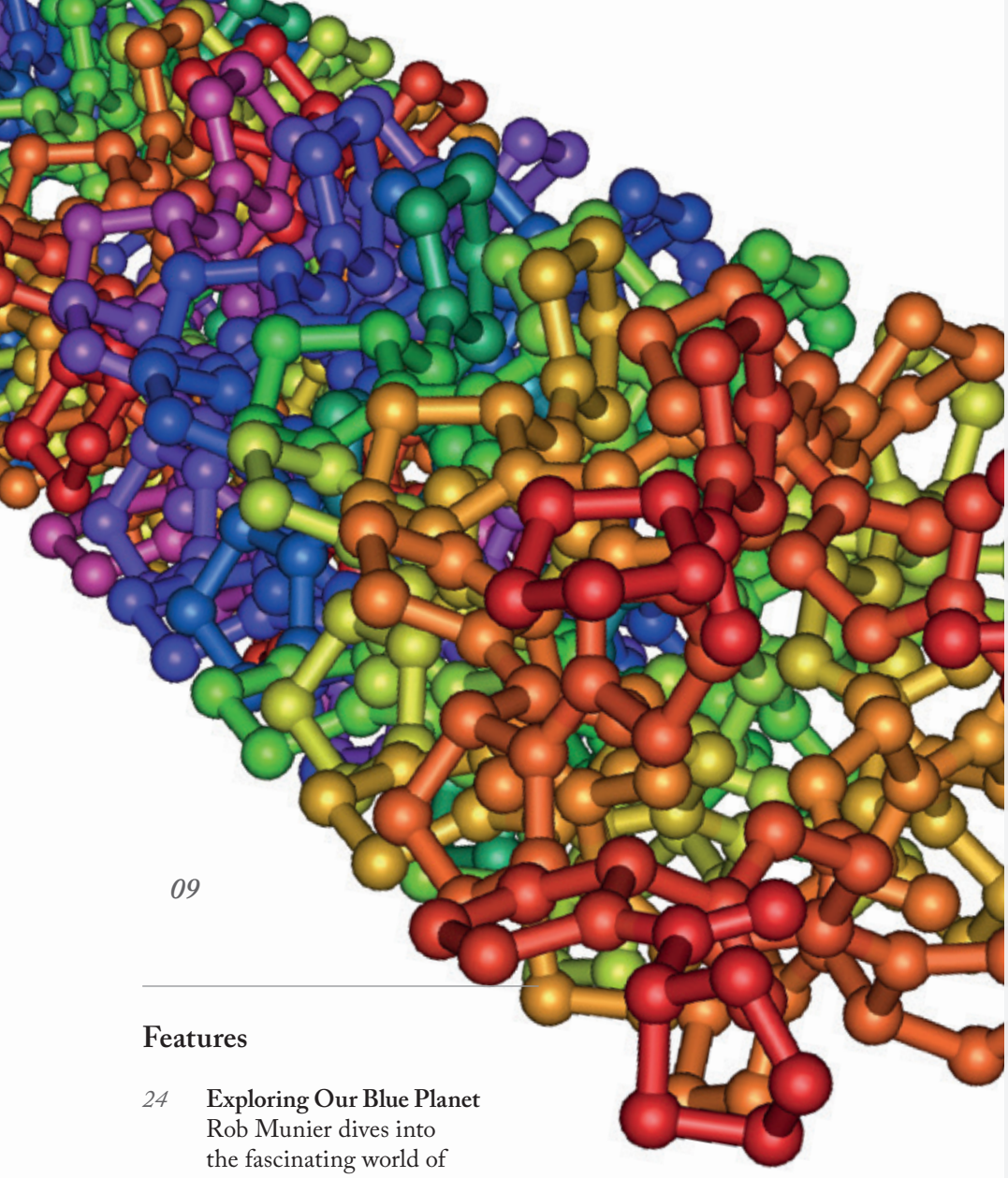


On The Cover

P Neumann and A Heater tend to Alvin. Photo by Chris Linder. Copyright © Woods Hole Oceanographic Institution

Upfront

In My View



09

Features

Reports

Departments

Sitting Down With

the **Analytical Scientist**

ISSUE 36 - JANUARY 2016

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Nice Measurement – Now What?!

Concerning disparity between measurements and decisions.

Analytical science has a unique vantage point of our world. From the oceans deep to the final frontier – and everything in between – we endeavor to make measurements that help unlock mysteries and provide enlightening information. Put another way, analytical scientists (as a collective) "see" a lot. But what if there is a disconnect between what analytical scientists see and what people want to hear?

Accurate measurements inform and drive science. Science should drive decisions. And then continued (hopefully) accurate measurements (built on "the science") should help steer humanity down a sensible path. Unfortunately, sometimes those in power need a different outcome and rewrite or simply ignore "the science".

I have long been interested in the analytical chain of custody, not just in terms of sample collection and laboratory workflows (clearly important) but – more importantly for me – what happens after the measurement has been made and documented. Where does it go, who acts upon it and what happens next? Does anything happen next?

In some cases, those who make the measurements are sadly too far removed from any decisions that result. Taking an extreme example, oceanographers like Rob Munier (page 24) are busily investigating ocean acidification (and its direct effects) around the globe – but the Kyoto Protocol will give you a clue as to how world leaders will react to "the other carbon dioxide problem"...

A striking example of measurements and decisions parting ways was found in Dick Pound's first World Anti-Doping Agency independent commission late in 2015, which resulted in Russia accepting a full and indefinite ban from world athletics for "state-sponsored doping". Pound said, "We've found cover ups, we've found destruction of samples in the laboratories. We have found payments of money in order to conceal doping tests."

Even more recently, it emerged that a letter from the International Association of Athletics Federations to Valentin Balakhnichev (then president of the All-Russia Athletic Federation) back in 2009 stated that blood levels of athletes were "putting their health and even their own lives in very serious danger" (1). How many analytical scientists were complicit?

Analytical scientists – with their unique vantage point – should always be the first to shout out when decisions appear to bear no resemblance to the measurement (or "the science"). If you need courage, check out Janine Arvizu's new-found Netflix fame on page 13. Whistleblowers unite!

Rich Whitworth
Editor

Reference

1. *http://www.bbc.co.uk/sport/athletics/35298526*

Upfront

Reporting on research, personalities, policies and partnerships that are shaping analytical science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email:
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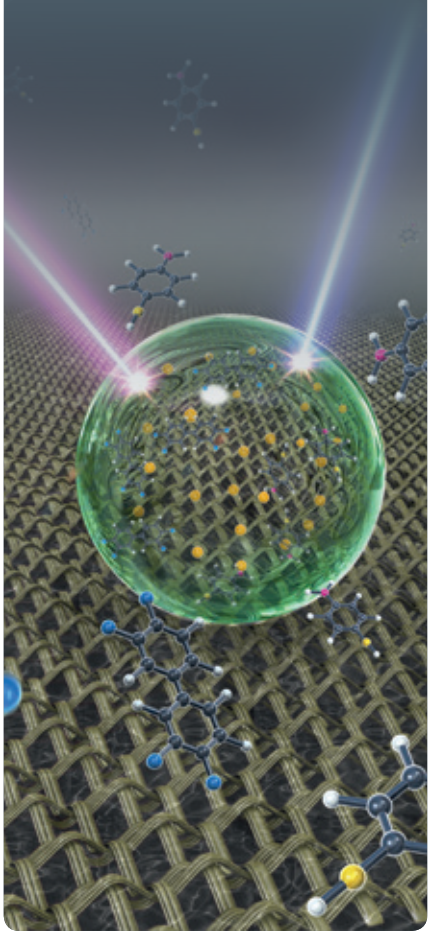


Courtesy of Shikuan Yang, Birgitt Boschitsch Stogin, and Tak-Sing Wong/Penn State

SERS SLIPS Ahead

Can a new slippery surface for Raman scattering open the door to routine single molecule detection?

Surface-enhanced Raman scattering (SERS) has high molecular specificity and high sensitivity, but its applicability is limited by a reliance on aqueous solvents for extraction and poor performance in heavily diluted solutions. Now, researchers from Pennsylvania State University have combined SERS with slippery liquid-infused porous surfaces (SLIPS) to create... You guessed it: SLIPSERS (1). We spoke with Tak Sing Wong, Assistant Professor of Mechanical Engineering and lead author of the study, to find out how they're addressing the limitations of SERS.

Why focus on SERS?

SERS is a very powerful platform for molecular diagnostics and analysis. However, there are two important roadblocks that limit its practical application. First, SERS detection in liquid media relies on highly statistical binding of analytes to the SERS-sensitive regions (or "hot spots") because of the diffusive nature of the analytes. As a result, it is very challenging to achieve single-molecule detection in highly diluted solutions. Second, many real-life analytes may be dispersed in liquid or gas phases or may be bound to solid substrates (for example, soil), which may require the use of non-aqueous solvents for extraction.

My postdoctoral researcher – Shikuan Yang – and I have come up with a solution to these problems. By allowing a non-aqueous/aqueous droplet containing the extracted analytes and the SERS substrates to shrink in an isotropic manner onto a solid surface, we can bring all the analytes to close proximity of the SERS hot spots. SLIPS is the perfect solid surface to achieve this because it can be designed to repel both aqueous and non-aqueous liquids.

What exactly is SLIPS?

SLIPS is formed by infusing a liquid lubricant on a functionalized micro/nanotextured solid surface. Once the SLIPS is prepared, we can place a liquid droplet that contains the targeted analytes and SERS substrates (for example, gold nanoparticles) onto the surface. By allowing the liquid droplet to fully evaporate, the SERS substrates and analytes will self-assemble into a microscopic aggregate, which can then be used for SERS analysis.

And how is SLIPSERS performing?

By using the SLIPSERS platform, we found that we can achieve limits of detection down to subfemtomolar level in both aqueous and non-aqueous liquids. This limit of detection level, particularly within non-aqueous liquid, is very challenging to achieve using conventional detection methods. In addition, the flexibility on the choice of extraction solvents also allows us to perform ultrasensitive detection of analytes from gaseous, liquid or solid samples.

How do you see SLIPSERS being applied?

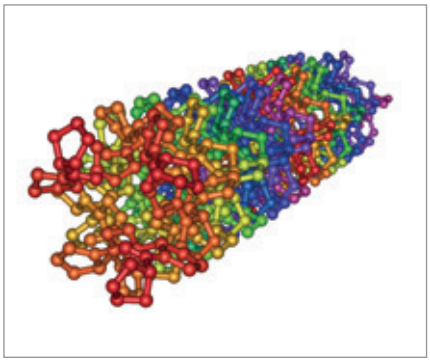
In the future, we hope to see SLIPSERS technology used for ultrasensitive molecular detection in applications related to analytical chemistry, medical diagnostics, environmental monitoring and national security. The ability to detect single molecules of any kind would revolutionize disease diagnostics and environmental monitoring.

Reference

1. *S Yang et al., "Ultrasensitive surface-enhanced Raman scattering detection in common fluids", Proc Natl Acad Sci USA, 113, 2, 268-73 (2016). PMID: 26719413.*

Characterizing Collagen at the Double

Two-dimensional mass spectrometry (2DMS) can be twice as fast as conventional methods in proteomic analyses



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Conceived in the late 1980s, two-dimensional Fourier transform ion cyclotron resonance mass spectrometry (2D FT-ICR MS) correlates the mass-to-charge (m/z) ratio of fragment and precursor ions in a single spectrum, but was put on the backburner until recent advances in computer technology and algorithms gave researchers the necessary processing capability. And though some studies have taken advantage of the new tech to analyze a handful of smaller proteins and peptides, no group has analyzed a protein nearly as large as collagen – the subject of a new study by researchers at the University of Warwick (1). The researchers found that 2DMS could "uncoil collagen" two times faster than conventional methods.

"Collagen is a big protein, about 400 kDa, with many modified amino acids and crosslinks," said Peter O'Connor, lead author of the study. "If we chop it into peptides using trypsin, and try to analyze the resulting mixture, there are thousands of peaks, and we can only assign a few dozen - the mixture is simply to complex."

The group were already involved in analyzing smaller proteins like cytochrome-c and calmodulin with 2DMS, and thought it might be worth trying 2DMS with collagen. "The results were pretty exciting and show that 2DMS can now be applied to a wide range of protein and proteomics samples."

"Not having to select a precursor prior to fragmentation means you remove that selection bias from the data," says O'Connor. "2DMS is also faster than a traditional LC-MS/MS run or an MS/MS run where every precursor is selected in series, at least for complex mixtures, for the same experimental resolution."

The researchers are now looking to apply 2DMS to cancer and diabetes research. O'Connor suggests that for cancer, 2DMS will allow more detailed study of the pathways activated by possible anti-cancer drugs and thus allow more evidence-based assessment of their efficacy. Whereas for type-2 diabetes, the method will allow better study of the protein aggregation pathways that result in loss of beta cells and triggering of insulin dependence.

"2DMS will allow better, faster, cheaper and a less biased analysis of all proteins in the sample, simultaneously," says O'Connor. "It will alleviate (though not eliminate) the need for reliable chromatographic separations, which are the slow and fiddly parts of a normal proteomics experiment [...] Overall, it's an exciting development that offers a new tool to biomedical chemists who are interested in detailed molecular understanding of disease". *JS*

Reference

1. *HJ Simon et al., "Uncoiling collagen: a multidimensional mass spectrometry study", Analyst, 141, 1, 157-65 (2016). PMID: 26568361.*

Faster (GC) Tactics

Five ways to speed up gas chromatography analyses using your current system

By Jaap de Zeeuw, Restek Corporation, Middelburg, The Netherlands.

Reducing analysis time is a constant priority. And it's sensible because it cuts costs per analysis and you get faster results. Here, I'd like to share some clever ways to reduce gas chromatography (GC) analysis time using existing instrumentation in two real-world situations.

Chromatogram peaks are well separated – we can shorten time of analysis by trading some efficiency through following these steps.

i) Use the column flow for higher velocity separations. Surprisingly, not many people use this powerful option, and yet you can use it immediately because all GC systems are equipped with electronic flow/pressure/gas-velocity controls. By separating at a higher gas velocity, it is possible to reduce the analysis time by a factor of 2–5. The system is not optimal with respect to plates, but because we have sufficient resolution, we can trade plates for time (see Figure 1). The additional advantage is that the fastest eluting peaks will be higher than the rest. If you do not want to change the injection conditions that you were/are already using, you can program the higher velocity injection to start after the first injection is complete.

It is very important that when linear velocity or column dimensions are changed, you also adjust the oven temperature to make sure that the components elute at the same temperature; if the elution temperatures are the same, the exact same chromatogram is obtained. To calculate the oven temperature program, you can utilize free software available as a web- or Windows application (1).

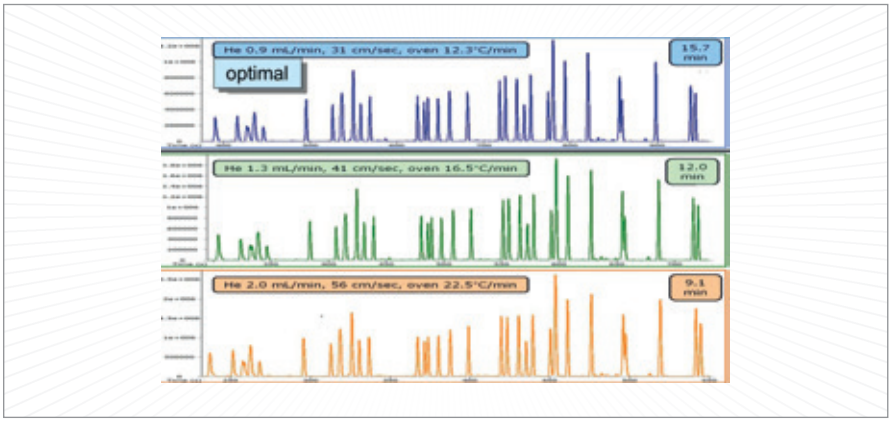

Figure 1. Separation of polybrominated diphenyl ethers at increasing gas velocity.

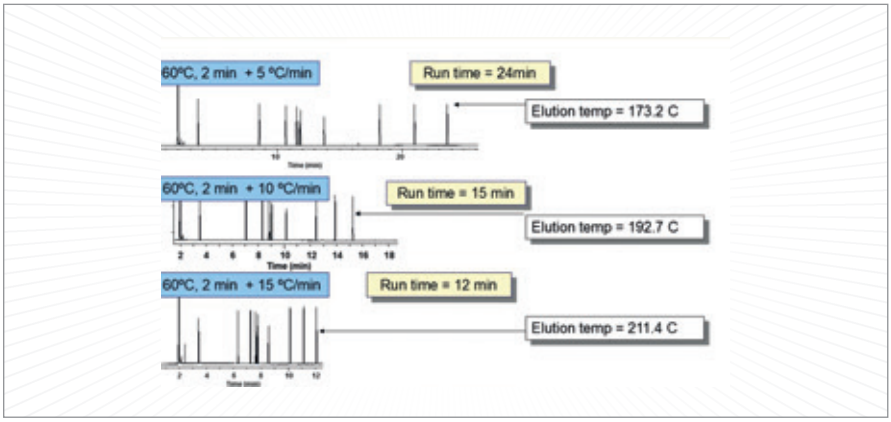

Figure 2. The effect of faster programming.

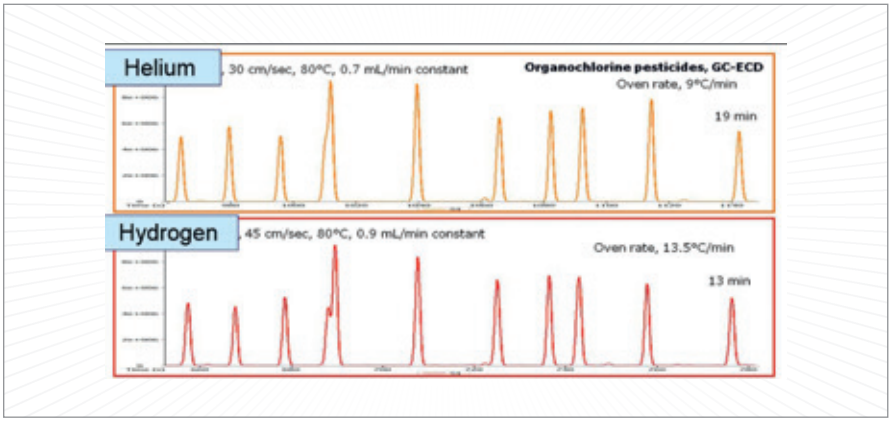

Figure 3. Helium versus hydrogen.

ii) Use a different temperature program. By using a faster program rate, peaks will elute sooner and at higher intensity (see Figure 2). Elution temperatures of components will change, which may result in a different separations. For very fast programs, you can use an "oven-pillow" to reduce the oven size.

iii) Use a shorter column. Shorter columns elute peaks faster but only reduce separation ever so slightly. My thinking is: "the better the chromatographer, the shorter the column". If you opt for this approach remember to adjust the temperature program using method translation to get the same chromatogram – you can use the free software again for this (1).

Note that using a system with less efficiency (plates), also means that peaks will run faster into each other during column ageing. The implication is that you'll need more column maintenance and the lifetime of the equipment is shortened. But that's the price you pay for faster results!

Existing peaks are just separated, and you don't want to reduce efficiency.

iv) Use a faster carrier gas. Hydrogen has about 50 percent higher optimal linear velocity compared with helium, which enables significantly faster separations while efficiency remains comparable (see Figure 3). As the linear velocity is changing, to get a similar chromatogram you have to adjust oven temperature using method translation (1). The great thing about hydrogen is that it is relatively cheap and you can make it in the laboratory using a generator. A potential downside with hydrogen is safety, but you can reduce the risk of hydrogen release by using hydrogen sensors in the oven, flow controlled systems, or metal (MXT) capillary columns. You can also use hydrogen with mass spectrometry (MS) systems, but there are some anomalies. Polar compound spectra may show +1 m/z fragmentation and the ion source can display some initial activity. And, when existing systems are converted to hydrogen, you may see some hydrocarbon background that can last for several days. Oils extracted from the "O" rings used in electronic pressure control systems cause this unwanted effect...

v) Use a smaller diameter capillary. Replacing a 30 m × 0.25-mm capillary with a 20 m × 0.15- mm capillary with the same phase ratio shortens run times by a factor of 2 but maintains efficiency while using the same carrier gas. Because the eluting peaks are narrower, you can reduce the amount of sample injected to generate the same signal. For a long time, we've known that 0.15-mm ID columns work well and they can be used in any type of GC or GC-MS system. They are available with different phase technologies and they are easy to install, as the OD is the same as a 0.25-mm capillary, so you can use the same ferrules.

For maximum impact, you can combine all these tactics to get the shortest possible analysis time!

For an extended discussion of all these options, see my blog (2).

References

1. *EZGC Method Translator and Flow Calculator. http://www.restek.com/ezgc-mtfc*
2. *J de Zeeuw, "Fast(er) GC: How to decrease analysis time using existing instrumentation? Part III: Impact of using faster temperature programming", Chromablography, August 10 (2011). http://blog.restek.com/?p=3414*

Gene Genie

Genomics platform director tops Thomson Reuters' list of highly cited authors

Stacey Gabriel, Director of the Genomics Platform at the Broad Institute of MIT and Harvard, has been named "hottest researcher" in Thomson Reuters' list of the World's Most Influential Scientific Minds 2015. Published annually, the list recognizes scientists (in 21 broad fields, see Figure 1) whose work has "consistently wielded outsized influence in the form of citations from fellow scientists" (1).

Thomas Reuters analysed citation data from its Web of Science and InCites platforms to determine which researchers' published works are most cited by their peers. 'Highly cited' papers comprised the top 1 percent, with the top 0.1 percent being 'hot papers'. As well as shining the spotlight on leaders and innovators, the list also highlights growing or emerging trends within the scientific community.

"We are pleased to recognize the world's most influential scientific minds; the innovators forging a path toward a brighter tomorrow," said Vin Caraher, President of Thomson Reuters IP & Science.

Gabriel, known for her work on the Cancer Genome Atlas (TCGA) project, came top for the second consecutive year with 25 papers cited. In fact, the field of genomics was particularly well represented, taking seven of the Top 19 spots – six of them from the Broad Institute of MIT and Harvard (see Table 1). Sadly, no chemists made it onto the leaderboard, but scanning the top-cited authors in the "Chemistry" section, we did come across a few names you may recognize from our own 2015 Power List (http://tas.txp.to/0116/PowerList): R Graham Cooks, Chad Mirkin, Joseph Wang and George Whitesides...

Are you or your colleagues listed? If so, let us know.

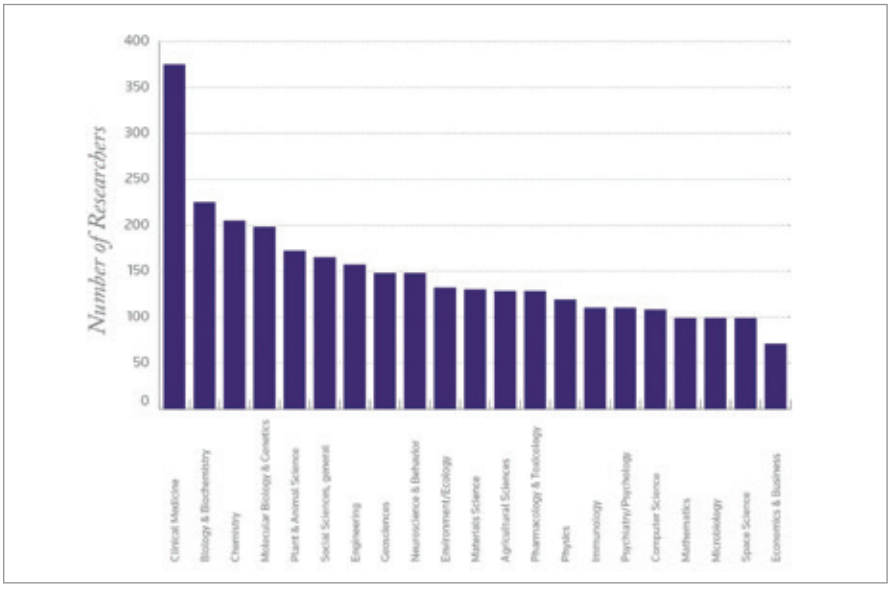

Figure 1. Highly cited authors broken down by field. Source: Thomson Reuters Web of Science & InCites.

Name	*Institution*	*Field*	*Number of Hot Papers*
Stacey B. Gabriel	Broad Institute of MIT and Harvard	Genomics	25
Henry J. Snaith	Oxford University	Physics/Materials	24
Christopher J. Murray	University of Washington	Global Health	22
Eric S. Lander	Broad Institute of MIT and Harvard	Genomics	21
Gad Getz	Broad Institute of MIT and Harvard	Genomics	20
Matthew Meyerson	Broad Institute of MIT and Harvard	Genomics	19
Michael Grätzel	École Polytechnique Fédérale de Lausanne	Materials	19
David (Xiong Wen) Lou	Nanyang Technological University	Chemistry/Materials	19
Alan D. Lopez	University of Melbourne	Health Metrics	16
Theo Vos	University of Washington	Global Health	16
Mohammed K. Nazeeruddin	École Polytechnique Fédérale de Lausanne	Materials	16
Hua Zhang	Nanyang Technological University	Materials	16
Mohsen Naghavi	University of Washington	Global Health	15
Yang Yang	Univ. of California, Los Angeles	Materials	15
Yi Cui	Stanford University	Materials	15
Michael S. Lawrence	Broad Institute of MIT and Harvard	Genomics	14
Scott L. Carter	Dana-Farber Cancer Institute	Genomics	14
Kristian Cibulskis	Broad Institute of MIT and Harvard	Genomics	14
Feng Zhang	MIT	Biomedical Engineering	14

Table 1. The 19 top authors with multiple "hot" papers according to Thomson Reuters Web of Science & InCites.

Reference

1. *http://stateofinnovation.thomsonreuters.com/worlds-most-influential-scientific-minds-report-2015*

Making a Murderer

Analytical science hits the mainstream with Netflix's hit documentary [spoiler alert!]

Filmed over 10 years, Making a Murderer follows the case of Steven Avery, a man who – having served 18 years for a murder he didn't commit – is convicted of a second murder. Did the police really frame him?

Ethylenediaminetetraacetic acid – more commonly known as EDTA – is used in blood collection tubes to prevent coagulation before analysis. EDTA is not naturally found in the bloodstream, so would its absence in bloodstains found in the victim's (Toyota RAV4) vehicle indicate that the evidence was not planted by police (as suggested by Avery's defense)? The court heard two testimonies – one from FBI Chemistry Unit Chief Marc LeBeau and another from now-legendary analytical chemist Janine Arvizu. The two witnesses had very different takes on what constitutes proof – and what it means to be an analytical scientist…

Defense lawyer: "Are you telling me right now that even though you never tested three other swabs of separate bloodstains found elsewhere in the RAV4 vehicle, that you're willing to express an opinion that none of those three swabs have EDTA either?"

Marc LeBeau: "I believe that to be true within a reasonable degree of scientific certainty, yes."

When expert witness Janine Arvizu was called to the stand she stated that there was nothing technically wrong with the procedure when it came to detecting and identifying EDTA. However, she went on to suggest that the potential for false negatives was another matter: "The problem really occurs when EDTA is not detected in a bloodstain [...] I don't know whether that's simply because they didn't detect it or because it wasn't there. [...] I don't know really what their method detection limit is."

Defense lawyer: "And looking at the data that is available in this stack, the validation tests that were done and those sorts of things, is there any indication that the FBI ever found out what the actual detection limit or method detection limit would be for this kind of a test?"

Arvizu: "No, there's no such indication in these data."

Defense lawyer: "And so from this data, can you express any opinion about whether the three stains examined by Mr. LeBeau could have come from the blood sample, the blood tube Q-49 that was also examined?"

Arvizu: "It's quite possible that those blood swabs could've come from Mr. Avery's blood tube, but simply not been detectable by the laboratory."

Defense lawyer: "So even having gone through this test, is it possible that EDTA is or was in those three RAV4 stains?"

Arvizu: "Yes."

Defense lawyer: "What about the three swabs from the RAV that were not tested by Mr. LeBeau? Can any conclusion be drawn on that?"

Arvizu: "I'm an analytical chemist. I'm not in the business of just guessing what's in samples. We have to test samples to decide what's in them."

Another victory for common sense. But the case continues. *JS*

End of the Road

Researchers use SPME and GC-MS to puncture claims that banned tire treatments are "undetectable"

When cornering at break-neck speeds, every bit of friction counts – and tire-softening agents can enhance grip, improve handling and shave precious milliseconds off lap times. But they are also prohibited in competition.

Stopping the cheaters in their tracks, researchers from the Indiana University–Purdue University Indianapolis (IUPUI) have developed a method to identify the banned tire softeners. Over the course of a year, the researchers tested 71 real tire samples from competitors in the United States Auto Club – 10 contained banned substances (1).

"The results weren't all that surprising to us," says William Kretz, lead author of the study, "But I think a lot of the people these products are marketed to might be surprised to find out how easily the treatments they apply to their tires can be detected and even identified. The companies that sell these treatments give their patrons a false sense of security in order to convince them to buy more merchandise. In actuality, it isn't difficult to detect these illicit treatments, if the tires are kept in a suitable environment and submitted for laboratory testing in a timely manner, the treatments show up clear as day."

The team used solid phase microextraction (SPME) ahead of GC-MS. "The first advantage of SPME was its sensitivity. By pre-concentrating the analyte on the fiber surface, we were able to see tire treatments at lower detection limits than what liquid injection would permit," says Kretz. "The second advantage was the lack of sample work-up. Because we were looking at the volatile compounds coming off the tire rubber itself, we didn't need to do a liquid extraction. We were able to analyze the tires directly, avoiding arduous preparation steps and losses due to partitioning, and so on."

Kretz believes the new method will help professional racing by providing critical information to race administrators, and thus enabling them to take corrective or disciplinary action against individuals who violate the rules. "It helps ensure the events are fair and contributes to the overall integrity of the sport," he says. *JS*

Reference

1. *W Kretz, C Carroll and JV Goodpaster, "Detection of prohibited treatment products on racing tires using headspace solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS)", Anal Methods, 8, 177-182 (2016). DOI: 10.1039/C5AY02657A*

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In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of analytical science. They can be up to 600 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

Seeing the Full Picture

The analytical world in general has yet to appreciate the power of FTIR spectroscopic imaging for process analysis. Here's why it should be in your toolkit.

By Sergei Kazarian, Professor of Physical Chemistry, Department of Chemical Engineering, Imperial College London, UK.

Fourier transform infrared (FTIR) spectroscopic imaging emerged two decades ago as a promising method for analyzing a range of different materials (1). Since then, it has been perceived mainly as a technique for obtaining chemical images of heterogeneous materials and samples, such as polymer blends or biological tissues. A chemical snapshot generated using FTIR imaging by recording many of IR spectra simultaneously is somewhat similar to a photograph from a conventional camera, the main difference being that it reveals features invisible to human eye – the images are based on chemical rather than visual differences. Thus, a chemical image is truly worth a thousand spectra!

However, spectroscopic imaging has even greater potential for chemical imaging of processes and systems that change with time. Surprisingly, this very exciting capability of spectroscopic imaging is underused. Modern FTIR spectroscopic imaging (using fast array detectors) offers itself as an experimental tool for studying dynamic systems. Indeed, a modern analytical chemist will often need to apply techniques in situ, online or inline and ultimately for industrially relevant processes. FTIR spectroscopic imaging usually can be applied in conjunction with different accessories – a microscope being one of the most familiar.

From the introduction of the technique, it was proposed that FTIR spectroscopic imaging may also work for macroscale studies (1) – one of the first examples being pharmaceutical tablet dissolution (2). Nevertheless, the combination of an FTIR spectrometer with a microscope became a commercially available system, while macro FTIR imaging of larger samples required the development of a macro compartment for accommodating accessories that would be suitable for dynamic experiments. In our laboratory, a number of such accessories and approaches have been developed for FTIR imaging experiments with systems that change over time. Importantly, using a range of spectrometers from different companies, we have been able to show that the combination of a spectrometer and macro compartment without a microscope provides a very reliable platform for the chemical imaging of dynamic systems (3).

However, the potential of studying dynamic systems in transmission mode was limited by the need for very thin samples. An attenuated total reflection (ATR) approach for macro FTIR imaging provides an alternative method that is particularly useful for the analysis of aqueous systems, as the strong infrared absorption of water does not present an obstacle. ATR-FTIR spectroscopy and imaging are often falsely perceived as surface techniques. In fact, when using ATR-FTIR spectroscopy one measures a layer of the sample, which is typically a few micrometers thick. Depending on the optics used and spectral region of interest studied, the probing depth

"Spectroscopic imaging has even greater potential for chemical imaging of processes and systems that change with time."

of ATR in mid-infrared range is comparable to that used in transmission infrared spectroscopy (which measures the absorption of IR light through thin samples with a thickness of 5–20 μm but usually requires microtoming of thick samples). The advantage of the ATR approach is that it provides the possibility of obtaining depth profiles within the measured layer of the sample by changing the angle of incidence or analyzing spectral bands at different wavelengths.

Macro ATR-FTIR spectroscopic imaging approaches using inverted prisms have been established for studies of tablet compaction and dissolution, diffusion of drug molecules into tissue, polymer mixing and polymer phase separation, dynamics of emulsion systems, systems under flow, mixing and reactions in microfluidics, protein aggregation and crystallization. The use of spectroscopic imaging to monitor the behavior of many samples simultaneously could be very valuable in high-throughput analysis, for example screening samples of different composition under identical conditions and controlled environment.

Seeing the actual process of tablet dissolution and drug release using FTIR imaging provides an approach for tablet optimization that is being employed in the pharmaceutical industry. Another example of a successful industrial application is the spectroscopic imaging of film formation in latex dispersions, which is related to important processes, such as paint drying. These examples should convince analytical chemists to adopt this chemical imaging methodology, not just for obtaining chemical images of static systems but also for use in understanding chemical processes.

The applications of macro ATR-FTIR imaging exist in other areas, such as the biomedical field, where it can analyze transdermal drug delivery and other processes. Even if ATR-FTIR imaging is yet to be exploited thoroughly in the medical field, it might well have very good potential for guiding applications of conventional ATR spectroscopy in medical diagnostics.

FTIR spectroscopic imaging could evolve into a powerful tool for routine online analysis by connecting flow cells to process lines that can be placed in the sample compartment of the imaging systems. It could also be used for inline analysis when suitable fiber-optics are available, such as bundling thousands of mid-infrared optical fibers with a focusing device on an array detector. With such advances, chemical imaging for process analysis will become even more useful and widespread – and then the full picture can be realized.

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A Tiny Sample Problem

A novel CE-MS technique shows great promise for sample-restricted metabolomics.

By Rawi Ramautar, Assistant Professor, Leiden Academic Center for Drug Research, Division of Analytical Biosciences, Leiden University, The Netherlands.

Metabolomics is a key discipline in studying molecular and cellular processes in living cells and organisms, with the ultimate aim of getting answers to biological and clinical questions. Today, state-of-the-art analytical techniques, such as nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) coupled to liquid chromatography (LC) or gas chromatography (GC), provide global and reproducible profiling of (endogenous) metabolites in biological samples.

Regardless of important innovations in analytical technology realized over the past decade, the current analytical toolbox still has difficulty analyzing ultra-small biological samples.

"The current analytical toolbox still has difficulty analyzing ultra-small biological samples."

Therefore, a significant number of crucial biomedical and clinical questions remain unanswered. For example, the analysis of (trace-level) metabolites in volume/material-limited biological samples, such as liquid biopsies, cerebrospinal fluid (CSF) from mice, cancer stem cells or even a single cell, remains a tremendous analytical challenge. To enable "sample-restricted" metabolomics, new miniaturized analytical workflows and technologies are needed urgently.

Over the last few years, a number of research groups have developed miniaturized analytical techniques for metabolomics studies of restricted sample amounts, proving that research in this particular field is very active (1–3). My own research is focused on the same goal, in particular using capillary electrophoresis (CE) because it is well suited for analyzing minute amounts of sample. Moreover, analytes are separated by their charge-to-size ratio making CE an attractive tool for profiling highly-polar and charged metabolites.

When it comes to coupling CE to electrospray ionization (ESI)-MS, I have used a novel sheathless interface design (invented by Mehdi Moini, George Washington University, Washington DC, USA) (4), which allows full exploitation of the intrinsically low-flow property of CE to significantly improve sensitivity and reduce ion suppression. The developed CE-MS platform has been used for highly sensitive analysis of ionogenic compounds in various biological samples (5, 6). A volume of only 2 µl was required in the sample vial for up to 10 consecutive injections of about 9 nl into the CE-MS platform, demonstrating the potential of the approach for analyzing volume-limited biological samples. For example, mouse CSF, of which only a few microliters are obtained under proper experimental conditions, could be analyzed directly with the CE-MS platform using minimal sample pretreatment (only 1:1 dilution with water). The approach retains sample integrity, which is essential for comprehensive profiling of polar and charged metabolites. I expect that the proposed technology will also offer a unique chiral perspective on the composition of ultra-small biological samples as the suppression effect of chiral selectors, such as cyclodextrins, may be limited under low-flow ESI-MS conditions.

In the forthcoming Microscale Separations and Bioanalysis symposium (April 3–7, 2016), to be chaired by Philip Britz-McKibbin (see page 19), special attention will be devoted to key developments in analytical technologies and workflows for sample-restricted biological/clinical problems. I'll be co-chairing the "Comprehensive Omics" session with Oleg Mayboroda (Leiden University Medical Center), which has the aim of providing the separations science community with a representative glimpse of recent achievements and innovations, as well as an interactive forum for discussing and exchanging ideas.

Overall, my view is that recently developed miniaturized analytical technologies appear to provide very promising results for sample-restricted metabolomics studies. But the next important step for these approaches is to show merit in large-scale biological and clinical studies. Such data are critical to promote these approaches further as a potential diagnostic tool. Additional work is also necessary on the pre-analytics side to effectively handle volume/material-limited biological samples and to efficiently transfer the analytes into the nanoscale analytical separation technique. Despite many analytical challenges in front of us, I anticipate that further development in this research field will have a major impact in metabolomics and bioanalysis in general as the new technologies continue to lead us in new directions, which is to say, towards a deeper understanding of biological functions in sample-restricted cases.

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MSI-CE-MS Simplifies Separations

Multiplexed separations in metabolomics: is there a faster, cheaper, and better way?

By Philip Britz-McKibbin, Department of Chemistry and Chemical Biology, McMaster University, Hamilton, ON, Canada.

The future of healthcare requires innovations in evidence-based medicine that promote disease prevention and healthy living on a population level. Nutrition, including diet, lifestyle and environment, play critical roles in chronic disease risk. However, they are not modeled adequately by genetic markers, and are poorly assessed by self-referral questionnaires when evaluating thousands of possible causative exposures, including over 25,000 different compounds in various foods (1). Since metabolites are molecular endpoints of gene expression closely associated with clinical outcomes and environmental exposures, untargeted metabolite profiling (metabolomics) offers a novel approach to decipher the complex and poorly understood determinants of human health (2).

A major challenge in mass spectrometry (MS)-based metabolomics is the low sample throughput, high costs, and complicated data processing, particularly when coupled to conventional separation systems. In this case, major infrastructure investment is required for large-scale epidemiological studies, which are often performed massively in parallel across multiple instrumental platforms. Alternatively, direct infusion and flow injection techniques coupled to high-resolution MS can greatly enhance sample throughput with appropriate sample pretreatment (3).

However, high efficiency separations are still needed for resolution of isobars/isomers, and minimizing ion suppression/enhancement effects while supporting the identification

Diaries at the Ready

On behalf of the organizing committee, I invite you to join us for the 32nd International Symposium on Microscale Separations and Bioanalysis (MSB 2016) being held at Queen's Landing in Niagara-on-the-Lake, Canada from April 3–7, 2016. MSB 2016 has evolved into the premier forum for the discussion of cutting-edge research on the frontiers of separation science. We invite you to browse our conference website (www.msb2016.org) to learn more about our exciting scientific program. If you are currently incorporating microscale separations in your research (for example, CE, micro/nano-LC, GC×GC, ion mobility spectrometry, microfluidic and lab-on-a-chip devices) then this is a meeting you will not want to miss!

See you at MSB 2016 in Niagara-on-the-Lake next April!

of unknown metabolites of clinical importance (4). These features are also critical for reducing false discoveries, as well as improving method accuracy and robustness – especially when analyzing large numbers of samples from biorepositories that vary widely between subjects, such as urine. As a result, various strategies have been introduced to shorten analysis times and duty cycle, including new column technologies operating at high flow rates, ambient ionization methods for direct sampling and ultra-fast separations based on microfluidic devices and ion mobility spectrometry. Yet, is there a cost-effective approach to boost sample throughput in discovery metabolomics without sacrificing analytical performance, peak capacity and data fidelity?

"Is there a cost-effective approach to boost sample throughput in discovery metabolomics without sacrificing analytical performance, peak capacity and data fidelity?"

Our group has recently introduced a simple approach for multiplexing separations in metabolomics (5) based on multisegment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS). In this case, seven or more discrete segments of sample can be injected in series within a single capillary that enhances sample throughput up to one order of magnitude without compromising separation performance and data quality. Moreover, MSI-CE-MS encodes spectral information temporally via signal pattern recognition that allows for unambiguous peak picking, feature identification and noise filtering within an accelerated metabolomics data workflow (6). Ongoing work is now focused on evaluating the long-term performance of MSI-CE-MS in validating lifestyle interventions that promote health on an individual level, as well as markers for improved screening and diagnosis of human diseases (7). Thus, new advances in separation science play critical roles in expanding the performance MS-based chemical analyses that are no longer limited by long elution times while analyzing "one sample at a time".

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Enlightened Experimental Design

The traditional approach of designing experiments by changing one factor at a time is outmoded.

By Phil Kay, JMP Systems Engineer, SAS, Marlow, Buckinghamshire, UK.

I hate to see people wasting their time – and money – which is why I am such a passionate advocate of statistical Design of Experiments (DoE).

By definition, DoE is the most efficient way to experiment to understand a process or system and it is extremely relevant for analytical science. Just enter "HPLC design of experiments" into your favorite search engine; you will find plenty of examples where scientists have optimized the resolution of HPLC methods using DoE. The authors will likely have listed the benefits of the approach: the reduced time, cost and risk; the increased understanding of the complex behavior of the system; the structure and rigor that it brings to the development process. They may also mention that they identified that some of the factors have important interactions. For example, they discovered that the best temperature was dependent on the flow rate. They will acknowledge that they would not have found the optimum using a conventional one-factor-at-a-time (OFAT) approach.

Those of you who have developed chromatography methods will probably have seen such examples and, if so, will be aware of the benefits. And yet many of you will not use DoE. And I would be willing to wager that a large number of the DoE case study authors still use OFAT as their default approach. Published examples, by their nature, are special cases. Nevertheless, HPLC development lends itself very well to DoE because experimentation is relatively cheap. A large experiment can be run with few human, equipment and material resources, for instance. In a setting where each run or trial is more expensive, my experience tells me that the scientist will be less likely to use DoE – despite the fact that the imperative for efficiency is even greater!

Working in the chemical industry, my job was to find out how to optimize and control complex and messy systems. I remember one particularly challenging situation where each trial preparation was very costly and we did not have the time for many of them because we were under pressure to get quick results. The system behavior was expected to be complex because of the large number of physical and chemical interactions. To add to this, both experimental and measurement repeatability were very poor. Given these problems, the chances of finding useful information with a designed experiment were not good.

One manager, who was normally a big fan of DoE, said we should just see if we are able to find something that works by OFAT. This was, of course, the wrong response to the situation. But heading down this route would have further lowered the chances of finding useful information, making it a good way to waste the time of many scientists. DoE is, therefore, a project management tool that enables rational decisions, making it about the most effective way to spend resource to maximize information and minimize risks. But people – even managers (!) – are not rational. When the pressure is on, we all tend to resort to our comfort zone, which in this case was OFAT.

So, how do we make DoE the comfort zone? Advances in methods and improved software for designing and analyzing experiments are enabling more people (in more situations) to consider DoE. Another part of the answer has to be education. The message that you should only change one thing at a time was ingrained in me from an early age and was never challenged throughout my years in school followed by higher education (my master's degree and PhD in chemistry). I don't remember hearing about anyone using DoE in my department during my undergraduate or postgraduate studies. Looking back, these methods would have been extremely useful. However, academic research is generally narrow in scope and focuses on simple, model systems. Real-life problems in industry are more of a challenge. So, like most scientists that I have spoken to, I only really found out about DoE when I started in industry.

Universities should be preparing scientists with the key skills that they need in industry. Things are changing. A small number of universities recognize that teaching DoE as part of undergraduate science courses gives students – and therefore the institution – a competitive advantage. Development of analytical methods would be an ideal context for undergraduate science students to learn about DoE.

Change cannot happen quickly enough. It pains me to think of the billions of hours (and dollars, pounds, Euros...) of wasted experiments.

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Exploring Our Blue Planet

The seven seas represent an extremely challenging and almost boundless environment that a secret army of passionate oceanographers nevertheless joyfully dive into. Here, I explain the role that analytical science plays in understanding the many mysteries of the deep.

By Rob Munier, Vice President for Marine Facilities & Operations Directorate, Woods Hole Oceanographic Institution (WHOI), Massachusetts, USA.

"How inappropriate to call this planet 'Earth' when it is quite clearly 'Ocean'." – Arthur C. Clarke

My interest in "watery" pursuits can be traced back to my youth and long summers on the eastern end of Long Island, New York, where there is a remarkably beautiful nexus of bays, creeks, canals and the Atlantic Ocean – all only 70 miles from New York City. I began SCUBA diving when I was 12 years old and have been diving ever since, both for research and for pleasure.

I became determined to study the ocean and found ways to use my love of diving as a research tool in both college and graduate school. I also vowed to make my vocation and avocation one and the same – as the saying goes: "If you do what you love, you will never work a day in your life." I have been reasonably successful in my vow and had the chance to be involved in some great projects and initiatives, including groundbreaking work in ocean thermal-energy conversion, an ocean-based solar energy process, and building undersea telecommunications systems that span the globe. I am now responsible for helping scientists and engineers gain access to the sea with ships and submersibles for one of the top oceanographic research institutions in the world.

Mysteries of the deep

The noted oceanographer Sylvia Earle said, "The most valuable thing we extract from the ocean is our existence." The seas occupy 71 percent of the world's surface – and the more we study them, the more apparent it is how little we know. Indeed, any expedition is capable of discovering a new species! The area of the seafloor surveyed with low-resolution sonars – basic bathymetry – is less than 15 percent. And less than 0.05 percent has been surveyed with high-resolution robotic autonomous undersea vehicles (AUV). Human-occupied submersibles have observed less than the area of the city of London (2.9 km2). Moreover, there are vast swathes of the ocean that have not been sampled directly. We have only been to the "hadal" zone (depths greater than 6000 meters and an aggregate area larger than North America) a handful of times. And there are entire areas of the Pacific that have no oceanographic observations other than from satellites.

The ocean is incredibly complex, with perturbations that lead to unexpected and dramatic impacts elsewhere. Little is understood of the unique ecosystems found at hydrothermal vents, natural seeps, and brine pools, which cause oceanographers to rethink conventional wisdom, including the origin of life on the planet. The ocean is also changing. So we need both a baseline of knowledge and ways to measure that change and provide the public and policy-makers with the data necessary to make informed decisions.

Oceanography and ocean engineering are exciting professions and our tools – high-tech ships, submersibles – combined with far-flung destinations would seem to be the perfect recipe for great public awareness and support. However, the public is generally under-informed about ocean issues. Perhaps this fact partially explains why oceanography is the stepchild to the space program (see page 34) in terms of priority and funding for research. Annual funding for oceanographic research in the US is about $1 billion – less than seven percent of NASA's budget. In light of the pressing ocean issues, this funding inequality needs to change – in a relative hurry.

Basic or applied?

It is true to say that oceanographers engage in basic research topics that interest them and that will attract funding. However, the roles of basic and applied research are actually on a continuum. Francis Bacon believed that true scientists should be like bees – extracting goodness from nature and using it to do useful things. Such a dynamic tends to focus research on societally important questions and creates a convergence towards the big issues of the day, such as climate change, sustainable fisheries, ocean acidification, pollution, natural hazards (earthquakes, for example), safe resource extraction practices, and delineating and understanding territorial seas and borders. Technology is also critical, and oceanographers routinely collaborate with engineers to develop the tools necessary to do their work. The harsh ocean environment presents unique challenges to access, sampling, and data collection, so the convergence of science and engineering is critical to success.

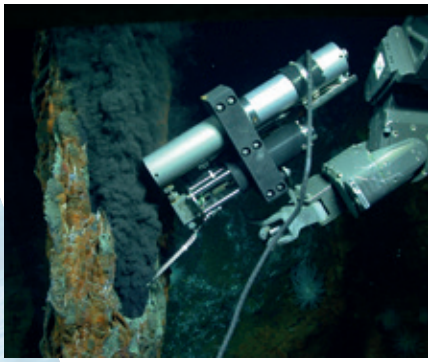
Oceanography also has a history of basic research, which has produced opportunistic breakthroughs and advances, enabling unexpected and important applications. A great example, which also demonstrates the science-engineering convergence, is the application of scientific methods and technologies developed to study hydrothermal vents to the Deepwater Horizon oil spill, including measurement of the oil-spill flow rate, the sampling of the effluent at the blowout preventer, and the mapping of a deepwater hydrocarbon plume. In that case, the fruits of basic science and research responded to meet an important societal need.

Ocean scientists and engineers who are engaged in basic research tend to refrain from involvement in advocacy, which ensures an unbiased and agnostic approach to questions – and maintains the integrity of the science. That said, the data obtained – and the outcomes achieved – are publically available for advocates and interest groups to cite for or against a particular position.

> "A host of analytical tools is used to analyze the cores, including X-ray fluorescence scanning to determine elemental chemistry and the detection of stratigraphic markers."

Data collection challenge

Key challenges to oceanography are the spatial and temporal domains. The ocean is vast and we have relatively few means of access and little persistence. Research vessels and underwater vehicles go out and scientists learn something about specific locations for the period of time they are there. Fixed "ocean observing systems" provide an extended time series of data but at one location. Unlike our pervasive weather systems on land, there are very few ocean observing systems, so the spatial coverage is very limited. Remote sensing systems, such as satellites, help to address the spatial and temporal problem,

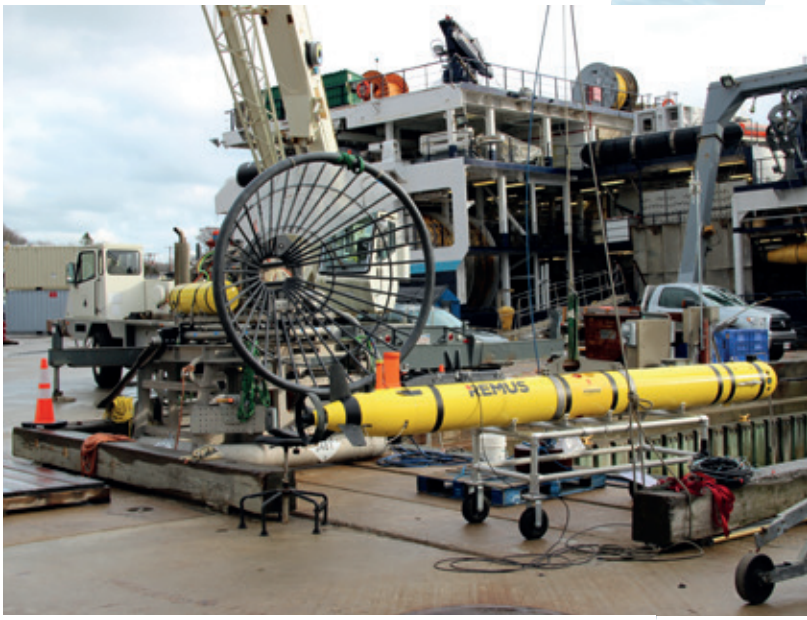


Clockwise from top left: An isobaric gas-tight sampler (IGT) sampling a black smoker vent [credit: photo by Chris German and the Jason Group, Woods Hole Oceanographic Institution]. Holger Jannasch works with a chemostat in his laboratory [credit: courtesy of WHOI Archives]. Rich Camilli holding TETHYS underwater mass spectrometer encased in its housing [credit: ourtesy of Rich Camilli*]. Deployment of Ocean Observatories Initiative (OOI) Pioneer Array surface buoy [Credit: photo by John Lund]. Rod Catanach working on autonomous underwater vehicle "Sentry" out on the deck of research vessel Atlantis. [credit: photo by Ken Kostel].

Woods Hole Oceanographic Institution

WHOI is a nonprofit institution based on Cape Cod in Woods Hole, Massachusetts, USA. Founded in 1930 with a grant from the Rockefeller Foundation, WHOI is the largest independent institution in the world dedicated to the study of ocean science and technology. With an annual turnover of over US $220 million, funding is obtained primarily through grants from US government agencies (such as the National Science Foundation and the Office of Naval Research), private foundations, philanthropy and industry. WHOI scientists seek answers to important questions, both fundamental and applied, and are leaders in their fields. Unique to WHOI is the strong coupling between the basic sciences, engineering and seafaring. WHOI also has a joint graduate education program with the Massachusetts Institute of Technology and awards PhD degrees in all of the oceanographic disciplines and ocean engineering.

To learn more about WHOI, visit: www.whoi.edu



Clockwise from top left: Stanley Watson working on research vessel Chain [credit: photo courtesy of WHOI Archives]. Rob Munier with autonomous underwater vehicle "Sentry" on research vessel Atlantis [credit: photo by Ken Kostel]. AUV docking station and REMUS 600 on the WHOI dock [credit: photo by Al Plueddemann].

but paint an incomplete, lower-resolution picture.

Oceanography brings together biology, chemistry, physics, and geology – and many areas of specialization within those disciplines. As such, the tools of the oceanographer are the tools of the scientist – although sometimes adapted for the harsh marine environment. Marine microbiologists need microscopes and chemical oceanographers need mass spectrometers. Oceanography presents a unique set of problems, however, because of the difficulties of access, remoteness, expense and risk. Samples collected at depth may change or disintegrate when brought to the surface. Also, samples collected aboard ship or a remote field site might be stored for weeks or months and may require special handling. This lag time prior to analysis may affect the quality of the results, so the traditional solution is to perform as much analysis as possible in the shipboard laboratory. Indeed, today's research vessels have well-equipped labs, so a lot can be done shortly after collection. The trend, however, is for oceanographers and engineers to find ways to make more measurements in situ. And the Holy Grail is to connect in-situ measurements via communication path to the ship or even ashore to obtain results in real time.

Fortunately, many of the most basic and important measurements in oceanography can be obtained accurately, as long as you can get there. These include temperature, salinity, pH, dissolved oxygen concentration, and fluorescence, among others. These are parameters important to all oceanographers and are routinely collected using sensors deployed from a ship, secured to a fixed mooring, or strapped to a submersible, regardless of any other more complex sampling objective. Growing use of robotic autonomous undersea vehicles (AUVs) and tethered remotely operated vehicles (ROVs) has caused sensor design to evolve to meet the stringent form factor, weight and power constraints of these unmanned submersibles.

What we measure and how we do it

Excess carbon dioxide is now a recognized pollutant and greenhouse gas. It is also absorbed by the ocean, which drives down pH and the saturation state of calcium carbonate, a process called ocean acidification (OA). Oceanographers are studying OA to understand the impacts on shellfish, plankton, and corals, whose skeletons are made of calcium carbonate. Key measurements of pH are made using in-situ sensors. Dissolved inorganic carbon (a proxy for calcium carbonate concentration) and pH are also determined by analyzing water samples with coulometric and potentiometric titrations, respectively.

Hurricane frequency and intensity are active areas of oceanographic research. Marine geologists such as WHOI senior scientist Jeff Donnelly have determined that sediment deposits from historical hurricanes can be identified in cores taken from coastal marshes. Data from these deposits are used to reconstruct the impacts, determine patterns, and put recent hurricanes, such as Sandy, in the context of the last several millennia. A host of analytical tools is used to analyze the cores, including X-ray fluorescence scanning to determine elemental chemistry and the detection of stratigraphic markers, such as Cesium isotopes using high-resolution gamma detectors. Accelerator mass spectrometry (AMS) is used for radiocarbon dating of fossils. In fact, we operate the National Ocean Sciences Accelerator Mass Spectrometer (NOSAMS) facility at Woods Hole, which is a resource used by the entire oceanographic community.

> "The underwater MS instrument developed to study natural seeps and asphalt volcanoes was used to identify and map (in 3D) the hydrocarbon plume that resulted from the continuous discharge of oil from the Macondo well."

As noted, oceanographers also study hydrothermal vents. These fissures that discharge geothermally heated water were first discovered in 1977 and opened a new "sea" of inquiry into chemosynthesis in the marine environment. Vent sites have now been found all over the ocean, primarily at tectonic plate boundaries, hot spots, and fault sites. They are known for their chemical and biological complexity, high temperatures, and ephemeral nature. Oceanographers have developed ways to collect and analyze samples at vent sites and these have increasingly enabled better understanding of their character, variability – and importance. For example, WHOI chemist Jeff Seewald developed an isobaric gas tight (IGT) sampling device to collect fluids flowing from hydrothermal vents at hydrostatic seafloor pressures, thereby retaining the volatile species in situ concentrations for later analysis ashore. Once in the lab, more traditional techniques such as gas chromatography (GC) and isotope ratio MS could be employed after samples were separated into gas, seawater and other fluid constituents.

Research on natural gas "seeps" and asphalt volcanoes led to the development of a mass spectrometer by WHOI researcher Rich Camilli that could be operated from AUVs, ROVs or human-occupied submersibles, providing the ability

to identify targeted compounds in situ. Some of this pioneering work was done off Southern California, where there are significant natural flows of both oil and methane gas from seafloor features. Submersibles are used to map and create photo mosaics of feature topography, to sample solid mounds of asphalt for traditional laboratory analysis, and to make in situ measurements of methane (or other targeted compound) distributions using mass spectrometry. Such an assembly of tools and analytical techniques has helped to further our understanding of these naturally occurring point sources of hydrocarbon in seawater, which are now known to account for half of the overall input into seawater worldwide.

Plankton represents the bottom of the food chain in the ocean, so understanding its biology is critically important. The traditional method for studying plankton is by towing nets behind a ship for occasional recovery, extraction of the plankton, preservation, and study in the lab. An early step in the lab is to analyze samples under the microscope for species identification and diversity assessment. While these methods are informative, they are obviously limited by the length of tow, sample size and most importantly, the destructive aspects of the hydraulic forces on the delicate tissues of the species being studied. Over the last decade, new devices have been developed for imaging plankton in situ, in real time. In effect, the microscope now goes underwater, continuously images plankton, and provides data back to a computer that can count individuals, identify species, and archive the data. Some systems can be towed behind a ship (video plankton recorder; VPR), some can be mounted at a fixed location (FlowCytoBot), others can be fixed to a ship's seawater system (continuous plankton imaging and classification system), and most recently others have been affixed to AUVs, dubbed PlankZooka and SUPR-REMUS. These devices take advantage of advances in imaging and computing power, since gigabytes of data can be generated. A VPR, for example, has been towed on record-breaking transits across the Atlantic and the Pacific, generating data that has changed our understanding of the concentrations of plankton in the ocean in both space and time.. With this technology, we now can detect blooms and water masses devoid of plankton that were never before identified, let alone understood, because of the "snapshot" nature of traditional methods.

Reacting to disasters

In 2010, the blowout of the Deepwater Horizon Macondo well was a call to action for oceanographers and ocean engineers. Many tools and techniques developed for basic research proved to have critically important applications and helped to measure the scale and impacts of the event. For example, the IGT sampler developed for hydrothermal vents was used to obtain the definitive sample of the effluent from the wellhead. The sample was analyzed to determine the gas/oil/seawater fractions and the composition of hydrocarbon gases and oil. Knowing the composition provided a means of correlating samples collected elsewhere in the Gulf of Mexico with the Macondo well, and helped scientists understand the fate of the oil in terms of transport and weathering. The underwater MS instrument developed to study natural seeps and asphalt volcanoes was used to identify and map (in 3D) the hydrocarbon plume that resulted from the continuous discharge of oil from the Macondo well. The MS was deployed both from a traditional "rosette" lowered by a wire from a research vessel and from an AUV. The MS was "tuned" to identify select hydrocarbon compounds, tracking 10 chemical parameters in real-time.

In three missions, the AUV was able to confirm the presence of a plume at 1100 meters depth that trended southwest from the well for 35 km. Water samples collected using traditional methods from research vessels were analyzed in the laboratory for dispersants using ultra high-resolution MS and liquid chromatography (LC) with tandem MS. The concentration of dispersants in space and time proved to be an important and novel proxy for the movement of Macondo oil and gas, and were shown to be associated with the oil and gas in the plume mapped using the in-situ mass spectrometer. These examples, among many others, demonstrate the resourcefulness of oceanographers; without compromising scientific rigor, dozens of missions went out to sea in the weeks and months following the blowout to measure the relevant parameters, to better understand the impacts, and to help inform decision-makers.

In the grand scheme of things analytical scientists working in oceanography aren't worlds apart from their land-based peers; we share similar goals in serving science and society. Author and marine biologist John Steinbeck wrote in his Log from The Sea of Cortez, "We search for something that will seem like truth to us; we search for understanding; we search for that principle which lays us deeply into the pattern of all life; we search for the relations of things, one to another..."

And so, the search in oceanography continues. There is much to be done and it starts with educating the public about the urgency of doing it. In the words of explorer and proto-environmentalist Alexander von Humboldt, "Weak minds complacently believe that in their own age humanity has reached the culminating point of intellectual progress, forgetting that by the internal connection existing among all natural phenomena, in proportion as we advance, the field traversed acquires additional extension, and that it is bounded by a horizon which incessantly recedes before the eyes of the enquirer."

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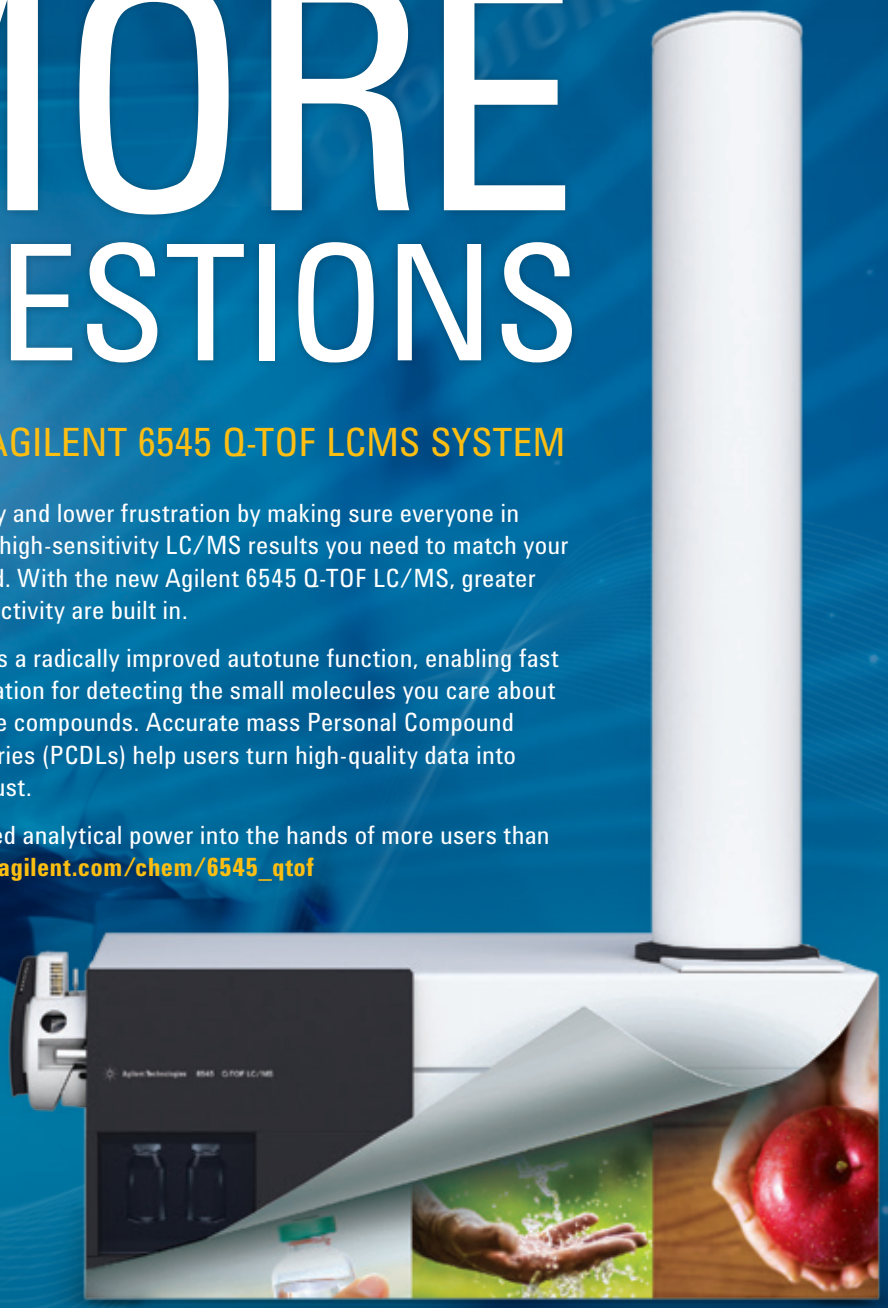

Agilent Technologies

GC-MS and the Art of POP Analysis

Persistent organic pollutants (POPs) encompass a wide variety of contaminants, but they share one thing in common: they have the potential to hit headlines and cause huge disruption to the food and feed industry. Fortunately, innovation does not stand still – and advances in mass spectrometry are opening up new vistas in POPs analysis. Here, I assess the complete range of GC-MS techniques used in POPs analysis, including the latest GC-MS instrument developments.

By Esteban Abad Holgado, Laboratory of Dioxins, Environmental Chemistry Department, IDÆA (CSIC), Barcelona, Spain.

I gave a presentation at the 10th International Symposium on Recent Developments in POPs Analysis, held in Prague, Czech Republic, earlier this year (you can see that presentation here: tas.txp.to/0116/Holgado). Why was I there? Essentially, to expand on an investigation that we presented at the 19th International Mass Spectrometry Conference in Kyoto, Japan, in 2012. Back then, we wanted to demonstrate some new ideas of utilizing mass spectrometry for the determination of dioxins in environmental and food samples. In particular, our project looked at the suitability of gas chromatography-tandem mass spectrometry (GC-MS/MS) for the analysis of dioxin-like persistent organic pollutants (dl-POPs). In Kyoto, we compared GC-MS/MS to the reference technique of GC-high-resolution (HR) MS – but the world of mass spectrometry moves very quickly indeed. Here, I share our work with the latest systems.

A short lesson

First, let me quickly go over the key groups of compounds that we are particularly interested in when it comes to POPs analysis: i) Organochlorine pesticides (for example, Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex and Toxaphene). Despite many of these compounds being banned, they remain a problem. ii) Polychlorinated biphenyls (PCBs). PCBs have many uses, including dielectric and coolant fluids in electrical apparatus, cutting fluids for machining operations, and in heat transfer fluids. iii) Dioxins. Despite also occurring naturally, dioxins are anthropogenic substances that get into the environment.

Why are we so concerned? Well, when these substances enter the food and feed chain, they generally hit the headlines as international scale incidents. There will be few of you who do not recall the scandal of dioxin contamination hitting the Belgian poultry industry back in 1999. And as recently as 2008, Italy had to recall mozzarella cheese products, while Ireland recalled pork products. The silver lining is that such failures in the food chain tend to trigger a positive response; for example, the global Stockholm Convention on POPs, and the EU's introduction of not only new regulations and limits, but also recommended protocols for sampling and analysis.

Reacting to new technology

Europe Commission Regulation (EU) No 252/2012 specified that GC-HRMS should be used for confirmatory dioxin analysis in food and feed, while GC-MS/MS was permitted as a screening technique. Jump forward to today and Commission Regulation (EU) No 589/2014 specifies the use of either GC-HRMS or GC-MS/MS for confirmatory dioxin analysis in food and feed, recognizing "technical progress and developments" in GC-MS/MS. Notably, the regulation does state that GC-MS/MS is "an appropriate confirmatory method for checking compliance with the maximum level", whereas GC-HRMS remains the recommended technique for "determination of low background levels in food monitoring, following of time trends, exposure assessment of the population". In short, this means Magnetic Sector HRMS has been recognized as delivering superior sensitivity, as required for low-level background studies. Additionally, Magnetic Sector GC-HRMS fulfills all requirements for all types of dioxin applications, and is considered the reference standard for dioxin analysis.

The EU regulations for food & feed have specific requirements for GC-MS/MS confirmatory methods.

i) You must monitor at least two specific precursor ions, each with one specific corresponding transition product ion, for all labeled and unlabeled analytes.

ii) There is a maximum permitted tolerance of relative ion intensities of ±15 percent for selected transition product ions compared with calculated or measured values (the average is taken from calibration standards), applying identical MS/MS conditions, in particular collision energy and gas pressure.

iii) The resolution for each quadrupole is to be set equal to or better than unit mass resolution (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart).

iv) For the limit of quantification (LOQ), the method must demonstrate that it is able to distinguish between the blank and cut-off value. A notification level needs establishing for samples that respond below this level.

v) For polychlorinated dibenzodioxins/polychlorinated dibenzofurans (PCDD/PCDFs), the limits of detection (LOD) should be in the higher femtogram range (10–15 g). For most PCB congeners, it is sufficient to set LOQ in the nanogram

range (10–9 g). However, to measure congeners similar to dioxin-like PCBs (in particular non-ortho substituted congeners) the lower limit of the working range must reach the lowest picogram (10–12 g).

In terms of workflow, Magnetic Sector GC-HRMS and GC-MS/MS are similar, following the same preliminary stages of sample preparation, extraction (manual or automated) and cleanup before analysis and finally calculating the toxicity equivalency (TEQ).

Gold standard

I've been working with HRMS - Magnetic Sector analyzer technology for more than 20 years. Currently, we are fortunate enough to enjoy state-of-the-art technology in the form of Thermo Scientific's DFS™ Magnetic Sector GC-HRMS system, which in my opinion provides gold standard sensitivity for dioxin analysis. Indeed, I think it's fair to say that it offers excellent performance in terms of sensitivity, robustness, throughput, and flexibility – and that's more than likely why it is being used by so many dioxin experts around the world.

When I started in the lab, Magnetic Sector GC-HRMS seemed out of reach because of the expense and degree of expertise required. I remember I was asked, "Why can't I analyze dioxins with electron capture detector (ECD)?" – after all, it is very sensitive and relatively cheap technology. I replied, "Go ahead, but you won't succeed." Two days later, I was able to see spectra with profiles like the Rocky Mountains, so they moved onto magnetic sector technology...

But that hasn't stopped me searching for alternative solutions. In 2007, we worked on a European Framework project with the University of Barcelona, Spain, using ion-trap MS/MS for analyzing PCDDs and dl-PCBs in food (1). Overall, the data was very good but we had to do a lot of sample clean-up work to get where we needed to be. We felt that it was not a satisfactory technique. Later, we did a collaborative study with The Autonomous University of Nuevo León in Mexico to analyze dioxins in beef samples using a low-resolution MS/MS screening method (a single quadrupole they already had in the lab) (2). It was certainly possible to look for contaminant data, but in the end, we once again concluded that it was not optimal for dioxin analysis.

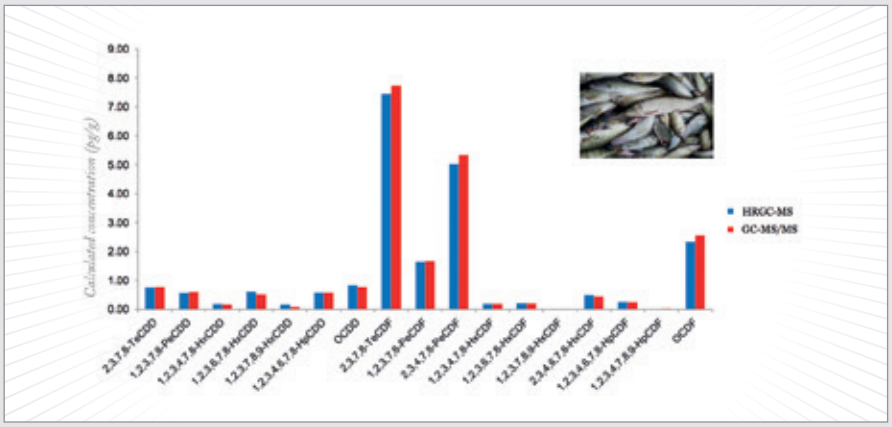

Figure 1. Comparison between GC-MS/MS and GC-HRMS for a fish sample.

Pushing boundaries, solving problems

Most recently, we have been working with Thermo Fisher Scientific in Manchester, UK, on the development of the TSQ™ 8000 Evo triple quadrupole GC-MS. It is a fast collision cell instrument with proprietary EvoCell technology for high-speed selected reaction monitoring (SRM), precision, and sensitivity. And it features a removable ExtractaBrite™ ion source for high matrix tolerance, as well as easy maintenance.

In evaluating its suitability for dioxin analysis, we have investigated various test samples – dried fish, fish, milk powder, feed, fly ash, and sewage sludge – and once again compared it with GC-HRMS. Figure 1 gives one example, and many more are shared in my presentation. How does the instrument measure up to the EU regulations for food & feed? Well, if you look back to the specific requirements for GC-MS/MS confirmatory methods noted earlier, it provides sensitivity that exceeds the rules.

So, while Magnetic Sector GC-HRMS is considered as the gold standard in Dioxin analysis with world-wide method compliance and due to its superior sensitivity for lowest level quantitative trace analysis in routine, GC-MSMS has been recognized to be a viable and cost efficient alternative for food & feed analysis in Europe. Moreover one thing is certain; nothing stands still in the world of food analysis and as a proof the most recent innovation to come over the horizon is GC coupled to Orbitrap technology – the Q Exactive™ GC! We've already achieved some interesting results and we are excited to see what else the future will bring. GC-Orbi technology will certainly be the new benchmark for powerful unknown compound identification for GC-MS, also in the field of POPs analysis.

"If you only have a hammer, every problem is a nail." Actually, not every problem is the same, is it? So for POPs and dioxins with Magnetic Sector GC-HRMS, GC-MS/MS and now even GC-Orbitrap there is a very complete range of powerful GC-MS technologies available. Each offers specific advantages to the Dioxin or POPs analysis expert, so that a real tailored solution for each specific analytic challenge is possible and can be found. In the end - given all the complex challenges in the world of analysis, you need more than a hammer.

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EXPLORING
THE
RED
PLANET

Scientists who are dissatisfied with the analysis of samples from our blue planet look to the stars – or rather, neighboring planets – to learn the mysteries of the universe. We speak with two such researchers – Roger Wiens and F. Scott Anderson – about analysis (not life) on Mars.

Curious About Mars

Roger Wiens, ChemCam Principal Investigator for the Mars Science Laboratory Rover at North America's Los Alamos National Laboratory in New Mexico, shares his experiences in extraterrestrial analytical science.

What is ChemCam?

ChemCam comprises a suite of remote sensing instruments on Mars for the Curiosity rover. It combines a laser-induced breakdown spectroscopy (LIBS) analyzer and a remote micro imager (RMI) telescope. ChemCam is not only able to determine if a rock has been altered by water, it is also able to look for the chemical ingredients of life – oxygen, nitrogen, carbon, and/or hydrogen. The inclusion of ChemCam on the mission means that Curiosity is the first Mars rover capable of detecting these elements remotely, that is, without coming in contact with the sample.

What has been your most remarkable discovery so far?

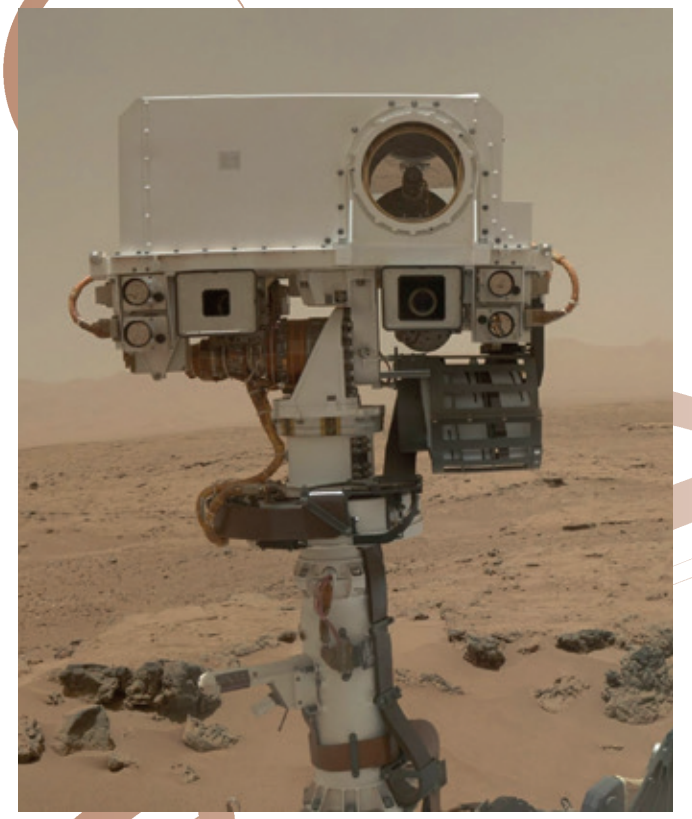
That's difficult to answer because Curiosity has completely revolutionized our understanding of Mars in a number of ways. She has discovered organic molecules including methane; we have seen the first extra-terrestrial riverbed rocks (conglomerates) as well as sandstones and mudstones formed at the bottom of a large lake. ChemCam specifically discovered that fine-grained Martian soils and wind-blown dust are hydrated. ChemCam also discovered a type of lower-density rock more reminiscent of those common on the terrestrial continents – more aluminium- and silicon-rich than were thought to exist in large quantities on Mars. We have also seen evidence of highly varying oxidation states in Mars' ancient groundwater.

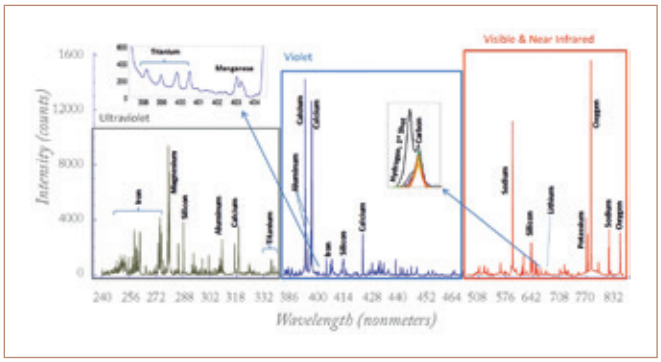
How will society benefit from your work?

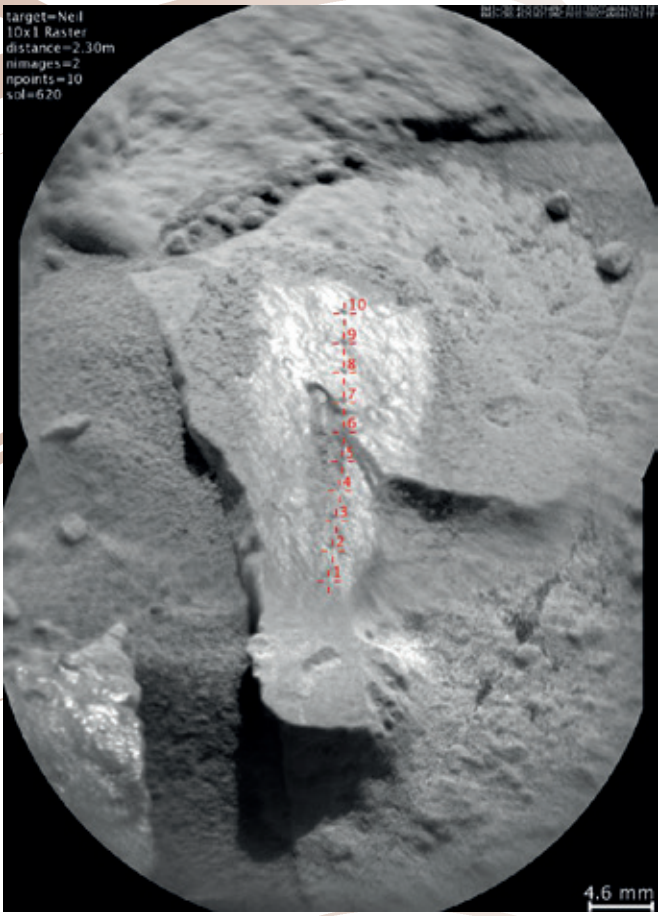
Miniaturization of instruments is the key to deploying them in the field, whether on Earth or on Mars. ChemCam leads the way in terms of applying LIBS to study field geology; similarly, there are now portable x-ray fluorescence (XRF) spectrometers that were inspired by the APXS (alpha particle x-ray spectrometer) instrument, now on its third generation of rover. Miniature gas chromatographs (GCs), similar to that on the SAM (comprising a quadrupole mass spectrometer, a GC, and a tunable laser spectrometer) instrument, are now available, and the same is true of field-portable x-ray diffraction instruments, thanks to the Chemistry & Mineralogy team.

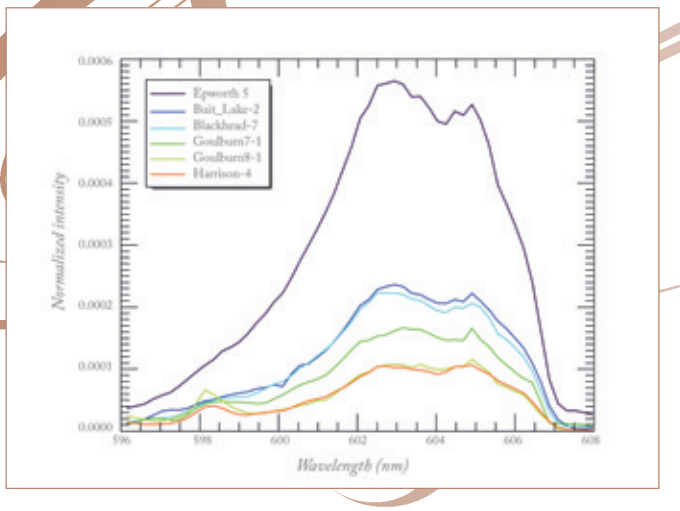
What are the challenges of conducting experiments on Mars?

The biggest challenge is that you can't go up there and fix a faulty mechanical or electrical component. Last year we had a small mechanical failure on ChemCam. We were very









Clockwise from top left:

Figure 1. The Curiosity rover mast with Mars scenery in the background. ChemCam's' Mast Unit is mounted inside the white box two meters above the ground; its 110 mm telescope, seen through the window, is the largest optical device on the surface of Mars. An inverted image of the Mars surface can be seen in reflection in the telescope's primary mirror. The stereo Mastcam and redundant stereo Navcams are seen below the ChemCam Mast Unit.

Figure 2. The first LIBS spectrum from Mars. The spectrum is combined from three (UV, violet, and VNIR) spectrometers. Insets show fine detail for atomic emission peaks of minor elements titanium and manganese (left) and hydrogen (center). The hydrogen inset shows spectra from individual laser shots, indicating that the first spectrum is rich in hydrogen, from the dust covering the rock; it is blown away by the laser shock wave, showing that the rock itself is low in hydrogen, as expected. The carbon peak shown in the inset is from the atmosphere.

Figure 3. ChemCam target Neil, showing a smooth surface with a manganese-rich layer. Ten observation points are marked. Dust was removed from the surface by the laser shock waves, exposing a clean surface for analysis. Repeated laser shots at each observation point revealed the thickness of the manganese layer.

Figure 4. Calcium fluoride molecular peak for several ChemCam targets. While most LIBS peaks are from atomic emissions, a few species, such as fluorine, are better observed via molecular emissions. The emitting molecule is formed within the LIBS plasma from elements present in the target. Ca-F emission peaks have helped the ChemCam team identify fluorapatite minerals.

fortunate to be able to work around it by applying a software fix. Another challenge is that everything must be done robotically. We only send up commands once a day, and we get the results back a day later. Both of these challenges force us to build a very versatile machine that is smart enough to do nearly everything on its own – that includes navigation and selecting targets.

How can you apply your discoveries on Earth?

We're living in an increasingly robotic world. So many of our activities on Earth, from astronomical observations to harvesting crops, are done more and more robotically. Exploring Mars robotically encourages us to take new steps in technology here at home.

But, we can't always measure the value of science by immediate benefits. Our understanding of Mars is important for longer-term benefits, whether it helps us understand climate change on Earth or leads to very long-term terraforming of our neighboring planet.

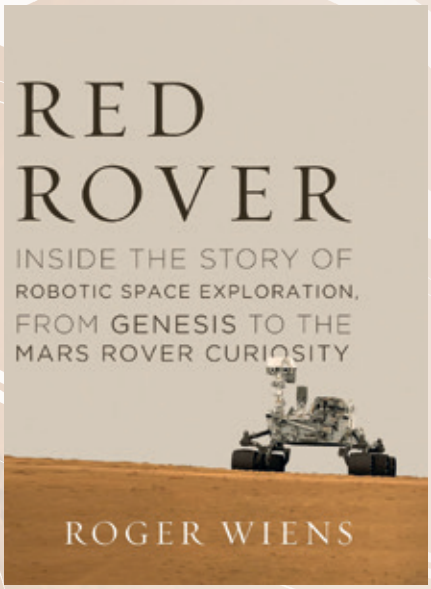
What about the political value of this research?

Curiosity showcases the prowess in space and robotic technology of the USA and its partner countries. If we can operate Curiosity remotely on Mars, think what we can do remotely in other places on Earth (or "Ocean" as noted on page 24). The mission has also helped forge strong bonds of partnership within the scientific community. ChemCam, for example, is a half-US, half-French endeavor for which both countries can claim credit. Other countries (UK, Canada, Denmark, Germany) have also joined our research and operations team. Each team member has been greatly enriched by the cross-cultural collaboration.

What is the next step for ChemCam?

In the short term, we are testing out new rover software that will pick our targets for us based on analysis of the rover's images. Up to now we normally get images down from the rover after it has stopped its drive, and we have to pick targets and uplink the commands with the right coordinates. Now we're training the rover to pick its own targets, so we don't have to wait until the next day to do the analyses. The AEGIS software package will save us a lot of time.

In the long-term, we are now developing the successor of ChemCam, called SuperCam, which will perform remote sensing on the next NASA Mars rover, scheduled for launch in 2020. Built on the ChemCam platform, SuperCam will add infrared and remote Raman spectroscopies. These techniques will provide high-confidence mineral identification in addition to the chemistry and high-resolution imaging now provided by ChemCam.



A revolutionary rover. A thrilling space exploration. A tale of human ingenuity and ground-breaking engineering…

In Red Rover, Roger Wiens paints an engaging and highly personal portrait of the Curiosity's ground-breaking trip to Mars, as well as his involvement as principal investigator for the ChemCam laser instrument on the rover.

Combining a remote micro-imager and laser-induced breakdown spectroscopy analyzer, the pioneering ChemCam suite is capable of detecting key chemical elemental ingredients remotely, going some way to answering the critical question: is this planet capable of supporting life?

From the Genesis mission that launched his career to the developmental and bureaucratic challenges of creating this one-ton nuclear-powered space laboratory, Wien's fascinating anecdotes punctuate the remarkable history of robotic space exploration. Both an uplifting tale of perseverance and an exciting account of scientific discovery, Wiens' memoir details the significant discoveries that characterize Curiosity's current journey, and assesses their impact on extra-terrestrial analytical science and beyond.

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ChemCam Highlights from the Gale Crater

1. The ChemCam team discovered with the very first laser shot on Mars that the soil and even the wind-blown dust is hydrated. The SAM instrument quantified the amounts, but ChemCam has shown the ubiquity of water in the soils and has helped constrain the mineral component in the soil containing the water.

2. ChemCam provides the first microbeam analysis of soils, showing for the first time that all soils analyzed so far consist of multiple components including contributions from the local rock types. The researchers can correlate these components with characteristic grain sizes.

3. In its first week of operation, ChemCam yielded the first high-silicon rock compositions. These compositions have been found not only in float rocks, but in the pebbles comprising the first conglomerates, and in the coarse soil grains. These were reported in the first Science papers and have been discussed in several papers since. The implication is that the igneous volcanism of Mars is much more varied, including much more evolved magmas than previously thought. Overall, the igneous rocks around Gale crater are more like Earth's continental crust than we expected.

4. Overall, ChemCam has returned more than 300,000 spectra from Mars, providing a comprehensive view of the compositions along the traverse in Gale crater. It has also provided more than 6,000 high resolution images.

5. ChemCam enables the analysis of fluorine for the first time on Mars – XRF-type instruments cannot observe elements with atomic masses lighter than sodium. ChemCam has made multiple observations of fluorine, which is relatively abundant in SNC (shergottite, nakhlite, and chassigny) meteorites. Its presence implies lower magma melting temperatures, and it is often found within alteration minerals.

6. The production of high manganese (Mn) concentrations requires a highly oxidizing environment, which currently does not exist on Mars. The discovery by ChemCam of a number of Mn-rich phases has powerful implications for the paleo-atmosphere of Mars.

7. LIBS is highly sensitive to alkali and alkaline-earth trace elements, so much so that the detection limit for lithium is ~5 ppm. These elements have generally not been quantified on Mars before and they each have implications for Mars geochemistry. For example, lithium is a strong indicator of alteration; rubidium, strontium, and barium each tend to be sequestered in different minerals: rubidium in anorthoclase, strontium in albite and more so in anorthite, and so on. The global rubidium:potassium ratio has important implications for planetary origins.

8. On the Bathurst Inlet sample, all five ChemCam observations showed surface enrichments in mobile elements, grading from the most mobile element (lithium) to the less mobile elements (for example, silicon and titanium). The gradient was consistent among all observed elements, definitively showing relatively recent rock surface alteration.

9. The micro-beam LIBS technique allows the ChemCam team to probe small areas, looking for interstitial material. In the first science paper on conglomerates, the researchers reported that one observation point showed the beam profiling through an iron-rich hydrated phase, which was interpreted to be an iron-rich cement binding the conglomerate clasts. The team also has evidence for this cement in the Rocknest rocks.

10. ChemCam was the first to observe and confirm the composition of the calcium sulfate veins in the Yellowknife Bay units.

11. ChemCam provided fine-scale geochemical constraints within the Yellowknife Bay formation: magnesium and lithium variations are correlated at the site of the raised ridge. Elevated magnesium (but not lithium) likely represents the outer layer of the cement, exposed on the dipping surface. ChemCam analyses of isopachous cements (cement of precipitated minerals that forms rims of nearly equal thickness around grains in a sedimentary rock) within early diagenetic (chemically altered) raised ridges indicate the presence of a manganese-iron-chlorine rich phase (or assemblage).

12. In addition to LIBS and high-resolution images, ChemCam also provides passive reflectance spectra. While the spectral range only covers the 0.4-0.9 micron range, spectral features consistent with hematite, pyroxenes/olivine, and calcium sulfate have been observed.

13. ChemCam measures the abundance of atmospheric water, oxygen and carbon dioxide, using passive-mode sky observations of columns.

14. The high resolution of ChemCam's RMI telescope shows rock textures in fine detail formed from sand and wind abrasion.

Dating in Space

F. Scott Anderson, a staff scientist at the Southwest Research Institute, Boulder, Colorado, USA, discusses the potential of in-situ resonance ionization mass spectrometry for understanding the age of planetary bodies.

What is it like being involved in NASA's space program?

I love it. It's a privilege and I'm always very excited to tell other people about it. I feel that I have an obligation to share my enthusiasm with the public, as they are the people who are funding the work.

What inspired you to get involved with dating space rocks?

I've been involved with studying Mars since graduate school, in particular the Valles Marineris and how this giant feature came into being. I discovered that the rate at which you model extension and applied tectonic forces can really change the ultimate shape of the canyon models you create – and those rates are controlled by the amount of time involved. As a student, I was surprised that planetary science didn't understand time very well, with geologic maps showing errors of up to 700 million years (or even a billion years in some instances) for estimates of canyon formation. A billion years is a quarter of the age of the solar system...

Hence, from very early on, I thought about how we measure the history of things. Of course, our understanding of the history of Mars ties with our understanding of the chronology of the moon and being able to obtain dates from areas with known numbers of crater counts. In fact, on the moon there is a significant gap in dated samples of known provenance from roughly a billion years ago to around 3.5 billion years ago. Because of this gap, we have trouble constraining the ages of various other planets within the same period.

Also, about a decade ago I was working at NASA's Jet Propulsion Laboratory (Flintridge, California, USA) where I became very interested in spaceflight hardware and building instruments. Back then, it was widely accepted that performing dating experiments on another planet was impossible and that you had to bring those samples back to the Earth – a very expensive proposition that might only happen once. Therefore, you had to pick the right sample – and characterizing a whole planet from only one sample location would be tricky. Even with the Apollo programs that brought back samples from a number of places (270 kg of rock), we still want to know how old the different surfaces of the moon are. In the 'Decadal Survey', NASA's blueprint for the scientific exploration of different planets, the number one objective for new lunar missions is to obtain more dates to help us understand not only the history of the moon, but also the solar system. During this time, I started thinking about approaches to miniaturizing a range of geochronology instruments.

So the idea of building instruments to obtain these important dates evolved from working at JPL and from thinking about how we can measure in situ instead of the very expensive and difficult sample return method. It's a political subject; I believe that we still need to do sample return in addition to in-situ dating, but a single sample return is not going to be enough to answer all the questions. With in situ analysis, which for Mars is approximately 10 times cheaper, you could fly missions to many different places for better characterization of a planet.

The final factor that inspired this project is my good relationship with Joe Boyce, an early leader of the Mars program at NASA. We talked a lot over beers and he once said, "Scott, you need to see if you can solve this in-situ dating problem using in-situ chronology." At the time, it was a crazy idea because no one really thought it was possible, but it inspired me to look into the laser ablation resonance ionization mass spectrometry technique. I actually looked at many different approaches and thought this was probably the best one. So we set out writing proposals and trying to figure out how to make it happen.

How does the technique work?

In all rocks, there are trace abundances of rubidium, including the 87 rubidium (^{87}Rb) isotope that decays into 87 strontium (^{87}Sr) radioactively over time. If you can measure how much ^{87}Rb and ^{87}Sr is in a rock, then you can determine how much time has passed since that rock was formed. However, these two isotopes have essentially the same mass, which makes dating difficult. To measure them with a mass spectrometer, you either need extraordinarily high resolution or you need to engage in some sophisticated tricks to separate the two.

To overcome the problem we use a multistep process. We put the rock in front of our mass spectrometer and our laser beam, and we ablate it to free up neutral atoms, amongst other things, from its surface, including those (Rb and Sr) that we want to measure. We illuminate the explosion plume from the rock's surface with lasers tuned to ionize the Sr atoms, a process called resonance ionization. At that moment, we use electric field pulses to push just the Sr atoms into the mass spectrometer. We repeat that process a fraction of a second later with lasers tuned for Rb atoms, and push those ions into the mass spectrometer too. Because there's a time gap between when we inject the Sr and the Rb, we can measure them separately in the mass spectrometer without them overlapping or interfering with each other, which provides an independent abundance measurement. To obtain good statistics, we repeat this one thousand times, while also measuring non-resonant backgrounds. To fully assess the variability in a rock, we ablate 300 different locations on samples of roughly 1–2 cm^2.

The only real requirement for sample preparation is a flattish surface or a known surface shape, such as a cylinder, which can be easily created in the lab. Those simple shapes are actually very consistent with existing systems for coring rocks, such as those used by the Mars Science Laboratory (MSL) or Mars 2020.

It takes two to three days to produce one of these measurements, but with the spaceflight designs we're working on, we think we can speed it up about 250 times, bringing down the time to two hours. The error bars that we get with these dates are typically about 200 million years, which was the case with Martian meteorite Zagami, and a lunar analog, the Duluth Gabbro (1, 2). Since we're looking at billion-year error bars for Mars and the moon, this 200-million-year bogey is good enough. With other samples we sometimes get this down to the 50 to 89-year range; however, those are for samples such as granites that are not commonly found on Mars, the moon or other planets. We are also always looking for ways to improve the measurement, and believe that the accuracy will continue to improve as we track down subtle instrumental issues.

How does the technique compare with other dating methods?

The pre-eminent method of dating in a lab is to use thermal ionization mass spectrometry (TIMS). This requires someone to take roughly three to 15 (sometimes more) small samples of a rock and grind them up, before processing them chemically to leech out the Rb and the Sr into a liquid state, which is then dried onto a filament. The filament is then placed in the mass spectrometer and heated up until the atoms boil off as ions, which are then measured independently in the mass spectrometer. This TIMS technique is exquisitely sensitive: typically, you see results as good as half a million years or so, compared to our 200 million years. In fact, these measurements are fantastic – and much better than we can do on-board a spacecraft. But...

There are some trade-offs with TIMS. Firstly, there's a lot of manual labor involved that can't take place on a spacecraft. Secondly, you're commonly looking at three to 15 measurements whereas we're looking at maybe 300 spot measurements. We acquire these 300 measurements in a rectangular pattern, forming an image. From each one of those pixels we get elemental abundance, the Rb and Sr isotopes, and the organics. The image also allows us to map the mineralogy in the rock, allowing us to assess potential biases such as thermally or aqueously altered minerals. For example, sometimes in terrestrial rocks we have observed that the isochron is well behaved for feldspars, but not for amphiboles. Thus, mapping of the minerals in the rock with our instrument allows us to see and understand any "misbehaving" minerals.

In contrast, when you only have the traditional three to 15 measurements, it's much harder to draw such conclusions. Interpreting the behavior of certain mineralogies in the rock helps us to understand how much heat was applied to it or how much water flowed through it during formation. It's much easier to do this using our technology than with traditional techniques.

So you're revealing the story of the rock?

I like to think so. And although the traditional techniques are much more accurate, a 100 to 200-million-year solution is probably good enough. A half-million-year solution in this case may actually be overkill. The real question is: can we answer scientific questions with what we're proposing? I think the answer is "yes", which is why this idea is radical and new – and why we're starting to get more attention!

What other challenges are involved in dating rocks from/in space?

Planets like the moon and Mars are easier to date because they don't really have plate tectonics or extensive weathering induced by water. Because of this, interpreting the rocks is often simpler. On the flipside, some terrains, particularly on the moon, are very low in Rb and Sr so are very hard to measure. But our recent progress measuring the Duluth Gabbro, a lunar analog (2), indicates that we are capable of addressing many of these terrains.

What were the main challenges developing the technique?

First, we spend a lot of our time focusing on understanding laser ablation, but it turns out there were other parameters we initially considered unimportant, for example, temperature stability in our laboratory. Lots of our lab equipment is sensitive to temperature changes.

Once we sorted out the lab equipment, we focused on laser ablation. Laser ablation is a very complex process, even though we're just using it to free the neutral atoms. It's not really the core of what is allowing us to measure the Rb and the Sr; in fact, it's the second step, resonance ionization (using the second set of lasers), that allows us to separate the Rb and the Sr. Nonetheless, laser

ablation is prone to fractionation and can even affect the production of neutral atoms. Scientists often worry about fractionating ions produced from a sample surface, and yet ions are actually relatively rare in this scenario – almost everything that comes off is neutral. While it's commonly thought that the neutrals are not fractionated, when you're measuring things at one part per million it turns out that those fractionation processes still matter. Thus, getting the laser ablation process to behave is very important.

Next, we needed to study the resonance ionization process – consisting of three lasers for Rb and another three for Sr – that allows us to separate and measure the abundance of Rb and Sr for each mineral. However, even with resonance ionization, it turns out that contaminants (mass interferences from molecules other than Rb and Sr) can still sneak in. They may be at very low abundance levels; however, even one part per million would still be enough to bias your answer. If there are background contaminants in the measurements, they may be there without any combination of the three lasers used for resonance ionization. To address this we take a background measurement whereby we turn the resonance lasers on and off and subtract this from the other measurements.

Finally, when developing a new technique, you just can't measure something once – you must do it many times to develop any kind of confidence in a result. Sometimes we get results that don't make sense, requiring us to go back and relentlessly study the issue until we can work the problem out.

Ultimately, we have worked really hard to change negative perspectives and more people are coming around to the idea that it can be done – maybe not as well as with the TIMS instruments, but well enough for spaceflight applications and some terrestrial applications.

Did the negativity make you more determined to succeed?

Yes and no. It's always very intimidating when an expert in the field and one of the 'old boys' tells you you're barking up the wrong tree. I would ask them why it couldn't be done. Then I'd take the opportunity to explain what we're doing. The story that I started to hear repeatedly was that no one else had thought about doing it in the particular way we were thinking of using. That's when I started to have hope.

Where to next?

Actually, there are additional steps that are closer to the science. Our real focus now is on measuring as many samples as we can, looking for any problems and showing the capability and limits of the technique. We have already identified a couple of areas where we think we can do better and we'd like to explore those to see whether we can improve the error bars that we are getting today. It seems that every year that goes by we seem to be able to do a little bit better than the year before. When we started, we could barely measure easy terrestrial granites, then after continuous improvements we found we could measure harder meteorites from Mars (like Zagami). After further improvements, we were able to measure difficult lunar analogs like the Duluth Gabbro. We continue to iterate on the ever harder samples, to improve our accuracy, and open more of the solar system to potential analysis using this technique.

What will be the challenges of getting the technology ready for spaceflight?

There are three. The first challenge is the laser systems. There are similar laser systems that exist and have been flown, but our exact laser system has not been built in its entirety. We have NASA funding to build it so we're thrilled to have the opportunity to do this kind of development. We have now demonstrated two of the lasers in prototype form, and are working on implementing the remaining systems. In the next couple of years, we plan to test these in simulated space flight environments.

The second difficulty is sample handling. I've said that sample handling is straightforward and that we believe that existing tools like coring-drill and the rock-abrasion tools will work, but doing it remotely on a different planet is always going to be a challenge. We are working with space flight tool manufacturers to overcome potential difficulties and to show how all the pieces work together. It's always important to fine tune the sample handling approaches and test them in real-world ways.

The third tricky issue is the politics of writing the proposals and going after this kind of science. Usually it hinges on the question of sample return versus doing measurements in situ. There are many people who feel they are inherent enemies of each other; I think in many ways it's because these two communities are afraid that one or the other of them won't be able to do the work over the long term. I think that's the wrong way to look at it because both of these measurements are very important, as I expressed earlier. Bringing back a sample allows you to do incredible work that you won't ever be able to do in situ. It takes that kind of insight to really characterize another world, so we need to stop having a debate over which one is going to win and instead talk about how we can use both approaches.

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Solutions

Real analytical problems
Collaborative expertise
Novel applications

Faster Chemical Separations

A 2015 Innovation Award-winning "fastGC" module enhances proton transfer reaction time-of-flight analyzer performance by adding an optional near real-time chemical pre-separation step. Here's the story behind its development.

By Lukas Märk, CEO, IONICON Analytik GmbH, Innsbruck, Austria.

The Problem

Although gas chromatography (GC) offers chemical separation and identification, it can take a lot of time to perform the experiment and analyze the results when compared with real-time gas analyzers, which provide dynamic information about a process. Is it possible to combine the key benefits of both technologies?

Background

With efficient, soft ionization and high mass resolution, proton transfer reaction time-of-flight (PTR-TOF) analyzers have become the de-facto standard for real-time monitoring of trace volatile organic compounds (VOC). In a fraction of a second, a large spectrum of VOCs can be analyzed right down to parts per trillion (ppt) levels. Such high performance is ideal for monitoring complex samples in a variety of applications, including environmental monitoring, food and flavor research, life science applications, industrial process monitoring and many others. With a high-resolution TOF mass spectrometer, isobaric compounds can easily be identified by their difference in exact mass. However, isomers (compounds with the same chemical composition and consequently the same exact mass) are not separated in a TOF. Using GC pre-separation in combination with a PTR-TOF resolves this problem, but at the expense of real-time analysis, since a typical GC run takes around 30 minutes – more than a thousand times longer than a PTR-TOF analysis.

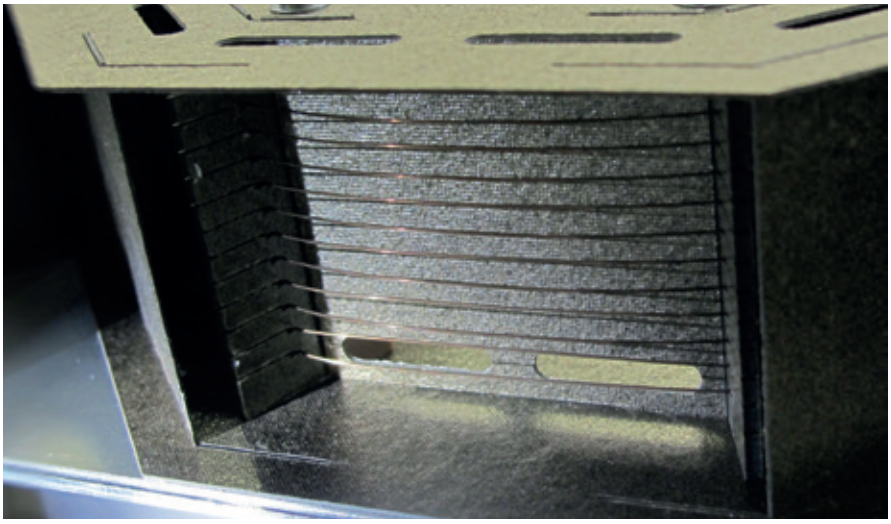

Figure 1. IONICON fastGC system, column and mount.

The run time of a GC begins at sample injection and finishes when the final compound elutes from the column, which depends on several factors – most importantly the column temperature. To speed up the process the column is heated (up to several hundred degrees), typically in an oven that represents the most significant contributor to the thermal mass of the system, which limits the speed of heating and also cooling. This is improved in fast-GC systems, which, in most approaches, reduce the thermal mass of the system (for example, by combining the column directly with a heating wire). Such systems reduce the runtime down to several minutes. A higher heating rate is usually achieved by applying more power – but a fast cooling rate is more difficult to accomplish. Of course, the cooling time sets a limit on when the next analysis can take place and therefore restricts the throughput.

Although combining a PTR-TOF with a GC pre-separation is tempting to get the additional chemical dimension, the

trade-off in time resolution is often too big a sacrifice, even at only a few minutes. The IONICON fastGC is designed specifically as an add-on for PTR-TOF trace gas analyzers to enable another dimension of chemical separation, mainly for separation of isomers and enhanced identification of compounds. The objective was to get very short run times, so that the real-time capabilities of our fast analyzers were sacrificed as little as possible.

The Solution

The fastGC system uses an innovative heating concept that allows much faster heating rates –more than 30K/s compared with 30 K/min in conventional GC systems. Most importantly, it also offers cooling rates of 30 K/s, which greatly increases the overall sample throughput.

For our fastGC, we use a commercially available metallic GC column, but trim its length to a few meters. By applying an electric current directly to the metallic column, it acts as its own heating wire. Here, we approach the lowest possible thermal mass, with only the column itself changing temperature and a resultant rapid heating and cooling response. Our concept demanded precisely controlled heating and the ability to regulate power so that the target temperature could be reached as quickly as possible. In essence, we needed to meet two major challenges preferably with a single solution. The first challenge is the regulation of the heating power; with an overall low resistance, a high current must be applied and regulated. The second (perhaps less obvious) problem is the measurement of the column temperature. In simple terms, a temperature sensor attached to the thin column would likely result in the column having the temperature of the sensor and not the other way around...

To resolve the first problem – controlling the heating power – we used pulse width modulation. Fast electrical pulses (several kHz) at variable lengths are applied to the column. The on-off duty cycle defines the average heating power. The voltage is constant, but the resistance of the column, which changes (slightly) with temperature, determines the resulting current. This also led to the solution of the second problem – the temperature measurement. Comparing the attained current to the voltage, we can calculate the column resistance, and from this the column temperature. To read the temperature in the absence of heating, we limit the minimum duty cycle to a pulse just long enough to measure the temperature correctly but resulting in negligible heating.

By placing the column in an oven, we can calibrate its resistance to temperature response. Since the change in column resistance with temperature is small, drifts and other resistances can affect the measurements. Therefore, we zero the temperature measurement at regular intervals, especially after start-up. We do this by cooling the column for an extended time to ensure that its temperature is at ambient. An independent sensor measures the ambient temperature. A built-in microprocessor determines and subtracts the offset resistance; the microprocessor also controls the heating power to quickly reach a set temperature or drive temperature ramps.

The column can be heated to several hundred degrees, limited by the maximum allowed column temperature. The column is fixed to a special mount, which is resistant to high temperatures, is non-conductive, and has a minimal contact with the column, as shown in Figure 1.

The whole setup is placed inside a housing. Air openings and a fan allow for a controlled cooling of the column with ambient air. Due to the low thermal mass, this allows cooling rates of 30K/s. Mounting it next to a PTR-TOF allows access to the ambient air for cooling on one side, while the other is in direct, cold-spot free contact with the heated inlet chamber, where the sampling gas arrives through

The Road to fastGC

1995:
The first PTR-QMS (quadrupole MS) was developed

1998:
IONICON founded as a spin-off of the University of Innsbruck, Austria

2007:
The first PTR-TOFMS (time of flight MS) was launched

2013:
fastGC development started

2014:
Launch of fastGC

2015:
fastGC first presented to the public at Pittcon 2015; fastGC receives "Golden Gas Award 2015" from Gases & Instrumentation International; fastGC receives "Innovation Award" from The Analytical Scientist.

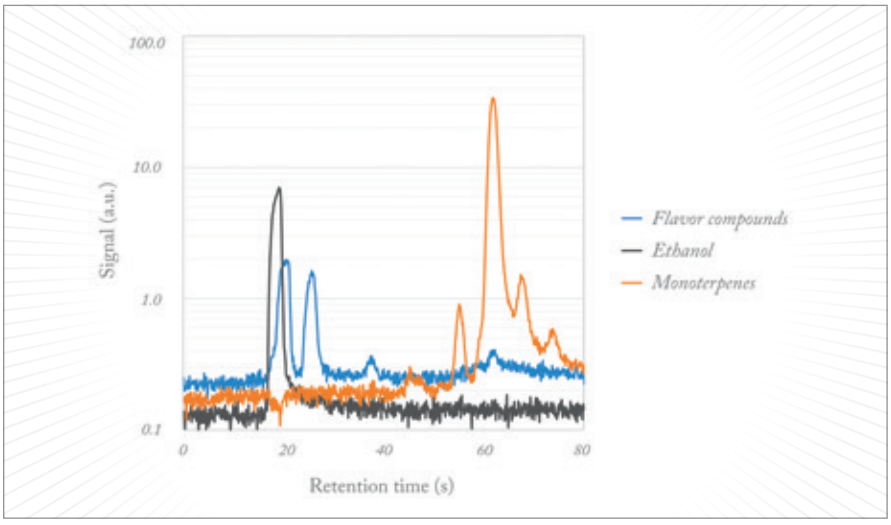

Figure 2. FastGC-PTR-TOF head-space measurement of a beer-lemonade mixture. Ethanol has eluted from the column before other flavor compounds and does not perturb their measurement. At least five different monoterpenes, with a dominant contribution from limonene, can be separated.

capillaries. For real-time trace gas analysis, the use of heated, inert wetted-surfaces is important to reduce surface interaction and retention effects.

We use an array of several micro-valves to configure the gas flow. A sampling loop (an inert capillary with an internal volume of several ml) draws the sample gas constantly. To initiate a fastGC analysis, gas from the sampling loop is injected into the column. By controlling the flow and the time, the amount injected can be varied. A digital mass flow controller regulates the flow of clean N2 carrier gas to push the sample through the column. When a lower flow is set than needed for normal PTR-TOF operation, additional N2 is used as make-up gas. The valves can also be configured to bypass the fastGC setup and perform normal real-time analysis with the PTR-TOF, which allows real-time monitoring with (automated) intermediate fastGC analyses.

Beyond the solution

The fastGC is available as a built-in option for our ultra-sensitive, real-time PTR-TOF analyzers, but can also be used to upgrade existing PTR-TOF systems. The key feature of the fastGC – adding a dimension of chemical separation to PTR-TOF data – has already been demonstrated in several publications; for example, the separation of several (isomeric) monoterpenes in biological samples (1). Monoterpenes are a group of biological VOCs emitted in high quantities by numerous plant species, particularly conifers and are associated with the typical scent in a forest. A PTR-TOF can record the combined contribution of these isomers, and – with the addition of the fastGC – the contributions of different monoterpenes can be monitored individually (see Figure 2 for another example).

Additional benefits of the fastGC add-on quickly became apparent in other applications, such as in the headspace analysis of alcoholic beverages, where the high ethanol concentration has so far compromised the measurement of other trace VOCs. Using the fastGC system, the ethanol quickly elutes from the column and subsequently does not interfere with the measurement (2). An example is shown in figure 2. This principle can be applied to other scenarios with high matrix concentrations.

The high rates for heating and cooling surpass those of other commercially available fast GC systems. And with minimal effort, the IONICON fastGC could eventually be adapted for integration with other gas analyzers. The swift acceptance and a rapid spread of the fastGC add-on that we have seen in PTR-MS user groups demonstrates great potential – and we expect to see its use spreading towards routine applications. Once established, we feel fastGC-PTR-TOF could go as far as replacing conventional GC-MS because the initially greater costs are balanced by higher throughput.

Currently, we are working on fully integrating the fastGC-PTR-TOF instrument with an auto-sampler set-up (soon to be launched for the IONICON PTR-MS products). Our ultimate goal is to develop an automated instrument that includes all components from sampling to chemical pre-separation to PTR-TOF analysis, followed by appropriate data processing – a challenge we are excited to tackle.

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Screening, Identifying, and Quantifying Potential Genotoxic Compounds with High Resolution LC/MS

Analysis of chlorhexidine drug substance using an Agilent 6545 Accurate Mass Q-TOF System and MassHunter Mass Profiler Software.

Syed Salman Lateef, Agilent Technologies, Inc., Bangalore, India

This study demonstrates a routine screening of drugs to identify and quantify potential genotoxic compounds. In this Application Note, we used an Agilent 6545 Q-TOF LC/MS system to acquire accurate mass data of samples containing chlorhexidine as the drug substance. Agilent MassHunter Mass Profiler software was used to mine the data and compare different samples to generate a differential list of compounds. An accurate mass database search against the differential list identified 4-chloroaniline, a potential genotoxic compound. All Ions MS/MS acquisition mode was used to confirm 4-chloroaniline by MS/MS library matching, and quantify it using external standards. This workflow is suitable for batch-to-batch sample analysis for detecting and quantifying known potential genotoxic compounds.
The full Application Note can be found online: tas.txp.to/0116/GenotoxicApp

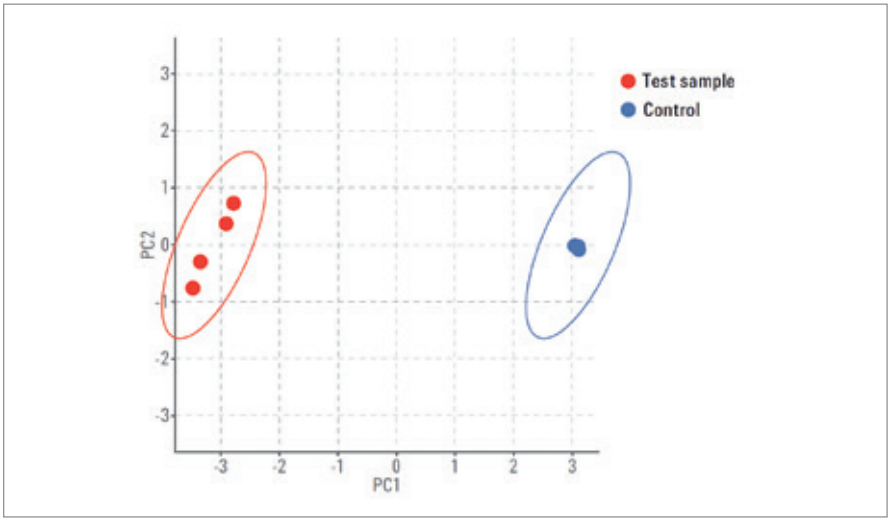

Figure 1. PCA plot showing different sample grouping. Red dots represent test samples and blue bots represent control samples.

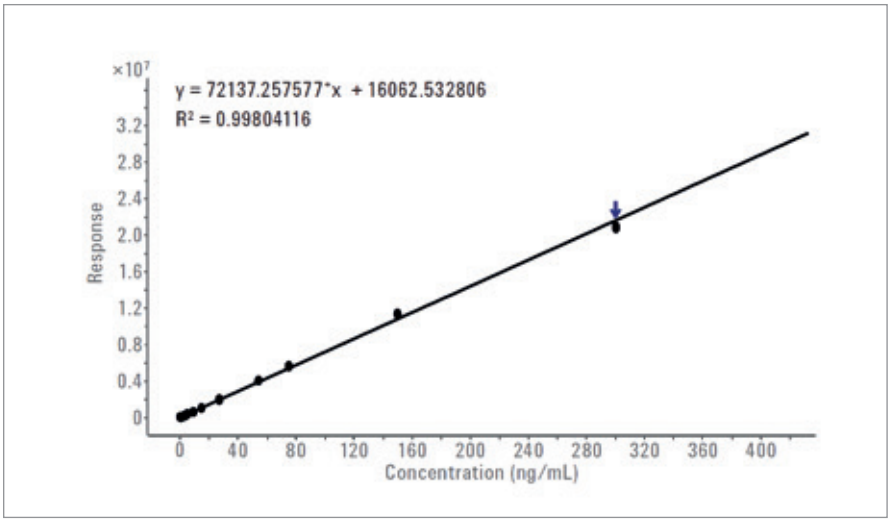

Figure 2. Calibration curve of 4-chloroaniline calculated using All Ions MS/MS.

Introduction

Drug substances may produce potential genotoxic compounds when they are stored for extending periods of time, or when they are stored inappropriately. Detection, identification, and quantification of genotoxic compounds is a time-consuming process. Regulatory authorities (1) require reporting of the formation of genotoxic compounds. Recent advances in software tools enables the fast and cost-effective detection of potential genotoxic compounds in complex samples. Agilent MassHunter Mass Profiler (MP) software allows the comparison of two sets of samples, and the determination of any significant differences between them. Principal component analysis (PCA) tools within MP assists the classification of compounds based on identified differentiation markers. A differentiation marker is a compound that exceeds a defined concentration, when compared to a control sample. A custom-built accurate mass database was used to identify the differences between samples. In this study, MP analysis of degraded and nondegraded chlorhexidine samples gave a list of statistically different compounds between samples. Using an Agilent ID

Browser feature within the MP software, these compounds were searched with a custom database containing potential genotoxic compounds. Compounds were further confirmed using accurate mass library matching, then quantified. Figure 1 shows the workflow used in this study.

Experimental

See full Application Note for details: tas.txp.to/0116/GenotoxicApp

Results and Discussion

Screening by differential analysis

The data files from the LC/MS analysis of degraded and control samples were processed using recursive molecular feature extraction in Mass Profiler software. Height filters of 4,000 counts for extracted compound features, quality score 100 and >4-fold change were used for statistical analysis. A greater than 4-fold change was applied to detect those features that differed significantly from control samples. See full Application Note for more details: tas.txp.to/0116/GenotoxicApp

PCA plot

The PCA plot reveals that the degraded chlorhexidine samples are different and distinct from the control sample (see Figure 1). This indicates that the degraded chlorhexidine sample contains features that are different from the control group. The control groups do not show significant separation, indicating no variation (blue dots) between samples.

Compound identification

A customized accurate mass database and library was created using standard compounds. The database also included literature reported mass, formula, and structures of chlorhexidine impurities. Post-statistical analysis, the differential list of compounds was searched against the accurate mass database using the ID Browser feature within Mass Profiler. The results indicated the presence of a potential genotoxic, 4-chlorhexidine in the degraded samples.

Feature summary of compounds

See full Application Note for details: tas.txp.to/0116/GenotoxicApp

Confirmation and quantification of potential genotoxic compounds

A shorter data-independent acquisition method was used for the targeted confirmation and quantification of 4-chloroaniline. In data-independent acquisition (All Ions MS/MS) of drug samples, both MS and MS/MS information are generated. The fragment ions in the MS/MS spectra of the personnel data compound library (PCDL) were used to extract ion chromatograms from the high energy channel. The extracted ion chromatogram (EIC) of the precursors from the low energy channel were aligned with fragment/production EICs to obtain the coelution score. The 4-chloraniline was confirmed based on accurate mass fragment matching and coelution of the precursor and product ions. 4-Chloroaniline was found with three qualified spectra in the library MS/MS spectrum where the fragments are selected from high energy MS analysis. The selected spectra were used with the qualifier and quantifier ions for the quantification method.

The qualifier and quantifier fragment ions, together with compound names, retention time, precursor ion, fragment ion, collision energies, and relative abundances were exported to MassHunter Quantitative Analysis software to set up a quantitative method. The most intense ion was used as a quantifier trace, while the less intense and unique fragment ions were used as qualifiers. A calibration curve with > 3 orders of magnitude was plotted from 0.1 to 300 ng/mL (see Figure 2). The 6545 was calibrated and tuned in high sensitivity mode. In addition, tuning for low mass (50–250 m/z) using Swarm autotune was enabled since some of the product ions for 4-chloroaniline were of low mass. The results of sample analysis showed an average value of 29 ng/mL in the degraded sample. Potential genotoxic compounds typically have a limit for reporting of 0.05 %.

When 1 mg chlorhexidine is dissolved in 10 mL solution, a 0.05 % limit would require quantitation down to 50 ng/mL. Therefore, any assay must be capable of a lower LOQ. The method developed in this study can detect impurities present at a concentration <1 ng/mL.

Conclusions

This Application Note demonstrates that potentially genotoxic compounds can be screened, identified, and quantified using high resolution LC/MS. A streamlined workflow was achieved by combining All Ions MS/MS data with Agilent MassHunter Mass Profiler software (Rev. 7.0). Automated differential marker analysis revealed significant differences between sample and control sets. The workflow also included the automated detection and identification of potential genotoxic impurities as target compounds using a PCDL. The All Ions MS/MS methodology was used to generate both quantifier and qualifier ions. This enabled the quantification of the target compound. The test sample processed with this technique was determined to be at a concentration of ~29 ng/mL or 0.02 % of 4-chloraniline (assay linear range from 0.1–300 ng/mL). This workflow can be used as part of routine drug sample analysis for the identification and reporting of potentially genotoxic compounds.

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Dioxin Determination in Air Filter Samples Using Pressurized Solvent Extraction (PSE)

Extraction of air sampling filters for the determination of dioxins using BUCHI's SpeedExtractor E-914

Claudia Blum, Susanne Feifel, and Maren Sander

This application note demonstrates the use of Pressurized Solvent Extraction (PSE) for the extraction of air sampling filters for the determination of dioxins. The extraction with the SpeedExtractor E-914 takes less than 1 hour and the results are comparable to those obtained by 16 hours of Soxhlet extraction.

Introduction

Dioxins (PCDDs) and furans (PCDFs) are persistent organic pollutants (POPs). Pretreatment methods are required to analyze dioxins, including extraction, purification and concentration prior to analysis by GC/HRMS.

Soxhlet extraction, required by official methods, takes up to 16 hours and is the "bottleneck" of the procedure. This application note shows the suitability of Pressurized Solvent Extraction for PCDD/F preparation for analysis in air filters.

Experimental

Quartz fiber filter paper (QFF) and Polyurethane foam (PUF) were placed in a high volume air sampler (HV-700, Sibata, Japan, flow rate 700 L/min) for 3 days on the roof of the Osaka City Institute of Public Health and Environmental Science.

PUF samples loaded in SpeedExtractor E-914 cell

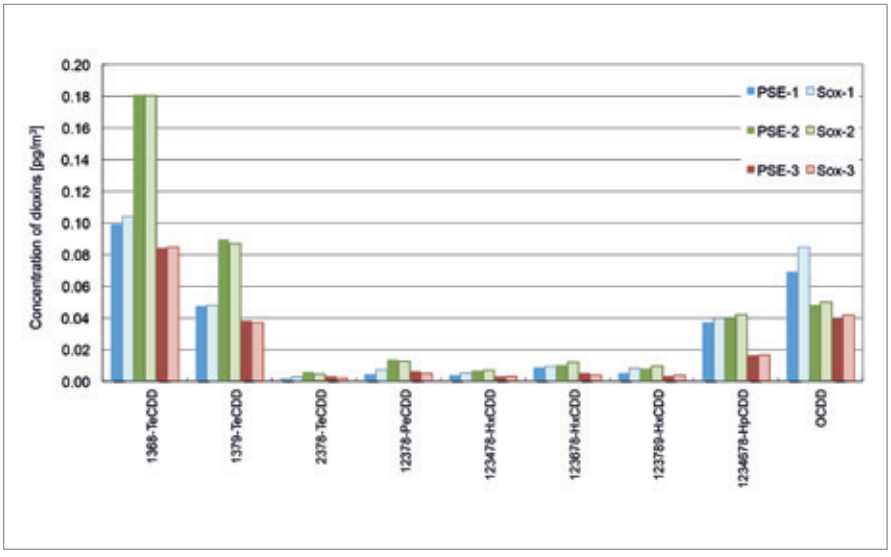


Figure 2: Concentration of dioxin congeners on PUF filters [pg/m³], extracted in parallel with PSE and Soxhlet

After sampling, load the filters into the SpeedExtractor E-914 extraction cells. The PUF filters are loaded as shown in Figure 1.

Extract the filters with toluene using the SpeedExtractor E-914 with the following parameters: Toluene at 100 °C (QFF) and 80 °C (PUF), 100 bar, for 3 cycles of 3 minutes each. The total extraction time was 53 minutes. In parallel, extract the filters with Soxhlet for 16 hours.

The cleaned-up and concentrated extracts can be analyzed by HRGC/HRMS.

Results and discussion

The comparison of three PUF filters extracted in parallel with SpeedExtractor E-914 and with Soxhlet showed, that the determined concentrations of dioxins and furans are comparable. Figure 2 shows the determined concentrations of dioxin congeners.

Conclusions

The determination of PCDD/PCDFs with the SpeedExtractor E-914 provides reliable and reproducible results. The determined concentrations with PSE are comparable with those obtained by Soxhlet extraction. The PSE method takes 53 minutes compared to the 16 hours for the Soxhlet extraction.

Small-footprint Absorbance System for Characterizing Heme Proteins

Compact Spark spectral sensor measures Vis-NIR absorbance of hemoglobin – and more

Yvette Mattley, Ph.D.

In this application note, we focus on the visible response of the heme group using the compact Spark spectral sensor to measure absorbance spectra for the heme proteins hemoglobin, myoglobin and cytochrome c.

The heme groups found in metalloproteins generate absorbance bands that vary based on the state of the heme group. These absorbance changes make UV-Vis spectroscopy a very powerful tool for studying heme proteins. Absorbance measurements can be used to study changes to critical protein parameters like protein conformation and to provide information on the binding and oxidation state of the heme prosthetic group.

Experimental Setup

We used a Spark-VIS spectral sensor with direct-attach cuvette holder-white LED module to measure the visible absorbance spectra for the metalloproteins hemoglobin, myoglobin and cytochrome c diluted in solutions. Spark integration time was set at 3. 5 ms, with 50 scans to average and boxcar smoothing width at 10. The solutions were prepared in water at a concentration of approximately 1 mg/mL. The samples were diluted as necessary to provide spectral data below 1 AU and pipetted into disposable cuvettes.

Results

The absorbance spectra measured for the metalloproteins are shown in Figure 1. The similarities observed in the spectral features for each protein result from the presence of the heme group in these proteins. Based on the shape of these spectra -- which change with the state of the heme group -- the iron atom in the heme groups of these proteins appears to have been oxidized and resulted in spectra consistent with methemoglobin and metmyoglobin (Figure 2), and oxidized cytochrome c. The spectra measured for these proteins would be very different if the iron atom was in a different oxidation state or had oxygen or some other gas bound to it.

Conclusions

The ability to study the state of the heme group using UV-Vis absorbance spectroscopy makes it a powerful technique for education, research and even clinical diagnosis. In the case of metalloproteins, the UV-Vis absorbance spectra provide detailed information about the heme prosthetic group required for the proteins to carry out their critical biological functions.

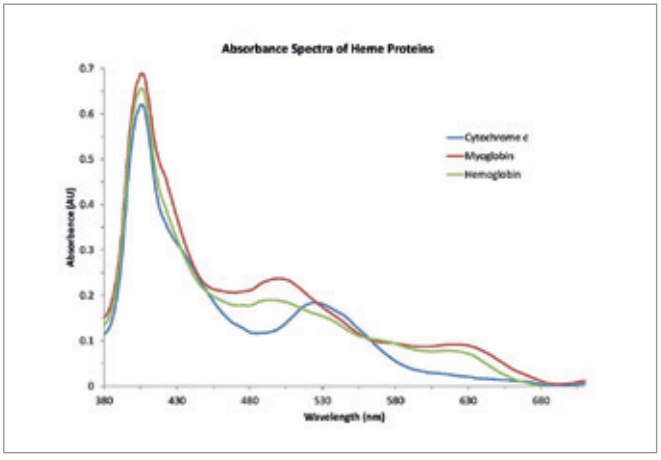


Figure 1. The Spark-VIS spectral sensor and its direct-attach cuvette holder-white LED module measured absorbance spectra of heme proteins.

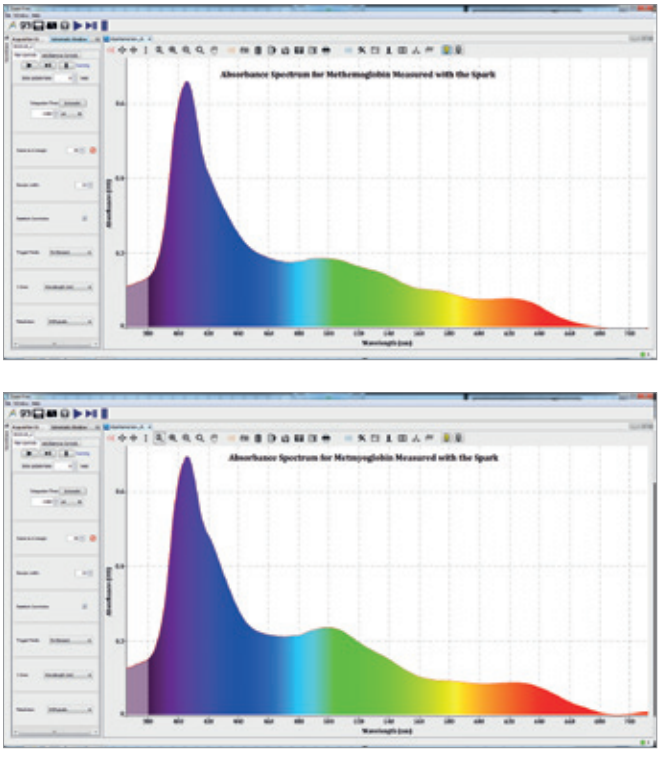
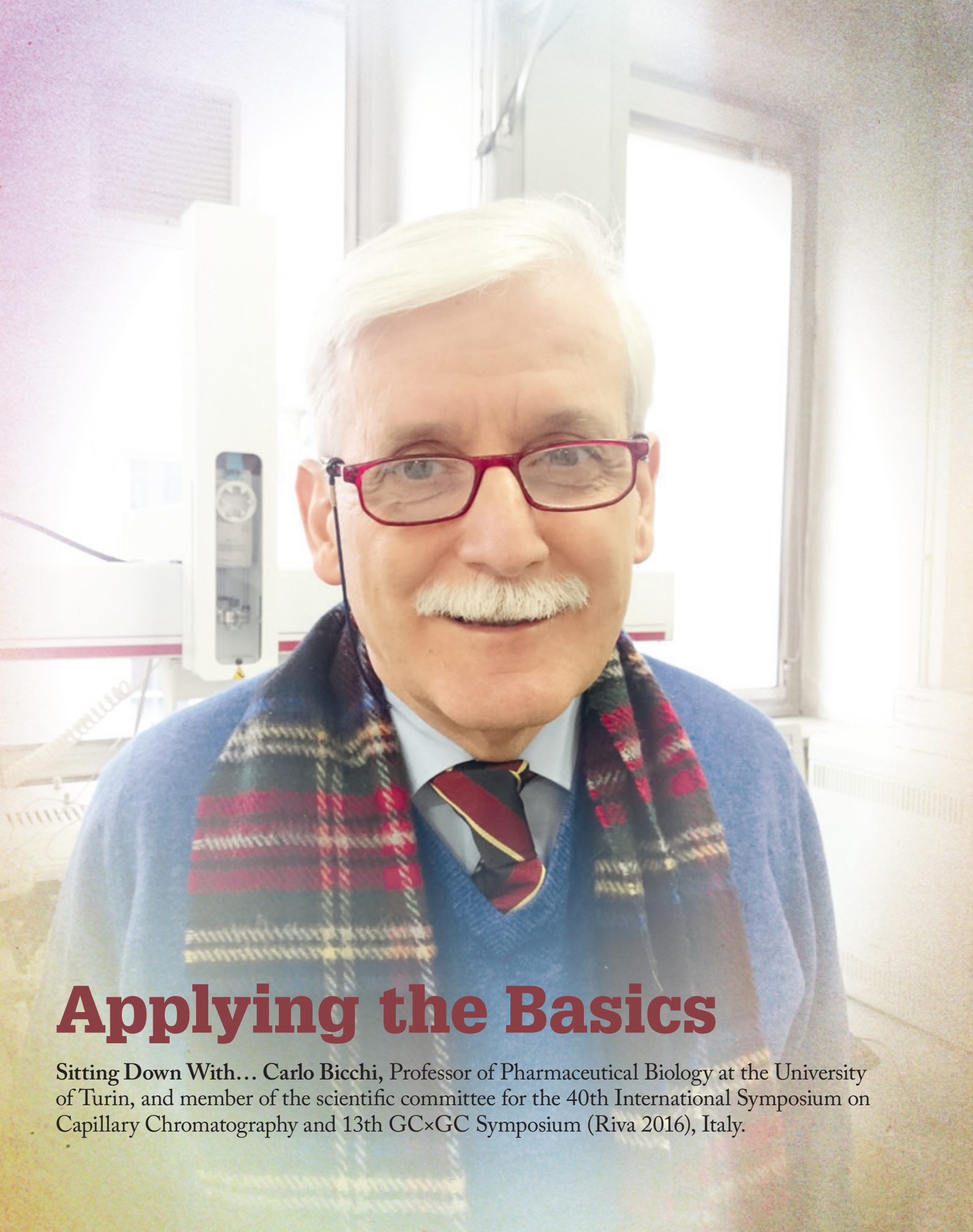


Figure 2. Oxidation of the iron atom in the heme groups of metalloproteins resulted in spectra consistent with methemoglobin (top) and metmyoglobin (bottom).



Applying the Basics

Sitting Down With… Carlo Bicchi, Professor of Pharmaceutical Biology at the University of Turin, and member of the scientific committee for the 40th International Symposium on Capillary Chromatography and 13th GC×GC Symposium (Riva 2016), Italy.

Why analytical chemistry?

Serendipity has played an important role in my career. After obtaining my master's degree in pure theoretical chemistry from the University of Turin, I was keen to continue in academia; however, PhD programs did not exist in the Italian university system back then. I asked several professors about potential scholarships and, eventually, an organic chemist with expertise in natural products and gas chromatography (GC) allowed me to join his laboratory – and the primary focus of my career hasn't really changed since then. Moreover, I never left the University of Turin! Right now, I am focused on the analysis of plant volatiles. From a technique point of view, I've extended the use of enantioselective GC and multidimensional GC to gain an exhaustive picture of the volatile fractions of plants.

Why plants?

I actually began with plants used in pharmaceuticals and now also work with plants used for food. Globalization is affecting the latter, in particular. Plant products arrive from all over the world – but sometimes the quality is second rate. Quality control within the pharma industry is very strict, but in other industries – food and cosmetics, for example – I believe there is room for improvement. For example, citrus fruit essential oils can often contain a mixture of different, cheaper, lower grade oils. Here, enantioselective GC is important as it allows us to determine enantiomeric excess or ratios, which are diagnostic parameters.

You've spent over 40 years in academia – what's changed in that time?

Currently, I'm a full professor of pharmaceutical biology, but I've also been the director of my department and dean of the faculty of pharmacy in that time. I have to say, I am pleased to see the back of bureaucracy – especially as I'm near retirement! Of course, I still teach and I run a research laboratory.

Things have certainly changed since the early 70s – some for the better and some downsides. One positive aspect is that it is much easier to tackle multidisciplinary projects; inter-laboratory collaborations bring together different skills and various specialists to deal with complex projects. But I've also noticed that increased flexibility can lead to a lack of identity or focus for some research groups (and research topics). In analytical chemistry, for example, I have seen too many people trying to do everything. You do need to have an open mind and wider knowledge of other analytical fields, but you also need expertise if you want to be (or stay) competitive. Another problem is the cost of research (and the difficulty in gaining finance) – a real challenge, particularly for smaller laboratories.

And what about the students?

I'm pleased to say we still have well-motivated students. Unfortunately, too few end up following academic career – instead, they are drawn by the allure of good industry salaries, which is not entirely bad – but it does mean we risk losing too many brilliant minds. Nevertheless, I have always believed that universities should work with industry in both basic and applied research; our laboratory has strong connections with various industries, which not only builds bridges for both applied and basic research, but also allows us to self-finance our laboratory.

What are the main challenges facing analytical chemistry, today?

In separations science, I don't think there is sufficient understanding of fundamentals by students or customers; too many people see chromatography as a black box into which you can inject a sample and just wait for a result. But chromatographers are not magicians and separations do not happen by magic (although it sometimes seems like they do)! In reality, you need to understand what you are looking for, which is why it is so important to teach basic concepts. Of course, universities might not be able to train students in all modern technologies used in separation sciences – and that's why collaboration between academic institutions and instrument manufacturers is so important.

What has been most rewarding?

I can't give you a specific answer. However, I can say that gaining a reputation for excellence in separation science for our laboratory is definitely on the list. When I started my career, the laboratory was good at natural product chemistry – but not natural product analysis. I decided to transform the laboratory and bring it to a level of excellence for natural product analysis (in particular, volatiles), which is why I chose to spend time with Pat Sandra (who has been very influential for me over the years) at the University of Ghent first and then at the Research Institute for Chromatography (RIC) back in the 1980s and beyond.

It's also very rewarding when I see people from our laboratory win international acclaim.

What can we expect from Riva 2016?

I believe the International Symposium on Capillary Chromatography (ISCC) and the GC×GC Symposium in Riva del Garda are important symposia because they allow you to keep a finger on the pulse. For example, two fast-growing areas that demand attention include miniaturized techniques (micro GC, micro LC, and lab-on-chip) and the impact of chemistry on biology. At Riva 2016, we'll see some very interesting new things in both areas, in particular for biological analysis – or rather chemical analysis applied to biology...

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