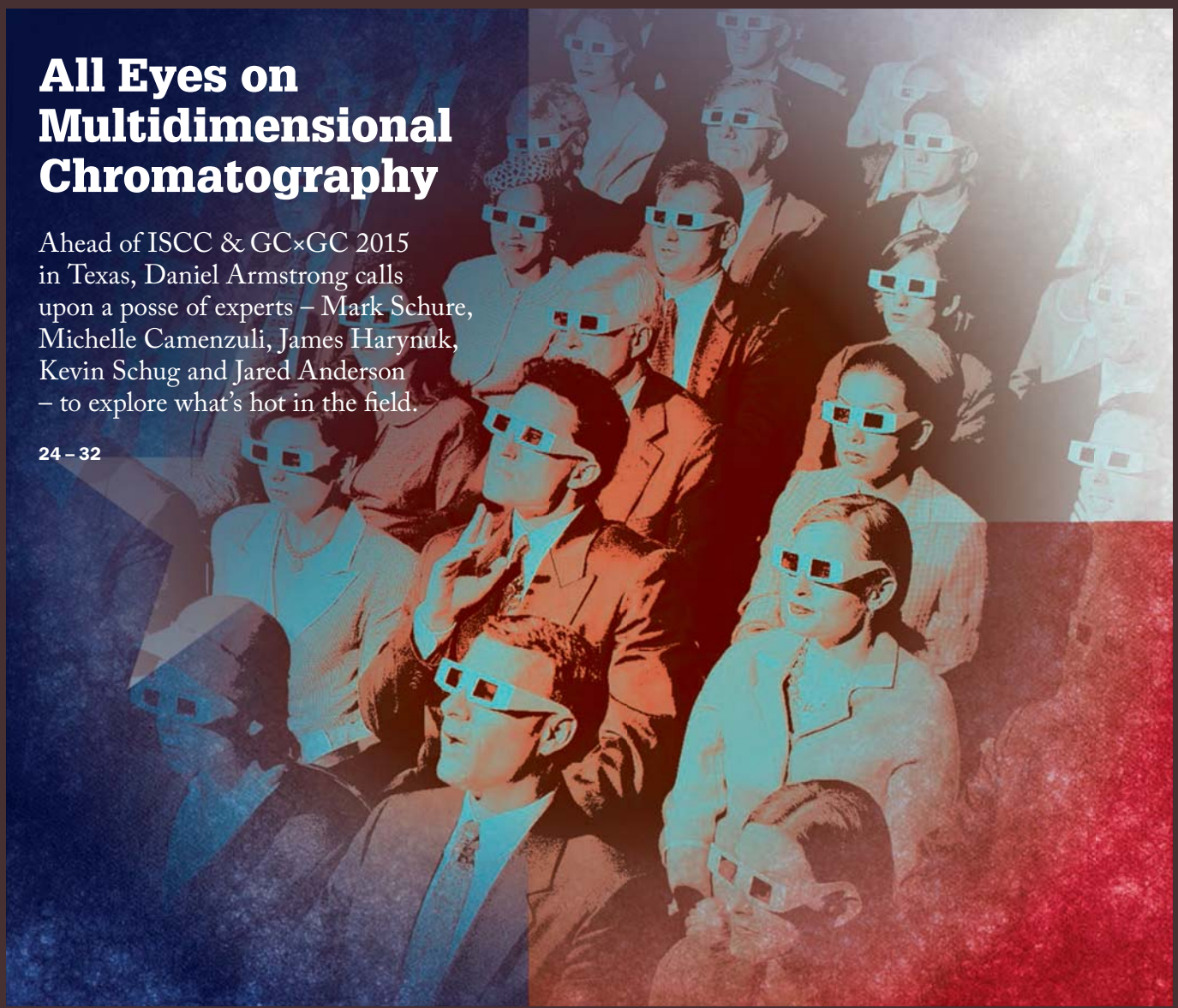
APRIL 2015 # 27

the Analytical Scientist



All Eyes on Multidimensional Chromatography

Ahead of ISCC & GC×GC 2015 in Texas, Daniel Armstrong calls upon a posse of experts – Mark Schure, Michelle Camenzuli, James Harynuk, Kevin Schug and Jared Anderson – to explore what's hot in the field.

24 – 32

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the Analytical Scientist

Andreas Seidel-Morgenstern (left), Peter H. Seeberger (right)

Meet the Winners

Peter H. Seeberger and Andreas Seidel-Morgenstern

Peter H. Seeberger and Andreas Seidel-Morgenstern of the Max-Planck Institutes in Potsdam and Magdeburg have been chosen as the winners of the inaugural Humanity in Science Award for developing a method for the continuous flow production and purification of cheaper antimalarial medicines using plant waste, air and light.

They were awarded with a humble prize of $25,000 during an all-expenses paid trip to Pittcon 2015 and their insightful essay will be published in a future issue of The Analytical Scientist.

Could it be you in 2016?

Analytical science has been at the heart of many scientific breakthroughs that have helped to improve people's lives worldwide. And yet analytical scientists rarely receive fanfare for their humble but life-changing work. The Humanity in Science Award was launched to recognize and reward analytical scientists who are changing lives for the better.

Has your own work had a positive impact on people's health and wellbeing? Details of the 2016 Humanity in Science Award will be announced soon.

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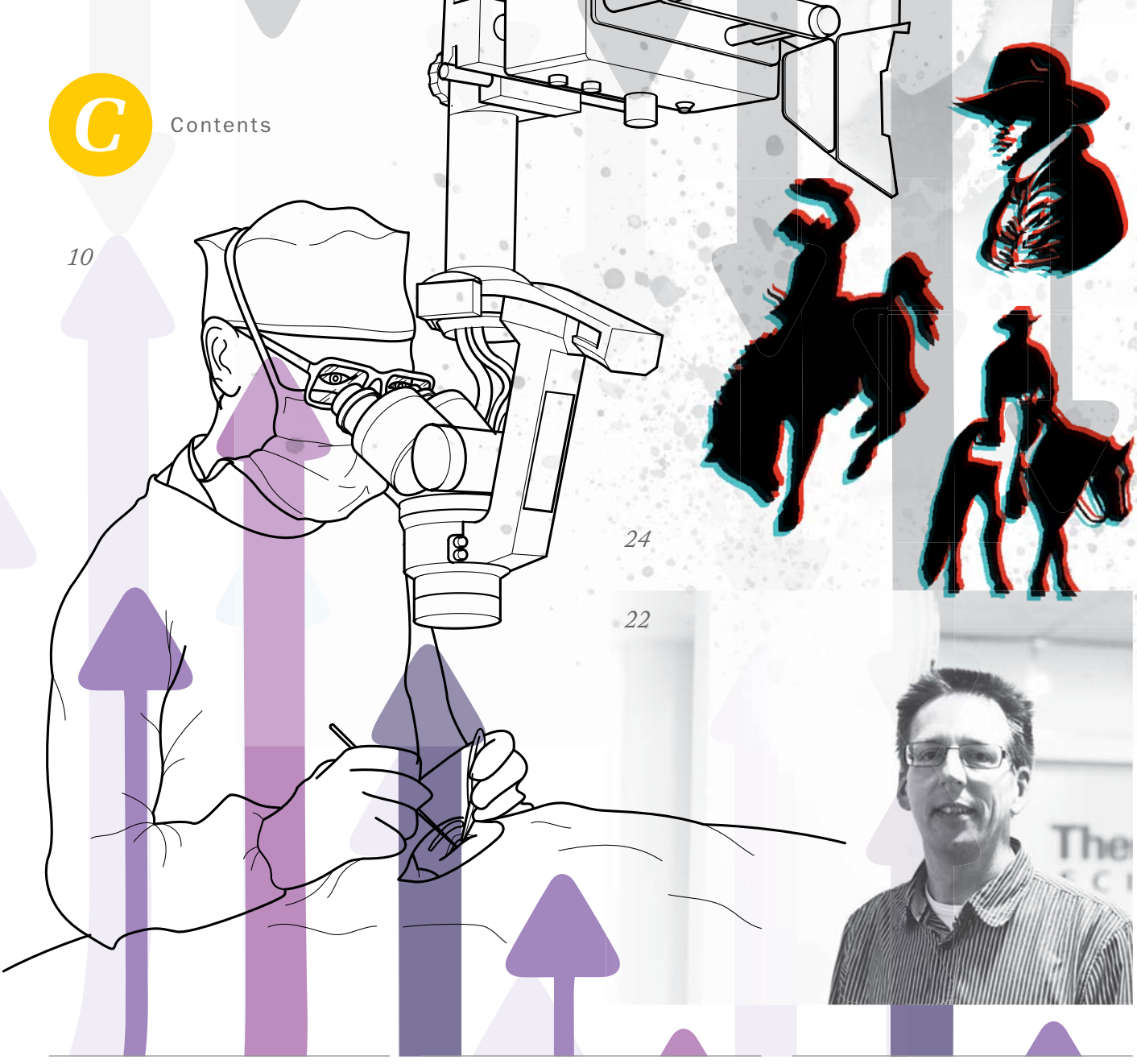

On The Cover

Everyone watches as multidimensional chromatography heads down to Texas, USA.

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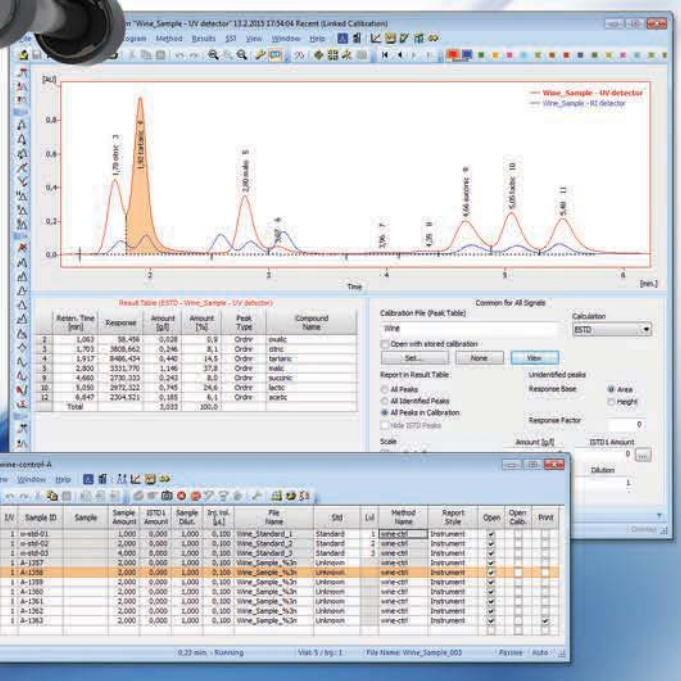

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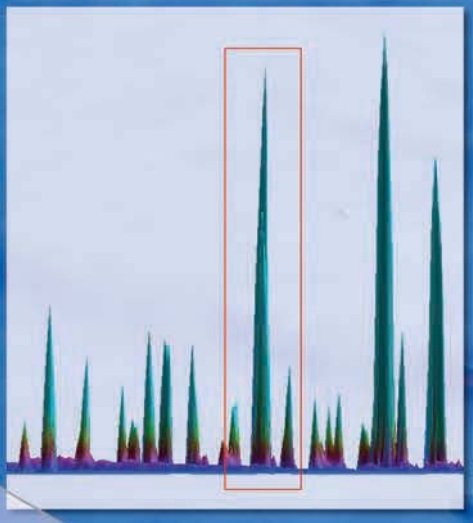

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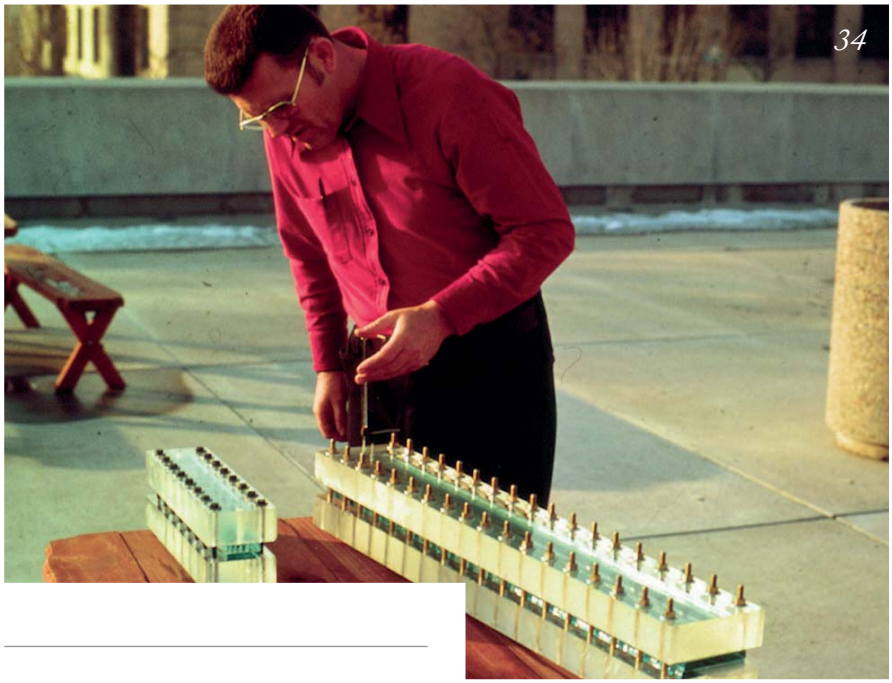

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the Analytical Scientist

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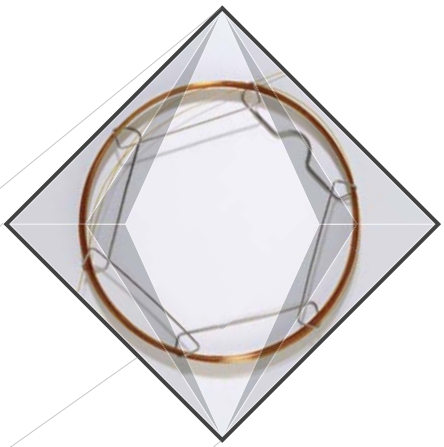

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Countering Commoditization

The moment we start taking technology for granted, we risk stifling future innovation and creativity.

As a musician, I've long been interested in the impact of commoditization on parts of life that should not be subject to simple (or complex) rules of economics. The moment music was digitized and freely shared, something changed. The value of recorded music started to erode. For a time, the awe and wonder of having 10,000 tunes in your pocket buoyed the music industry, which scrambled to clamp down on illegal music sharing services. Today, companies such as Spotify grant unlimited access to music on a (low cost) subscription basis. Other media faces a similar situation. Simply put, creative content is no longer scarce, and its value has been diminished accordingly. Why spend $10 on a Blu-ray disc or CD – or a downloaded copy for that matter – when you can spend the same amount on unlimited high-definition or high-fidelity streamed content from a "service" provider. Of course, new releases still garner interest, but as profits erode, how will it affect the creative process – or innovation?

Luckily, from a musical perspective, live performances are the new "premium" content – after all, you can't digitize an experience... yet. Clearly, we do recognize value in certain aspects of music...

Yesterday, I spoke at length with John Yates – a proteomics pioneer based at The Scripps Research Institute (if you didn't see our first Power List). We touched upon the demise in recognition for the importance of proteomics and mass spectrometry – the essential workhorse technology – in certain studies. Why, he asked, are certain technologies or techniques openly acknowledged in research papers when others are not? The answer is perhaps commoditization. To quote Wikipedia (commoditized knowledge anyone?): "Commoditization is defined as the process by which goods that have economic value and are distinguishable in terms of attributes (uniqueness or brand) end up becoming simple commodities in the eyes of the market or consumers." Though it seems strange that this should or could happen to a field that is by no means mature, John clearly recognizes a problem and is fearful that the lack of recognition for proteomics could send it on a downward spiral from a funding perspective.

Look out for a feature on the life and work of John Yates in an upcoming issue. In the meantime, may I suggest that we reconsider treating technology, techniques, processes – and creativity – as commodities and give them the recognition that they deserve, lest we stifle the next generation of pioneers?

Rich Whitworth
Editor

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:
rich.whitworth@texerepublishing.com

Surgical Spectroscopy: the Race is On

Researchers working on Raman spectroscopy based surgical tools sprint towards clinical applications.

The teamwork of surgeons and analytical scientists became a hot topic in our February issue (tas.txp.to/0215/precisionmed). From the efforts of the Maastricht MultiModal Molecular Imaging Institute to technologies, such as the iKnife and Verisante's 'parking sensor' technology, it seems that the race is on to bring analytically-enhanced tools into clinics and operating rooms, particularly those tools that can differentiate between cancerous and normal tissue. Now, two more research groups have published work describing Raman-based technologies and how they can potentially aid cancer removal.

Skin Cancer Spectroscopy

At Florida Atlantic University, a team of researchers has focused on non-melanoma skin cancer (1). The treatment of choice at the moment is Mohs micrographic surgery (a specialized excision), but the researchers argue that this is not always necessary or feasible. Their suggestion is to combine $C0_2$ laser ablation (see Figure 1) with Raman spectroscopy. Laser ablation offers precise tissue removal, with the potential for less scarring and almost bloodless surgery, but it is difficult to confirm if all the cancerous tissue has been removed. Raman spectroscopy, however, could be used in situ following partial laser ablation.

The study has been a success, with the team developing a spectral classification model based on principal component analysis and binary logistics regression that could correctly identify squamous cell carcinoma tissue with 95 percent sensitivity and 100 percent specificity following partial laser ablation. The group hopes the work will clear the way to bringing guided laser-ablative procedures into the clinic. Andrew Terentis, lead scientist on the study, tells us more.

How did you get involved in this work?

I have to credit Hugh Beckman (co-author on our recent paper) for the original idea of combining laser ablation with Raman spectroscopic diagnosis. Hugh is an ophthalmologist who has worked with various forms of laser treatments over the years. He'd heard about Raman spectroscopy and wondered whether it could be useful when combined with laser ablation. He searched for a Raman spectroscopy expert that could test the feasibility of this idea and eventually came into contact with me. Since my group was already working on the use of Raman spectroscopy to diagnose skin cancers, it was natural for us to pursue this project. John Strasswimmer is the other crucial partner in this research since he is the clinician that provides the patient access and skin cancer specimens we need for study.

Any outstanding work from other groups?

We are familiar with the excellent work being conducted by the groups in Canada, which you covered in February (tas.txp.to/0415/surgical). I also believe that Gerwin Puppels' group in the Netherlands has done transformative work in the area of adapting Raman spectroscopy for use in surgery. In the UK, Ioan Notingher's group has also done some nice work with using Raman spectroscopy to diagnose skin cancers. There are other groups as well, but these are the ones that stand out for me at present.

What are the main challenges?

The real challenge is yet to come: developing the technology into something clinically useful for the future. Raman spectroscopy is useful because it provides

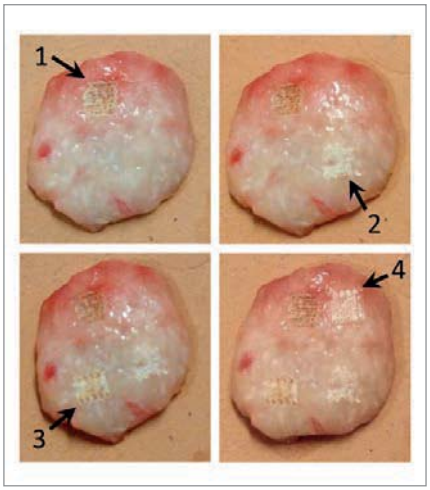


Figure 1. Squamous cell carcinoma showing different areas of ablation treatment (numbered). Treated areas were probed with laser Raman spectroscopy to assess the residual tissue. The ablation laser works in a grid pattern and pictured in the photos are the results of four different ablation treatment levels on 5mm^2 treatment areas. Area 1 was treated with the highest amount of total energy, followed by 3, 4, and 2.

a lot of structural information – a "fingerprint" pattern of the biochemical content of the tissue. However, normal Raman spectroscopy is a comparatively low sensitivity technique and it does take a long time to scan a large area of tissue. If this is to be implemented clinically in the future, the speed of real-time Raman data acquisition and computer processing of the data needs to be improved and optimized. Thus, there are a multitude of engineering as well as computational challenges involved.

What does the future hold?

We are currently consulting with possible industry partners that can work with us to develop a clinical prototype.

And although more technologically challenging, the treatment of any type of internal cancer using robotics to guide the ablative/cutting laser and a Raman probe to differentiate normal from cancerous tissue could be feasible in the future.

Probing Brain Cancer

Meanwhile, researchers at the Montreal Neurologic al Institute and Hospital, McGill University and Polytechnique Montréal, have focused their work on an intraoperative probe for use during brain surgery designed to detect cancer cells at cellular resolution and inform surgeons whether the removal of cancerous tissue is complete (2). The probe relies on Raman spectroscopy and is about to enter a clinical trial in Montreal. One of the study's senior authors, Kevin Petrecca from the Brain Tumour Research Centre at Montreal Neurological Institute and Hospital, is already using the probe routinely. Frederic Leblond, co-senior author and Professor in Engineering Physics at Polytechnique Montréal, answers our questions.

What is fueling recent interest?

The development of Raman spectroscopy as a diagnostic tool for cancers and other tissue abnormalities has held great promise for decades, but the field has been prevented from reaching its full clinical potential by significant technical challenges. The Raman effect that allows us to use optical properties of tissue to distinguish pathologies is very subtle, requiring very sensitive instrumentation, long acquisition periods and advanced classification algorithms. Until now, the use of Raman spectroscopy has been limited to pre-screening diagnostics, pathology samples and animal models.

How did you get started?

Grade 2-4 gliomas are inherently invasive cancers. As the decreasing gradient of cancer cells invades the brain, it is not possible to distinguish a boundary between cancer and normal brain, leading to local recurrence in around 85 percent of cases.

Our group has extensive prior experience in the development of image-guided neurosurgical techniques, including magnetic resonance imaging and fluorescence, and the limitations of existing technology prompted us to consider newer, more specific and sensitive molecular detection techniques. This led us to investigate the integration of Raman spectroscopy into the neurosurgical workflow.

Cancer is a very heterogenous disease and specific and sensitive tissue classification requires techniques that are able to highlight several molecular processes simultaneously. Raman spectroscopy potentially allows the identification and quantification of a very large number of molecular species and represented an excellent candidate for brain tumor detection. We have now already been able to demonstrate clinical proof-of-concept using the probe intraoperatively – in vivo - for over 30 patients with varying tumor grades and types.

The probe is also easy to use not only because of its small footprint but also because inelastic scattering signal detection is in real-time taking a fraction of a second, i.e., 200 milliseconds. Moreover, the tissue volume that is interrogated by the probe is approximately 0.1 mm^3, which is consistent with the volumes of tissue removed with the microsurgical dissection techniques used in neurosurgery.

Could the probe be used in other types of tumor surgery?

Yes – we have data to demonstrate that this technology is not restricted to gliomas. It can be adapted to function in various clinical settings and can be used to identify different pathologies in real-time.

References

1. *S.A. Fox et al., "Raman Spectroscopy Differentiates Squamous Cell Carcinoma (SCC) from Normal Skin following treatment with a high-powered CO2 laser," Lasers Surg. Med., 46, 757–772 (2014).*
2. *M. Jermyn et al., "Intraoperative brain cancer detection with Raman spectroscopy in humans," Science Translational Medicine, 7 (274) (2015).*

Beer Ahoy!

When bottles of beer from the 1840s were found on a shipwreck, there was only one thought on the minds of analytical scientists: what chemicals do they contain?

Scientists from the Technical Research Centre of Finland (VTT) and the Technical University of Munich have analyzed two bottles of beer found in the cargo of a shipwrecked schooner, which is estimated to have sunk in the Baltic Sea in the 1840s.

"In many ways, the beers were similar to modern beers," says Brian Gibson, a senior scientist at VTT and one of the study leaders. "They clearly contained malted grain and hops and were fermented with Saccharomyces yeast. They appeared quite clear suggesting some form of filtration, and the presence of iso-α-acids indicated that they had been boiled prior to fermentation, as is the case with all modern beers."

Gibson and his colleagues performed a number of analyses on the beers using a variety of chromatography and spectrometry techniques (1). No live yeast cells were found in the bottles, but they did find live, mainly lactic acid bacteria, which are typical beer contaminants. "These bacteria had apparently survived for 170 years without any additional source of nutrition. These long-lived strains are currently being studied at VTT and we hope to learn more about their biology and in particular to understand how they survived for such a long time. This information will hopefully help us to more effectively control brewery contamination levels in the future," says Gibson.

Find out more in our infographic. *SS*

Reference

1. *J. Londesborough et al., "Analysis of Beers from an 1840s' Shipwreck," J. Agric. Food Chem., 63 (9), 2525–2536 (2015).*

CSI: Hair Dye

Can surface-enhanced Raman spectroscopy (SERS) help catch vain criminals?

Hair is commonly found at crime scenes and DNA analysis can certainly help identify suspects. But DNA analysis of hair is limited to samples with intact bulbs and the technique is time consuming, not to mention that there are always hundreds of thousands of samples waiting to be processed...

Looking for alternative methods, researchers from Northwestern University, Illinois, USA, believe that surface-enhanced Raman spectroscopy (SERS) could be used, at the very least, to help pin down suspects that use artificial dyes. SERS can be used directly at crime scenes using portable units to detect hair that have been dyed, as well as exactly what brand was used (1). Gold nanoparticles adsorbed on hair amplify Raman signal from dyes, which are present on the hair surface. Dyes with different chemical structures yield different SERS spectra, which can be considered as 'dye fingerprints'. Since SERS is non-destructive, samples could still be subject to DNA analyses in the lab.

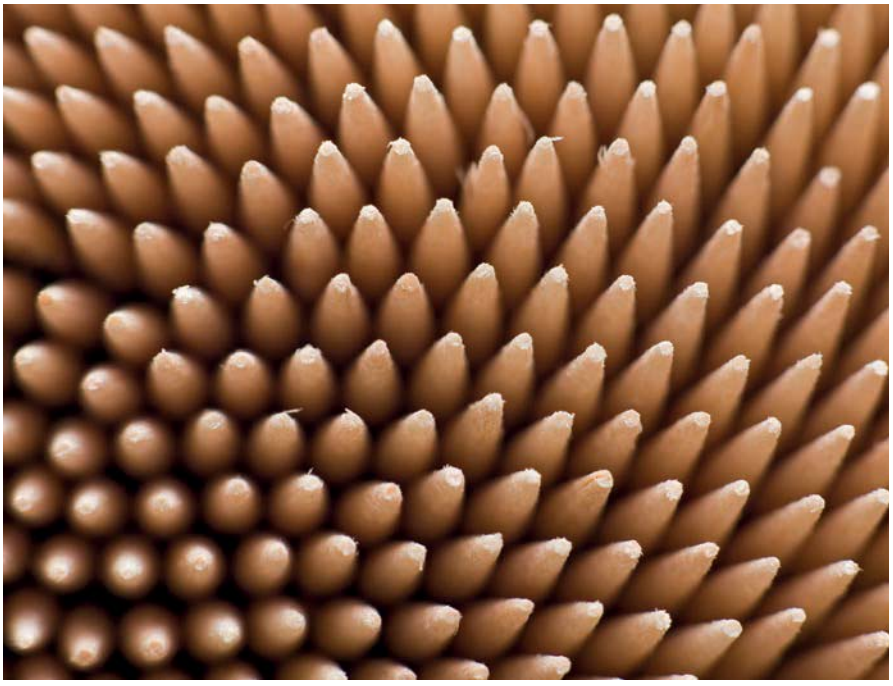
"The technique is so precise that we can even identify distinct brands of dye that were used to color hair. Moreover, only a few microns of hair is required for this analysis," says Dmitry Kurouski, lead author on the paper. "It was previously shown that SERS can detect chemical analytes down to the single molecule level. On average, people who artificially dye their hair apply the dye every two month and we expect that artificial dyes can be detected with SERS on hair that was colored more than four weeks prior to the analysis."

Besides the actual dye, commercial colorants contain numerous ingredients, such as lauryl, cetearyl, myristyl, and stearyl alcohols, fragrance, sodium sulfide, ammonia, and detergents. SERS can selectively see the dye rather than pick up signals from all the additional components. At the moment, the difficulties with DNA analysis lead many forensic investigators to use a microscopic to visually compare hair from crime scenes to known samples, which isn't always conclusive. Liquid chromatography and mass spectrometry can also be used to detect warfare agents and numerous drugs in hair, but they destroy the sample in doing so.

Richard Van Duyne, the discoverer of SERS and co-author on the paper, adds, "Next, we will explore uncharted territories of potential applications of SERS, as well as study the fundamental principles of the technique. Almost all things that surround us contain dyes. SERS can also be used to detect dyes in food products, plastics, metal and wood paints. For example, SERS analysis of car paints could potentially help in police investigations of car incidents and crashes." *SS*

Reference

1. *D. Kurouski and R. P. Van Duyne, "In Situ Detection and Identification of Hair Dyes Using Surface-Enhanced Raman Spectroscopy (SERS)", Analytical Chemistry, DOI: 10.1021/ac504405u (2015).*



MS Toothpicks

Can wooden toothpicks be the key to improving detection of drugs of abuse using mass spectrometry?

A group of researchers sponsored by Hong Kong's Beat Drugs Fund have demonstrated that a technique called "wooden-tip electrospray ionization mass spectrometry" can be used to detect four common drugs of abuse in biological fluids, such as urine and oral fluid. Current methods already exist for detecting drugs of abuse, but the group say these are time consuming (involving a preliminary screening to deal with a large number of samples, followed by the confirmatory analysis) and can generate false results.

"Real-life biological samples are complex and can contain a large number of various compounds, thus detection of target analytes could be susceptible to severe matrix interference," says Zhongping Yao, an associate professor in the Department of Applied Biology and Chemical Technology at The Hong Kong Polytechnic University, and one of the lead researchers on the work.

"Electrospray ionization mass spectrometry (ESI-MS) is a commonly used analytical technique, but it often requires extensive and time-consuming sample preparation and chromatographic separation steps for sample clean up and reduction of chemical complexity prior to ESI-MS analysis. In the last decade, various efforts have been made to enable direct analysis of raw samples using ESI-MS-based methods. We reasoned that as a wooden tip has a hydrophilic and porous surface, it could act as a medium for absorbing common polar interfering substances. In addition, wooden tip is a solid substrate, thus not vulnerable to the clogging problem encountered in conventional capillary-based ESI-MS."

The reasoning led to the development of wooden-tip electrospray ionization mass spectrometry (WT-ESI-MS). It makes use of normal wooden toothpicks for loading and ionization of samples. A wooden tip is directly mounted to a nano-electrospray ionization mass spectrometry source and connected with a high voltage for ionization of samples (see image). When a raw urine or oral fluid sample is loaded onto the wooden tip surface, drug analytes in the sample can be ionized and detected with mass spectrometry, without the need for sample pretreatment and chromatographic separation. A sample can be analyzed within a few minutes.

Yao adds, "Interestingly, the dimension of commercial wooden toothpicks is compatible with the commercial nano-ESI ion sources, so WT-ESI-MS requires no hardware modification to the mass spectrometers and can be easily adopted by various users. It is encouraging and surprising that the analytical performance, including limit-of-detection, linear range, accuracy and precision of our technique is comparable to conventional mass spectrometric methods for drug analysis."

Initially, the technique was developed to detect ketamine and its metabolite norketamine in urine and oral fluid, but this has now been extended to methamphetamine, MDMA and cocaine. "So far, it can only be used in the laboratory and it's not a silver bullet as it's not working very well for some drugs, such as cannabis and heroin," says Yao. "At the moment, we're working to develop more sensitive methods with different sample loading media, including surface-modified wooden tips and solid phase microextraction tips."

Eventually, Yao envisions integrating the technique with a portable mass spectrometer to develop a device for on-site drug analysis. And he doesn't intend to stop at drugs; he believes that the technique could also be applied for direct analysis of various raw samples, including clinical, pharmaceutical, food and environmental samples. *SS*

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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
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Greening Analysis with SFC

Supercritical fluid chromatography started out as anything but green. The road has been bumpy, but the modern technique cannot be ignored from an environmental perspective – despite its slight identity crisis.

By Eric Francotte, Novartis Distinguished Scientist and executive director in Discovery Chemistry at the Novartis Institutes for Biomedical Research, Basel, Switzerland.

Supercritical Fluid Chromatography (SFC) was first applied in 1962 using exotic supercritical fluids for a very specific application. Back then it was certainly not green; it used fluoro-chlorinated organic solvents that are now banned because of their negative impact on the atmospheric ozone layer.

Today, the great majority of SFC applications use carbon dioxide (CO_2) as the supercritical fluid and most users implicitly mean SFC with CO_2 when they refer to the technique. There is also a debate about the correctness of the term SFC, as – in practice – conditions are probably often subcritical rather than supercritical (see Tea With Pat Sandra: tas.txp.to/0415/patsandra); however, this debate does not affect the green aspects of the technique.

Although CO_2-SFC has been used for decades, it is only recently being recognized as a real alternative to classical chromatography (normal or reversed phase). There are several reasons why the technique has not had a smooth ride over the last 30 years, among them: (i) the lack of robustness of the instruments, (ii) the cost of CO_2 delivery infrastructure, (iii) the limited interest in environmental considerations, and (iv) regulatory constraints in some countries.

Now that our society is becoming more aware of environmental concerns – global warming, wasting energy, the production of greenhouse gases – there is a consensus that we must reduce our reliance on combustible fuels of fossil origin and protect our environment. SFC can contribute to this objective even though it may seem paradoxical given that modern SFC does not seem green because it uses CO_2 as the mobile phase. But actually, SFC does not produce CO_2 – it makes use of available CO_2 which is a byproduct of various industrial chemical and biological processes.

The re-emergence of SFC was driven by the particular application of chiral separations, which are mostly performed under normal phase conditions. The switch was relatively easy; SFC is also a normal phase mode of chromatography and most solvents used in chiral HPLC are alkanes, which have similar chromatographic and physical properties to those of supercritical CO_2. The switch has allowed users to diminish the amount of organic solvent used for chromatographic chiral separations by about 60 percent, not only reducing the direct emission of organic solvent into the atmosphere but also reducing the amount of organic waste that has to be burnt, producing additional CO_2. Moreover, for preparative applications, in which the amount of organic solvent to be evaporated is considerably less, the

"The switch has allowed users to reduce organic solvent usage for chiral separations by about 60 percent."

energy for evaporation of the fractions is significantly reduced. In this respect, CO_2-SFC can be considered as a successful example of 'green switching'.

The argument has motivated interest in the application of SFC as an alternative to reversed phase chromatography (RP-HPLC), which uses acetonitrile as the most common organic solvent component. In reality, for reversed phase chromatography, there were additional factors, including the high toxicity of acetonitrile, which rapidly metabolizes to cyanhydric acid after inhalation or skin penetration. It has now been demonstrated that SFC can also replace RP-HPLC in about 75 percent of the applications related to small molecule purification. The application range has dramatically expanded within the last two years and now covers drug molecules and intermediates, natural products, metabolites, small linear and cyclo-peptides, pesticides, lipids, fatty acids, carbohydrates, steroids, hormones...

The application diversity is rapidly growing for analytical purposes in classical small molecule analysis, such as drug analysis, bioanalysis, drug abuse, food and perfume industry. It goes without saying that this green switch has been made possible thanks to the high dedication of a number of instrument manufacturers.

SFC use at the preparative scale for purification is also attracting more and more attention. Even though the green impact of the switch is smaller for RP-HPLC compared with normal phase applications (the amount of organic solvent is only partially reduced because acetonitrile is replaced with methanol), it still consumes about 20 percent less organic solvents on average. Moreover, in RP-HPLC preparative applications, the evaporation of aqueous fractions by lyophilization is an energy consuming process that requires about seven times more energy compared to fractions produced by the SFC approach.

Times have changed and, wherever possible, SFC should be the preferred technique considering the incontestable environmental advantages and cost benefits. SFC is unlikely to become the universal separation technique, but where it does not yet fit the purpose, we should at least explore its potential.

Further use of CO_2-SFC at larger scale, pilot, production, or flash chromatography should be strongly encouraged. In short, CO_2-SFC can help make our world greener.

Why Not Excel in Data Analysis?

There are a plethora of useful software packages available to analytical scientists for processing experimental data and results. At the top of the list – or pretty darn close to the top – is Microsoft Excel. Why?

By Mark T. Stauffer, associate professor of chemistry, University of Pittsburgh at Greensburg, USA.

Excel can do a variety of mathematical and statistical operations, such as one- and two-way analysis of variance (ANOVA), multiple linear regression, and even some matrix functions, thanks to a slew of readily available functions that cover a wide range of mathematical, statistical, and organizational operations that analytical scientists perform frequently (or infrequently). Excel allows users to design macros to perform user-defined operations. Over the past decade, software innovations have enabled expansion of its many existing capabilities and introduction of new capabilities, such as in some areas of chemometrics.

Importantly, Excel will interface with many well-known software packages for data analysis, and a number of software packages have been developed to specifically interface with it to expand Excel's already wide range of data analysis functions and tools. In short, Excel possesses great versatility for analytical data treatment as well as the potential for further expansion of its many capabilities and is already one of the most widely used spreadsheet software packages on the planet.

I will be the first to admit that

Excel cannot do everything; however, it does have a wide range of existing data analysis capabilities, and is highly versatile and adaptable to allow development of user-defined routines for data analysis. Undergraduate and graduate students in technical programs will encounter Excel at some point during their studies and in the workplace beyond.

For example, I require students in my undergraduate analytical chemistry and instrumental analysis lecture and laboratory courses to use Excel extensively. It's great preparation for data analysis they might perform in the post-baccalaureate workplace. I have also introduced first-year students to Excel in their second-term general chemistry courses, and my undergraduate research students must use the software for the various types of data analysis they perform. Again, the focus is on the students' job functions in their future workplace roles.

There are probably numerous reasons not to use Excel, but I have to say it serves the purposes of many of us analytical scientists well and is in my humble opinion, an excellent (no pun intended) tool for performing many types of analytical data treatment. So, I ask: why not Excel?

Trust in Quantitative Analysis

The ageless wisdom, "imitation is the sheerest form of flattery," once again holds true. Unfortunately in quantitative analysis, imitation comes with real consequences.

By Richard C. King, PharmaCadence Analytical Services, LLC, Hatfield, PA, USA.

I am concerned about trends I see in analysis that stretch the definition of quantification. A mentor of mine once pointed out that quantitative analysis is binary. You know the distribution of values around the true value or you do not. You know the certainty with which a measurement represents the true value or you do not. Your analysis is quantitative or it is not.

The above definition places no restrictions on what the precision and accuracy are, it merely states that both accuracy and precision must be measured for an assay to be quantitative.

Quantitative chemical analysis is a vital part of our world. Rules and regulations that protect and preserve our natural resources and environment are based on quantitative analyses. Food we eat and medicine we take are determined to be safe based on quantitative analysis. Tests used by physicians to diagnose and treat disease and illness are based on quantitative analyses. Implicit in this testing and measuring is trust – trust in the value and validity of the measurements.

This trust is important because the majority of people making decisions and experiencing the consequences do not perform the analyses themselves. Instead, they place their trust in analytical labs who, in turn, trust each analyst to act with integrity at all times.

The importance of quantitative analysis has created a growing, undeniable demand for more and better chemical information all at cheaper costs with faster turnaround times. This requires decreased costs and increased number of measurements per unit time. The potential for growth as quantitative chemical measurements make their way from research labs into production labs, from specialty practitioners to technical users, creates a picture ripe with possibilities to improve everything from health to energy and the environment. We can take the next step and start imagining quantitative chemical measurements making their way into consumer and do-it-yourself markets, enabling innovations such as checking the purity of water in your water bottle and monitoring your own blood chemistries.

"The importance of quantitative analysis has created a growing, undeniable demand for more and better chemical information."

As with all visions of brighter futures, there are threats that must be addressed.

Today, there are two big threats to quantitative analysis and both impact the quality of data we generate. One encroaching trend that may erode the trust earned from our past successes

are imitations. That is to say, assays, methods and procedures that claim to be quantitative, but are not. "Semi-quantitative" is a term that makes little sense. Fit-for-purpose is a strange thing to think of as new: instrumental analysis that is not calibrated using the substance being measured cannot demonstrate accuracy, single points cannot demonstrate reproducibility, predictions and assumptions are not measurements.

It is up to all of us who practice quantitative analysis and who are trusted to provide valid, actionable results to reject these imitations.

The second threat to trust placed in quantitative chemical analysis is apathy among the people who make the measurements. If you design experiments, collect samples, conduct analyses, process data, or report results used in a quantitative sense to make a decision, you are being given an important trust.

Too often, I find people who neither acknowledge nor value this trust. Trust takes a long time and a lot of hard work to earn, but takes very little to lose. It is vital that the trust placed in our quantitative measurements be protected and nurtured. Nowhere is this more important than with the people performing the analysis. Nothing but the strictest integrity can preserve the trust earned by the prior success of quantitative analysis. The attention to detail, the effort put into excellence, and the recognition by all involved of the importance of each and every quantitative measurement is required to bring the bright future we can imagine into reality.

"It is up to all of us who practice quantitative analysis and who are trusted to provide valid, actionable results to reject imitations."

How to KISS (Keep it Simple, Stupid)

Consider designing the readout first when developing point-of-need assays.

By Scott Phillips, associate professor of chemistry, Pennsylvania State University, University Park, Pennsylvania, USA.

When I describe proof-of-concept results for a new point-of-need assay platform, people rarely ask about operational simplicity. Instead, they question me about cost, stability, mass production, and other issues that, which at least at the outset, are irrelevant to whether the assay is easy to perform and evaluate. In my view, these latter two metrics ought to be the highest priority at the proof-of-concept stage.

Ideally, a point-of-need assay will require only a single step by the user (adding the sample), function without specialized electronics or instruments, cost very little, provide sensitive and selective results in minutes, and give completely unambiguous readouts to anyone, anywhere, at any time. Rather than being a product of remodeling and simplifying existing assay platforms, perhaps this ideal type of point-of-need assay is achievable by first inventing new readouts for assays. After all, an assay that only a few trained individuals can interpret will have a much smaller global impact than an equally good assay with an output that is exceedingly simple to read and understand.

The need to improve the readout is especially true for quantitative point-of-need assays. The standard outputs of color, fluorescence, and electrochemistry are susceptible to contaminants in the sample that affect their sensitivity and reproducibility, and all require specialized electronic readers to enable quantification (specialized readers can be a deal breaker for end users in some point-of-need environments). New outputs, on the other hand, may offer new opportunities (1).

Distance-based measurements (for example, the distance that a colored sample travels on an assay platform) (2), bar-based measurements (for example, the number of bars that change color during an assay) (3), and time-based readouts are all emerging as alternatives to more traditional analytical signals for use in point-of-need assays (4, 5). Such readouts are simple and clear – the user need only compare distances, count colored bars, or measure time. They also take advantage of straightforward and familiar tasks, do not require specialized instruments to obtain a quantitative result, and are in the process of being generalized for a variety of classes of analytes.

Time-based readouts are exciting due to the high-resolution and accuracy of the quantitative measurement. Indeed, our research group has developed time-based readouts for paper-based assays, where the time for color to appear in one region of paper relative to another region correlates with the concentration of a target analyte. These time-based assays include sample pre-processing steps, signal amplification for trace level detection, and normalization of assays for effects of humidity and temperature on sample distribution rates in paper devices. The user does not need to know that these features exist, since he or she must only add a sample (of nearly any volume) to the paper, and then use a stopwatch to measure the time for two regions to become colored relative to one another. The assay is very easy to perform and the readout is straightforward. Moreover, the approach is compatible with detecting and quantifying small molecules, inorganic ions, enzymes, and proteins, and is capable of femtomolar detection limits. In addition, there are further opportunities for continually improving sensitivity through additional research.

Our time-based assay platform still is at the proof-of-concept stage, but it illustrates the new capabilities that can emerge when one designs the readout before developing the entire assay platform. For standard laboratory settings, it is logical to continue improving upon existing readouts and assay strategies. But the unique challenges associated with simple, inexpensive, point-of-need tests requires new – perhaps even backwards – approaches to designing assays. The concept "invent the readout first" is unconventional, but just might be backwards enough to circumvent intellectual or technological traps that often inhibit the development of effective point-of-need assays.

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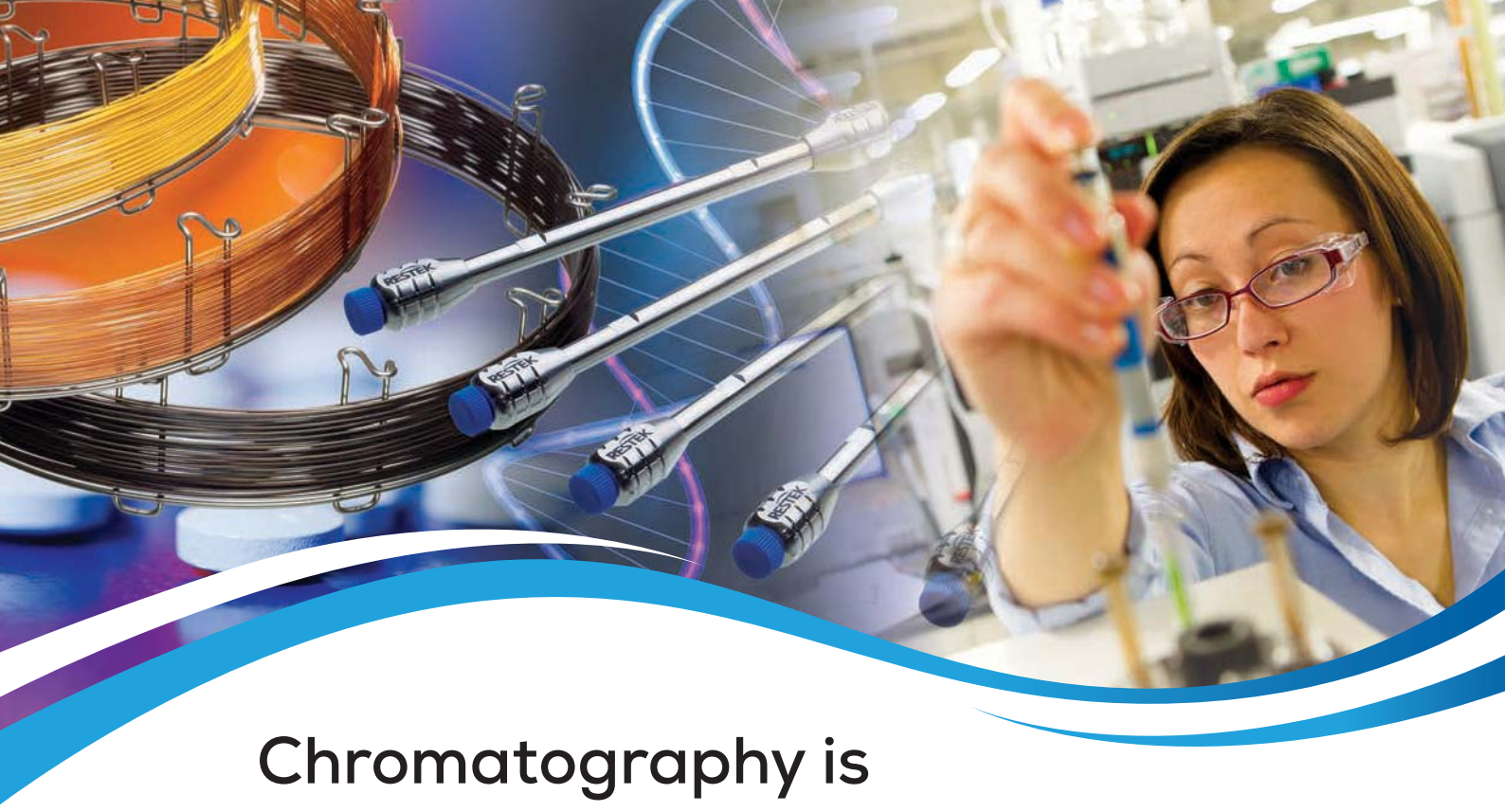

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The Frontier of Gas Chromatography

When I was asked to evaluate a brand-new instrument with disruptive potential in my field, I did not spend long thinking about the answer. Here, I share a little background and my first impressions.

By Hans Mol, Group leader Natural Toxins and Pesticides, RIKILT Wageningen UR.

I've been working for nine years at RIKILT-Wageningen UR in the Netherlands, predominantly working with the government on aspects of food and feed safety. For that reason, we are always interested in evaluating new instruments and techniques that can address the current – and future – challenges facing us. As such, I am very pleased to have a pre-production version of the much-anticipated GC-Orbitrap sitting on my lab bench...

Looking back, I've often been fortunate in finding myself at the cutting edge of GC.

My analytical journey really began when I did my masters project in Udo Brinkman's group at the Free University in Amsterdam – he was well-known and respected, and my interest grew. I continued onto a PhD at the Technical University in Eindhoven under professor Carel Cramers – a couple of years behind Hans-Gerd Janssen (who actually supervised my PhD). My research was very much focused on large volume injections (for residue analysis), using programmed temperature vaporizing (PTV) injectors. PTV is commonplace now, but this was in the early 1990s – and it was somewhat disruptive technology back then, competing with retention gap, on-column-type of large volume injections from other groups. We were pretty sure early on that, in routine applications for food and environmental samples, PTV would become the industry standard.

I finished my PhD in 1995 and continued on as a post-doctoral research working on GC coupled to both MS and an atomic emission detector (AED). I then worked for about 10 years for a contract research organization offering analytical services for food, (agro)chemical and pharmaceutical industry. Importantly, we did a lot of method development work on LC-MS, GC-MS – and myriad other techniques – and I gained a great deal of experience. And that brings me to RIKILT.

There have been many technological advances over the past 20 years or so. The availability of LC-MS for food and environmental analysis was a huge milestone. When I started, the field was very GC oriented. If compounds were not amenable to GC, we would use derivatization to make them amenable. LC was a last resort in some ways – until the commercialization of electrospray ionization. As the instruments became increasingly affordable (they were already in use in big pharma with its big budget) – they changed the field.

Another step change was the introduction of high-resolution MS (HRMS) techniques (time-of-flight (TOF) or Orbitrap instruments) to LC-MS; indeed, in certain applications these are now replacing triple quadrupole instruments.

But what about similar progress in GC? Much of the effort from instrument suppliers seemed to be focused on LC (remember the pharmaceutical industries big budget?) and GC – despite its utility in persistent organic pollutants and pesticides – was left behind. Until now.

I expect the new GC-Orbitrap instrument will count itself among the aforementioned milestones and redress the imbalance!

GC-Orbitrap Technology lands

Just this very week (at the time of writing), one of the first GC-Orbitrap instruments was installed in my lab. Ahead of installation, the space we created raised a few eyebrows with certain visitors (other instruments had to be relocated). Anticipation has been high and so keeping the secret has not been easy.

Previously, we had the opportunity to see the instrument at Thermo Fisher Scientific's operations in Runcorn, UK, and it looked very promising. And while it's still early days, I have high expectations – as do my colleagues, who have formed a relatively orderly line, samples in hand! Over the next few months, we will be putting the instrument through its paces.

The main challenge in my particular field is the sheer number of pesticides of interest – around 1400. The question is relatively simple: "are there any pesticides in this sample, and if so are they above the maximum residue limit (MRL)?" For targeted analysis, you can use a triple quadrupole MS system, but you're limited in terms of scope, because you are only measuring pre-defined compounds. If you want to look for something new or different, you need to go back to your sample and re-run the analysis.

Conversely, with full-scan methods, you inject your extract, measure the compounds of interest but have the option to look back into the raw data for other analytes. Moreover, the number of compounds that can be measured in a single run is much higher than a triple quadrupole. Using a dedicated triple-quad method, you can routinely target 100-150 compounds (instruments have improved here as well – shorter dwell times potentially allow a slightly higher number to be squeezed into a given method). But with full-scan analysis, you measure everything – and there are 700-800 pesticides that are amenable to GC. That's a gain we are excited about.

From a method development point of view, there are also advantages to full-scan analysis because the conditions can be quite generic. In fact, there's little optimization needed at this stage – that's addressed in the data handling. In contrast, in GC-triple quad methods, you have to set acquisition windows and if you want to add compounds you need to optimize the transitions for each of those compounds. In simple terms, it takes more time.

Hands on – first impressions

In terms of resolution, the GC-Orbitrap is clearly a major step forward, outperforming everything on the market. And so in Runcorn, we were more interested in assessing sensitivity and selectivity. We ran a calibration curve in a more difficult matrix (a leek sample) and were impressed by the sensitivity, which was actually better than the triple quadrupole instrument in our lab. However, our instrument is previous generation, so the next question was, how does it compare with the current generation of triple quads? Fortunately, we were able to perform that experiment in Thermo's lab, which had the two set ups side by side. For the analytes tested, comparable results were obtained.

Maintaining sensitivity while adding the full-scan capability (and the advantages that come with it) is a big plus point. Selectivity is equally important but, to be honest, I think that's much more difficult judge – we need to run more samples and look at more analytes to form a fuller picture on how HRMS compares with MS/MS, which also has limitations, especially in terms of electron ionization (fragments of fragments become less and less specific after all).

Complex samples, such as food supplements, are perfect to test the true capability of GC-Orbitrap. Feed ingredients are also very complex (essentially they are manufactured from any food industry output that holds nutritional value but which cannot be used for anything else). Traditionally, such samples present real challenges in terms of detection limits, demanding more attention and time on sample preparation and method development. Broadly speaking, the GC-Orbitrap will help; we can use fewer methods because of the selectivity, and the sensitivity will allow us to reduce injection volumes (from around 5µl down to 1µl) or to use less concentrated samples. By introducing fewer co-extractants in this way, we can reduce deterioration in GC performance.

One of my colleagues works on forensic-style analysis and has expressed particular interest in the GC-Orbitrap. The samples in these 'cold cases' are 'suspect' but we don't know why – has something toxic been added at some point in the supply chain? Alternatively, there may be a dead animal and a big question mark. Different procedures apply in this field because the analysis needs to be as unbiased as possible. Samples must be screened and then cross-referenced against very large NIST libraries to find a match. Alternatively, comparative analysis against known reference products can be useful to assess which samples are deviating from 'normal' by overlaying profiles and identifying suspicious peaks. Up to now, this type of work is being done with comprehensive GC (GC×GC) with a nominal mass (low-resolution) MS system. We are very interested in the potential of doing the same analysis using one-dimensional GC coupled with high-resolution (Orbitrap) MS.

Surveying a changing landscape

I'm not one to make sweeping predictions, but I expect that targeted methods with triple quads will be phased out as time goes on. Full scan instruments are just as capable – and even if you don't get sufficient selectivity, with Q-Orbitrap or Q-TOF you have the ability to do MS/MS as well. At a certain point, the question will become: why do I still need a triple quadrupole instrument? I can only think of one reason: its highly stable quantitative performance – and that's another area I am very interested in exploring with the Orbitrap.

Will the transition from triple quadrupole methods happen overnight – or in five years? Well, even if the instrument far exceeds all our expectations, there will be a considerable lag in wider adoption. After all, our lab is working at the cutting-edge – we're much quicker to evaluate and embrace the great and the good. In more routine analysis, extra time will be required for general acceptance – and established procedures must be challenged and changed. After all, the GC-Orbitrap is something very new and different indeed.

Video interview with Hans Mol:
tas.txp.to/0415/HansMol
To find out more:
thermoscientific.com/HRAMGCMS

All Eyes on Multidimensional Chromatography

Ahead of ISCC & GC×GC 2015 symposium, we bring together a posse of multidimensional ranchers for a glimpse of what's going on in the field – and what to expect at the Texan conference.

the new sheriff in town

Daniel Armstrong, Chair of the ISCC & GC×GC 2015 symposium and Robert A. Welch Chair in Chemistry at the University of Texas at Arlington explains the broad significance of multidimensional techniques and the value of riding down to Texas in May.

It has been recognized for decades that the capability of multidimensional separations – in terms of peak capacities and the analysis of complex samples – is unsurpassed by other analytical approaches. Early on, planar chromatographic and later, slab gel electrophoresis methods were used to separate complex samples in space, as opposed to time. Indeed, the importance of uncorrelated or orthogonal separation systems and much of the basic theory regarding peak capacity, peak overlap and resolution were understood even before the advent of practical comprehensive chromatography technology. Over the last several years, proper hardware (especially effective modulators) plus the software and algorithms needed to handle, treat and display the enormous amounts of data involved have been developed. The comprehensive 2D approach, where the entire first-dimension separation is subject to the second dimension separation absolutely demanded the aforementioned advances in hardware and software. Today, multidimensional chromatography has advanced to the point where it has become a useful and often indispensible technique to the average practitioner.

It is probably not a coincidence that the rapid growth of multidimensional separations has coincided with the tremendous expansion in "omics" research. Most analytical journals are currently publishing numerous papers involving proteomics, genomics lipidomics, foodomics, petroleomics and so on. In some fields, environmental or petrochemical for example, complex samples are best analyzed by comprehensive GC. In other areas, comprehensive liquid-based separation methods are required – genomics or proteomics, for example. In many cases, the method of detection (mass spectrometry, diode array, etc.) adds yet another dimension to the analysis, further enhancing the amount of information obtained.

Comprehensive GC became a practical commercial method well before comprehensive LC – or any other combination of liquid-based techniques. Indeed, comprehensive GC can make use of several different effective modulation approaches and the software and data handling capabilities have evolved to the point that they can nearly be considered user friendly (or not, depending on the individual you are talking to). We can expect the same advances to be introduced for comprehensive liquid systems as time passes.

And so, from May 16, 2015, Fort Worth will become the world's focal point for all forms of multidimensional separations, capillary and fast separations. The concurrent 39th International Symposium on Capillary Chromatography & 12th GC×GC Symposium will provide an exceptional venue for basic education in the area of comprehensive separations with short courses on GCxGC, ionic liquid stationary phases for GC, and sample preparation methods. Leading researchers will present their latest work in all areas of comprehensive separations. Instrument and column manufacturers will present "lunch included" workshops for interested scientists. And the work of many young scientists will be featured and there will be scientific poster awards. Moreover, it couldn't be easier to get to.

Last but not least, no one will want to miss the exciting plenary lectures. George Whitesides will present "Simple/Low-cost Bioanalysis: Health-care in the Developing World" and Sandy Dasgupta will share his "Mission to Mars: A Chromatograph for Extraterrestrial Explorations". We hope to see you there!

The 39th International Symposium on Capillary Chromatography & 12th GC×GC Symposium will take place May 16–21 in Fort Worth, Texas.
For more information, visit: www.isccgcxgc2015.com

wrangling orthogonality in multidimensional chromatography

By Michelle Camenzuli, van 't Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands.

The quest for greater separation power, in terms of peak capacity, has inevitably led to the development of multidimensional chromatographic techniques. However, the increase in separation power comes with a cost: more time and/or effort is required to fully optimize multidimensional chromatographic systems. Much attention has been given to developing protocols and techniques for optimizing instrumental aspects, particularly modulation between the separation dimensions and the optimization of gradient elution profiles to increase selectivity. And though it is clearly important to improve our understanding of these aspects, it is arguably equally important that we understand and appreciate the role of orthogonality.

The concept of orthogonality refers to the combination of separation modes – or selectivities – that are independent from each other in terms of their retention mechanisms. The use of independent separation mechanisms allows different properties of the sample components to be exploited thus improving our ability to separate them. Truly orthogonal two-dimensional systems provide the potential to gain access to the theoretical maximum possible peak capacity as given by the equation from Giddings (1):

$$^{2D}n_c = {}^{1}n_c \times {}^{2}n_c$$

In practice, a number of factors prevent us from reaching the "Holy Grail" of theoretical maximum peak capacity, and so variations on the above equation have been proposed to take such factors as dilution during modulation and difficulties in coupling truly orthogonal selectivities into account. However, such discussions are outside the scope of this article. The "take-home" message here is that to move towards the theoretical maximum, we need orthogonal selectivities.

The consequence of combining similar (or worse, identical) selectivities is that sample components will cluster along the diagonal of the separation space and thus not utilize the full peak capacity potential. Therefore, we must understand orthogonality and consider it fully during optimization. Orthogonality metrics are useful for this, particularly in industry, where method development is often guided by the need to achieve specific values for system suitability parameters. The problem lies in the choice of orthogonality metrics. I have read of numerous instances where an analyst has not fully understood the concept of orthogonality and has used the wrong metrics, leading to a biased measure of orthogonality. Even worse is when an analyst makes an incorrect claim of orthogonality – for example, stating that IMS is orthogonal to MS in IMS/MS. That is simply not true. Both IMS and MS involve a mass-based separation despite the fact that IMS also separates in terms of shape. These separation mechanisms are correlated and therefore not orthogonal, which is why peaks in an IMS/MS spectrum cluster along the diagonal.

Many orthogonality metrics exist and unfortunately there is inadequate space here to review them fully; however, the criteria I personally use to determine the best metric are as follows:

1. Easy to implement
2. Relates physically to the concept of orthogonality. That is, it should directly link to the concept of orthogonality; not model or describe orthogonality based on descriptors that do not make physical sense
3. Freedom from user bias
4. Independent from the number of components within the sample
5. Should be precise
6. Clustering of peaks around the upward leaning diagonal is not assumed
7. The metric should be insensitive to region where there are no peaks
8. Should describe the separation in question (not probe solutes).

In my opinion, there are only two orthogonality metrics that come close to satisfying these criteria completely: the bin counting

methods developed by Gilar, Stoll and colleagues (2, 3) and the recently introduced asterisk approach, which I developed with Peter Schoenmakers (4). The main advantages that the asterisk approach has over the bin counting methods is that it is not necessary to know the number of sample components beforehand and it is easy to implement. Conversely, the advantage that the Gilar, Stoll bin methods have currently over the asterisk approach is that they are applicable for samples containing less than 25 components.

Whatever the multidimensional separation technique used, it is essential that we fully understand orthogonality and choose an appropriate metric to guide our method development.

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reining in multidimesional data

By James Harynuk, University of Alberta, Canada.

My group is working on several new things for GC×GC right now. Firstly, we have added in peak width predictions to our retention time predictions. Importantly, obtaining the data to make these predictions can be done at the same time as obtaining the data to get the thermodynamic parameters for retention times. That development is very exciting because now we can start to work on optimizing separations automatically, which is something that you cannot really do if you do not know how wide your peaks are.

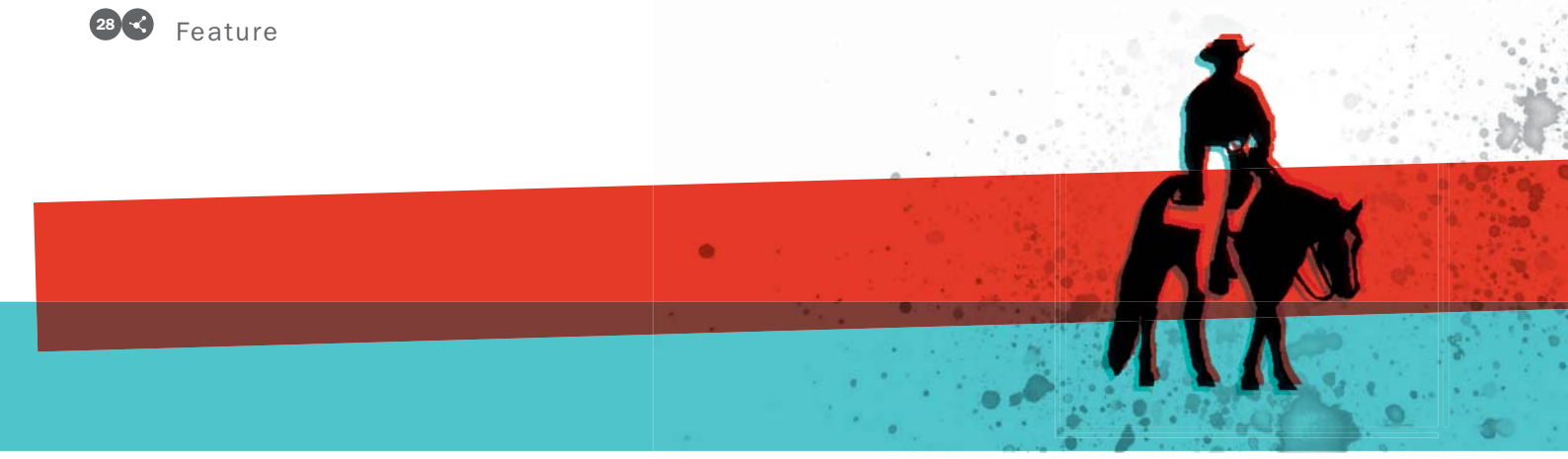
Our online website for doing these calculations (retention times only first – with the peak width module to come) will be up and available for general use soon. I'll admit that it has been "coming soon" for a long time. As many readers will know, there is always something more urgent that comes up, either in the lab or with the two co-stars in my photo. However, I really expect to have it up and fully running over the summer.

We are also working quite diligently at expanding our unique ion filter (which we published in Analytical Chemistry last summer) to two dimensions for handling GC×GC-MS data. There, the data reduction is well over 99 percent, and it really speeds up the data processing and model quality as opposed to when we use the entire raw data set. Notably, there are some dangers with the alignment algorithms in 2D when using this ion filter. When moving peaks around on the retention plane, spectra from different regions will be blended, possibly creating artefacts. We have some ideas about how to get around this, but we aren't quite ready to talk about those just yet...

Perhaps the most exciting thing for me right now is that we have one of the new vacuum-UV detectors from VUV Analytics to play with for a little while. It is a very interesting piece of hardware – and we are still working on what we can and cannot do with it in GC and GC×GC modes – for more on that, check out Kevin Schug's article (page 28). We will certainly have something to show (I don't know what yet) using this detector with GC and GC×GC at ISCC & GC×GC 2015.

A final thing that many users of GC×GC instruments will be interested to see from our group in Texas is our poster that shares how we redesigned the source for our Pegasus. Short version: ours was dirty and not tuning properly, we couldn't afford to replace the whole thing, so we broke it even more and then rebuilt it. The new version works great and we can clean it again if we ever need to.

I look forward to seeing you in Texas!



vacuum-uv lone star versus ms

By Kevin Schug, University of Texas at Arlington, USA.

Recently, a vacuum ultraviolet (VUV) detector for gas chromatography (GC) was introduced into the market, taking a top five spot in The Analytical Scientist Innovation Awards 2014 in the process. From the stand point of current detector technologies available to the end user, I believe VUV technology is the only worthy alternative to mass spectrometry (MS) when one wants to obtain both qualitative and quantitative information on analytes eluting from a GC column. VUV detection has already been demonstrated for use in analyzing petrochemicals, drugs, pesticides, fatty acids, permanent gases, and other various analytes that are GC-amenable (1, 2, 3). It is a universal detector. All chemical compounds absorb light in the 115 – 240 nm wavelength range probed by the detector, and to this point, all tested analytes have unique gas phase absorption features. Of course, the degree of absorption varies depending on the nature of chromophores on a given compound. The VUV detector is also very fast; it can record full wavelength-range spectra up to 100 Hz, making it quite amenable for use with GC×GC and fast GC applications.

So, the big question here: can VUV realistically compete with MS as the premier comprehensive GC detector? I honestly think it can. I believe it will take some time to understand the potential for de novo compound identification or classification, in the way that it is considered well accepted and commonplace by MS. Yet, the major difference between the two is that MS has a well-developed library; for the VUV detector, researchers are in the early stages of assembling that resource.

For a complete unknown, electron ionization – MS (EI-MS) can provide some significant hints regarding the functionality of the compound. But MS has never been considered a structural identification technique – it can only help aid and confirm assignments. Interpretation of EI-MS mass spectra is a practiced art, much like interpreting an NMR spectrum. It is arguable that VUV spectra provide similar fingerprinting capabilities, where various classes of compounds are accompanied by specific features in the measured absorption range. In fact, for a proposed structure, VUV spectra can be readily predicted using theoretical computations. Mainstream tools to calculate the EI-MS spectrum based on an inputted structure are hardly popularized and well known, to my knowledge.

Still, significantly more research will be needed to understand class- or substituent- specific features in VUV absorption spectra, and to assess whether these can interpreted for practical or routine use. Until this is established, the growth of a VUV spectral library will provide equally convenient use of GC for qualitative analysis as enjoyed by GC-MS users who rely heavily on their MS library.

Finally, VUV detection has the potential to address analytical problems where MS detection fails. MS can have notable problems for the analysis of some isomeric, highly labile, and very low molecular weight analytes. As we have continued to expand the application base of VUV, it has been proven to fill those gaps. Both VUV and MS detectors have excellent sensitivity. MS sensitivity can depend on operation mode (for example, selected ion monitoring in quadrupole) and the distribution of fragment ion intensities for a given analyte. VUV sensitivity depends on chromophores; aromatic

"So, the big question here: can VUV realistically compete with MS as the premier comprehensive GC detector?"

compounds have the highest absorption in the ~160–180 nm range. Our ability to apply spectral filters (projection of response based on a selected wavelength range) either pre- or post-run using VUV detection is largely akin to producing extracted ion chromatograms for MS. However, deconvolution of signals from co-eluting compounds is arguably more easily addressed by VUV detection; the general additivity of distinct high-resolution gas phase absorption spectra is a problem that is easily solved to determine contributions from individual components to an observed peak.

Overall, MS has many more years of development and application than VUV detector technology. I do believe that objectively comparing the two provides a nice perspective on some of the similarities associated with the information they provide. A closer look shows the potential where VUV can accommodate some applications that MS cannot. Perhaps the discussion comparing the two will ultimately devolve to price... There are arguments for both sides on that aspect, but I'll allow you to research that for yourself.

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rodeo champ

2015 Dal Nogare and Uwe Neue award winner Mark Schure is an old hand at 2D-LC – he's experiencing déjà vu while other riders are playing catch up.

For most of my industrial career, I've been a modeler and have had to deliver practical results. I could use any approach I thought would shed light on the problem and that generally entailed using a lot of computing power.

Two-dimensional LC (2D-LC) is just one of the many very interesting techniques that I used at Rohm and Haas. In fact, we unleashed a great many experimental techniques from size exclusion chromatography to matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to figure out polymer structure and quantitative aspects of block copolymers.

But for 2D-LC, I collaborated with two very good experimentalists (one of them was Robert Murphy) to develop the technique. This was in the early days for commercial applications, although polymer 2D-LC first appeared in the 1960s. I remember some of the initial work done in Canada was very interesting and it was all done by reinjection after collecting fractions. But people weren't investigating hundreds of samples, they were focusing on just one or two. We wanted to go beyond the primitive 2D-LC stage and get into comprehensive 2D-LC, where you take a fraction of the first dimension and shoot it into the second dimension; you keep doing this synchronically through the first dimension chromatogram. However, we had to work out how to do it first... and that entailed establishing the method and development cycle before getting into the sampling problem: how many samples do you take from the first dimension into the second?

One day I was sitting down thinking about the sampling issue, as it was uncharted territory. Could I develop a mathematical theory for it? I thought about it for several days and then I realized the essence of the problem was analogous to Nyquist sampling in signal processing, where you need to work out the number of samples you can take across a signal without losing fidelity. Applying this to 2D-LC, you have to discover how many samples you need across the first dimension peak to prevent losing fidelity in the second dimension, but without distorting the first dimension. I worked it out (it's relatively simple mathematics and I talked about this at Pittcon 2015). I explained that if you're simply trying to identify a sample, such as a peptide sequence using a mass spectrometer, then 2D-LC is very good at reducing the saturation in your chromatogram, and you don't have to sample as fast as you have to when using the technique for quantitative applications. You simply select some of the first dimension to go through to the second dimension, use a mass spectrometer as a peptide filter and use a database to identify the proteins.

Déjà vu – it's 2000 all over again

Interestingly, all of the early 2D-LC sampling work I did has cropped up again. At Pittcon, there was a talk on 2D-LC and the presenters said that they had a particular liking for papers on sampling and dilution, the latter being important because in 2D-LC you dilute samples by the product of the dilution factor in both dimensions. This multiplicative dilution causes detector sensitivity problems.

We published on this in 1998 and it is in fact the second most quoted paper after Michelle Bushey and James Jorgensen's work on computer-controlled 2D-LC.

There was a publishing hiatus after about 2000 – nothing else appears in the journals before 2010. So, I would say we were pioneers; we wanted to use 2D-LC for complex polymers and we needed to develop the basic science. Of course, application of techniques and developing the basic science was one of my jobs throughout my industrial career, the purpose being to give the company a competitive edge. Fortunately, we were allowed to publish technique-oriented papers so that we could enlighten others, although as you can see from the aforementioned dates, it took people a while to catch on to what we were doing...

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young guns: ionic liquids for gc×gc

By Jared Anderson, Iowa State University, USA.

Comprehensive two-dimensional gas chromatography (GC×GC) is a step up from standard GC and involves connecting two distinct columns each possessing stationary phases with different selectivities to achieve high resolving power.

My laboratory is interested in designing new generations of stationary phases that can be used for targeted analyte separations. In the pursuit of new stationary phase materials, there are a number of properties that must be met. The stationary phase must exhibit high thermal stability in order to provide low column bleed at high temperatures. This feature is particularly important when coupling GC to mass spectrometry detectors. The phase material should be chemically inert, ideally possess high viscosity, and be capable of effectively wetting the surface of fused silica capillary columns.

While the basic features above are important, the resulting separation selectivity is the most important property exploited by the separation scientist. Polysiloxanes are the most popular GC stationary phases due their tunable polarities and excellent long-term stability. My laboratory has been involved with the synthesis and development of ionic liquid (IL) stationary phases in GC×GC. In particular, we have focused on employing these materials towards complex samples, particularly petrochemicals.

ILs are interesting because their overall physico-chemical properties are dependent on the cation and anion combination. I like to think of ILs as non-molecular solvents that contain two personalities (cation and anion). When analytes solvate into ILs, they can interact through hydrogen bonding interactions, dispersive-type interactions, π–π interactions, and dipolar interactions. These interactions originate from the cation and/or anion and can be highly customizable. Our understanding of how ILs solvate analytes has been greatly improved using linear free energy relationships, such as the Abraham solvation parameter model.

My view of ILs has morphed considerably in the 15 years that I have worked in the field; at first, most of the research was based on a collection of nearly a half dozen ILs. As our understanding of IL structure/separation selectivity has improved, my group has ventured to exploit our synthetic capabilities to rationally design new IL structures that possess the aforementioned essential properties but that also include structural motifs that impart the desired selectivity to the separation. Let's consider the separation of kerosene by GC×GC. The most common approach would be to employ a nonpolar primary column (5% phenyl, 95% methyl polysiloxane [HP-5]) and a polar second dimension column (polyethylene glycol [PEG]). Such a column set can often provide good selectivity of the nonpolar, aliphatic compounds while also providing good resolving power of aromatics. However, when higher temperatures are required, most standard PEG columns begin to show unwanted activity above 270°C. You might expect that the high thermal stability of ILs could address this issue presented by PEG-based stationary phases. However, while ILs can generally be regarded to as "polar" stationary phases, most general IL stationary phases provide good separation selectivity of aromatic compounds, but provide little to no separation of the aliphatic compounds. This result can be advantageous if you are seeking to utilize the separation space for the resolution of aromatic compounds. My group was interested in exploring the structural characteristics of ILs that might impart the needed selectivity to also separate the nonpolar fraction of kerosene.

In recent work from our laboratory, we have shown that the cationic component of the IL plays a vital role in providing the separation of nonpolar aliphatic molecules. Specifically, we synthesized highly alkylated phosphonium and imidazolium cations (monocationic and dicationic platforms), paired these with a number of different anions, and observed that ILs possessing low cohesive forces generally provided the necessary selectivity to separate the nonpolar aliphatic molecules in GC×GC. The role of the anion in the GC×GC analysis of petrochemicals is not as pronounced as the cation, but choosing weakly-coordinating anions that produce low melting salts is important. We have identified monocationic and dicationic ILs that have increased the thermal stabilities well above 300°C, but we are not yet satisfied. We will strive to increase thermal stability while preserving selectivity. We are also interested in exploiting this "structural tunability" feature of ILs and expanding it to the separation of other complex samples.

If you are interested in IL stationary phases for GC×GC analysis, please remember that, just like polysiloxanes, no two ILs are the same. As the popularity of these unique stationary phases expand, I am confident that new generations will be designed to meet the needs of GC×GC users. Applications involving IL stationary phases in GC×GC are still in their infancy but I believe that their future is very bright and that this class of stationary phases will find an important niche in the separation scientist's toolbox.

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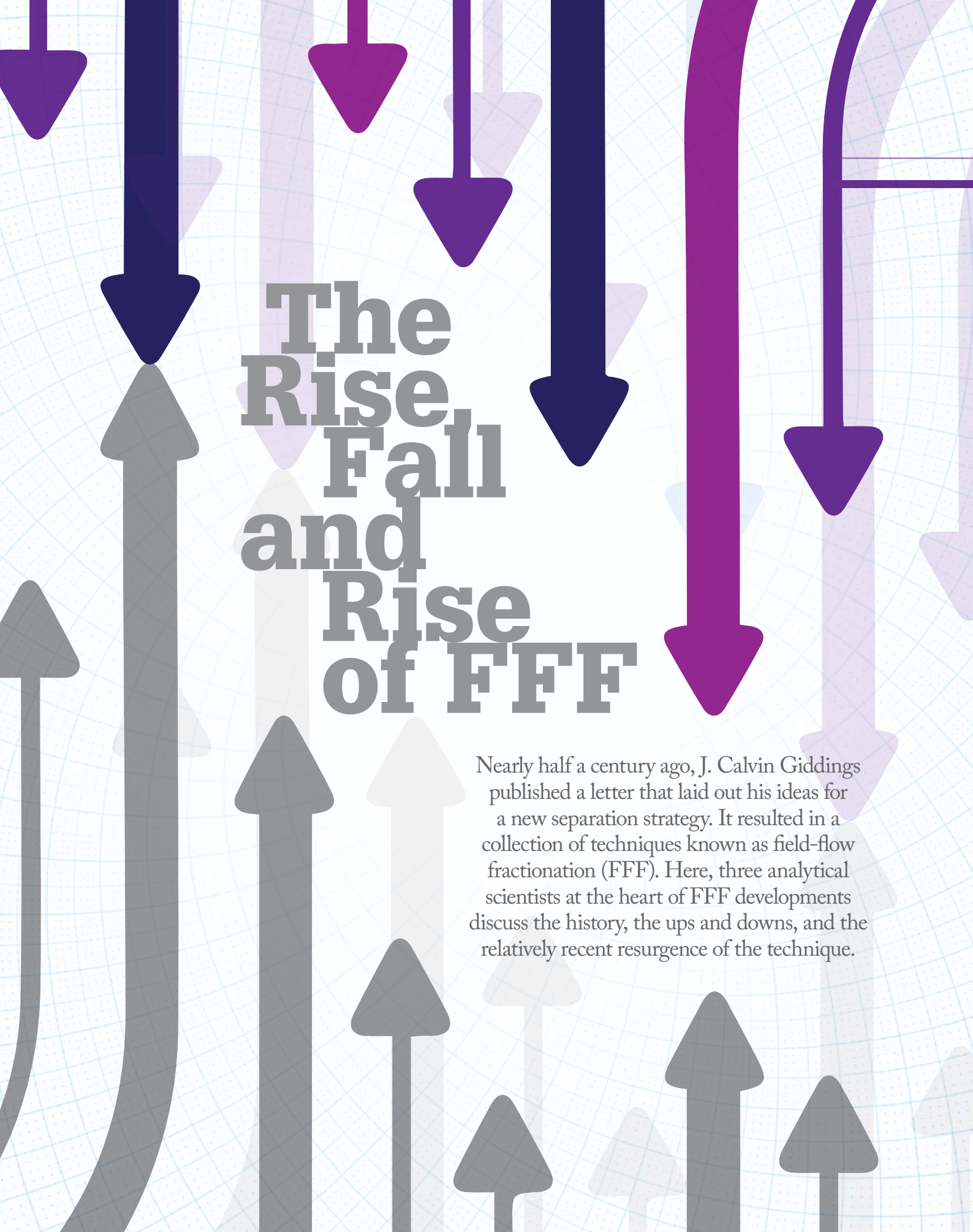

The Rise, Fall and Rise of FFF

Nearly half a century ago, J. Calvin Giddings published a letter that laid out his ideas for a new separation strategy. It resulted in a collection of techniques known as field-flow fractionation (FFF). Here, three analytical scientists at the heart of FFF developments discuss the history, the ups and downs, and the relatively recent resurgence of the technique.

AF4's Time in the Sun?

By Wim Kok, Faculty of Science, the Van't Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands.

In 1966, J. Calvin Giddings published "A New Separation Concept Based on a Coupling of Concentration and Flow Nonuniformities" in Separation Science (1). His innovative idea suggested transporting a mixture of different analytes through a thin channel using a laminar (Poiseuille) flow of a carrier solution. If you 'force' specific analytes into particular flow lines in the channel, using an external field perpendicular to the flow direction, the differences in velocity between the flow lines will separate the analytes much faster than the field itself.

Enter flow FFF

For many years, FFF remained the playground for a limited number of research groups. Sub-techniques with different types of fields were studied (gravitational/centrifugal, thermal, electric, magnetic), and some of these were used in commercial instrumentation and niche applications. However, only one of the sub-techniques – flow FFF – has seen a breakthrough into the real world – and that was only recent.

In flow FFF, a second flow through the porous wall(s) of the channel creates the field for separating the analyte particles. This second or cross flow is perpendicular to the direction of the main flow. The cross flow drives analytes to the accumulation wall, which is covered by an ultrafiltration membrane through which the carrier solution passes while retaining analyte (macro) molecules and particles.

The Giddings group's original ("symmetric") design incorporated a channel with two porous walls for flow FFF (2). Later, Karl-Gustav Wahlund proposed an asymmetrical system, with only one porous wall, to simplify flow control (3). In this asymmetrical flow field-flow fractionation (AF4) mode, the carrier solution is pumped into the channel and splits into the cross flow, passing through the porous wall, and the channel flow transports the analytes to the detector. The accumulation of analyte particles on the wall resulting from the cross flow is counteracted by diffusion, and each analyte ends up in a diffusion layer close to the wall and has a specific characteristic thickness. Because the cross flow is the same for all analyte molecules or particles, the thicknesses of the layers for different analytes is determined purely by their diffusion rate, which is governed by their size.

Figure 1 illustrates the principle of AF4. It shows analyte particles with higher diffusion coefficients enter faster channel flow lines and are the first to elute. AF4, therefore, separates analytes by hydrodynamic size and by the effective size of the molecules/particles in solution.

Strengths and weaknesses

There are a number of alternatives to AF4 for determining size distributions of molecules and particles. But, what are its strengths and weaknesses? One of its often-quoted strengths is that it is a soft separation method, with little surface interaction and shear stress. However, its best asset in my (and Giddings' [4]) opinion, is its ability to handle a wide range of particle sizes, from those retained by an ultrafiltration membrane, to micrometer-sized solid particles. AF4, therefore, covers the gap adequately between size exclusion chromatography on one hand and various particle-sizing methods on the other. And, the experimental conditions can be adapted easily to fit the expected size of the analytes. Moreover, it is possible to analyze flow-programming samples containing components of varying sizes in one run. Figure 2 shows an example of this from our own laboratory.

AF4 also has weaknesses. It is not a very selective or efficient separation technique. This is because of slow equilibration within the diffusion layer – the main contribution to peak broadening.

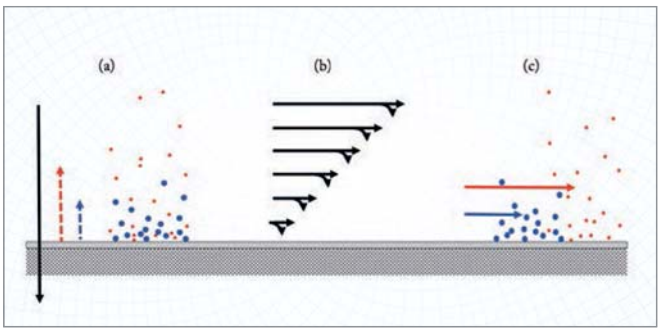

Figure 1. The principle of AF4; (a) selective concentration of analyte particles on the accumulation wall, (b) flow lines near the wall and (c) selective transport of analyte bands in the channel.

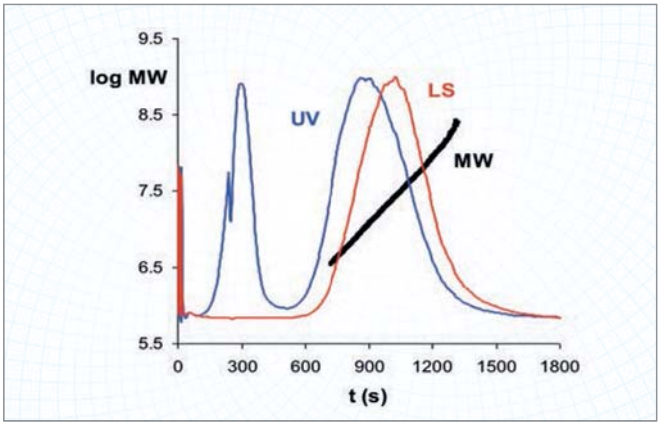

Figure 2. Aggregation of milk proteins using AF4 with flow programming showing UV and light-scattering detector signals – the molecular-weight distributions are calculated from these.

J. Calvin Giddings, the inventor of field-flow fractionation.

There is only one remedy to this problem: miniaturizing the channels. Also, there is often a delicate balance between overloading effects and detection sensitivity, because the compounds of interest in the sample solution are highly concentrated in the accumulation layer first, and upon elution highly diluted in the carrier solution.

Growing interest in AF4

There has been a noticeably significant increase in the use of AF4 for research and routine analysis over the last few years. So, 50 years after Giddings' initial ideas, and 40 years after the first experimental demonstration, flow FFF is finally taking off. The slow start was probably due to the lack of suitable instrumentation in the early years. However, several conceptual improvements and innovations in AF4 instrumentation (flow programming options, frit inlet and outlet systems, hollow fiber channels) have since been introduced. But, the big step forward is the availability of more robust and reliable instruments.

Another explanation for the increasing interest in AF4 is demand. For example, macromolecular biopharmaceuticals are the future for the pharmaceutical industry; in polymer science, ultra-high molecular weight polymers attract attention; in materials science, chemical technology, and in environmental and health studies, nanoparticles are hot. AF4, therefore, may offer solutions to some of the separation and characterization issues that arise in all of these areas.

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In the FFF Hot Seat

Christoph Johann, founder and MD of Wyatt Technology Europe, the company that originally introduced field-flow fractionation (FFF) instrumentation to Europe's analytical scientists, looks at the history of the technique and shares his involvement with the field.

How did it all start?

As noted by Wim Kok, Calvin Giddings invented FFF. He was a chemistry professor at the University of Utah (Salt Lake City, Utah, USA) and an expert in white water kayaking. According to legend, he had the idea for FFF when he noticed how different water currents penetrate each other as water flows along a river. His observations inspired him to design a separation technique using a force field applied perpendicular to a transport flow within a channel.

My personal involvement in the technique and with Calvin Giddings began in 1990, when I signed up for an FFF workshop at Pittcon. Attendance was poor because FFF wasn't well known or popular. Nevertheless, I was attracted to FFF because of its ability to separate particles (which isn't possible with size-exclusion chromatography) and I saw it as a potential tool to use with multiangle light scattering (MALS) from separated samples for particle characterization.

Eventually, I ended up as a distributor for FFFractionation (now Postnova Analytics), the spin-off company from the Giddings group. The instrumentation was primitive and even for the 1990s not state-of-the-art. The flow FFF instrument had no software; the scientist using it was expected to control the flow rates with a stopwatch and a burette, and to run samples by manually flipping

switches. I developed my own software that enabled automated flow FFF experiments.

In 1995, I sold 15 symmetrical flow FFF instruments, but I didn't sell any the following year because most customers were simply unable to use the system; it was too complicated and tedious. For example, replacing a membrane took almost a full day to do and it was an art to reassemble the channel without trapping bubbles or having leakages. The experience taught me that ease of use and robustness are the most important points to consider in developing FFF instrumentation. And, this user-friendly focus has led to the technological breakthroughs we have seen in the last decade in this field.

Why do you think FFF has suffered ups and downs?

FFF has been 'down' in the past because the excellent results published, mostly by the Giddings group, raised high expectations from scientists. Unfortunately, others could not reproduce similar results, mainly because commercially available instruments at that time were impossible to use. As a result, the general opinion about FFF was that it looks good in literature, but it cannot be used in practice. As always in such cases, it takes a long time to change perceptions and this is why it has taken years to convince people to try FFF using one of the new generation of FFF instruments.

I think the biggest challenge today is the complexity of the analytical problems that tend to need FFF as a solution. Easy samples are analyzed using column chromatography and only applications that cannot be managed using traditional techniques are considered for FFF separation. A growing number of excellent publications highlight the progress made in recent years. Applications that work exceedingly well with FFF include virus-like particles (VLP), vaccines and nanoparticle characterization for environmental applications.

How important is FFF today?

In my opinion, there is no doubt that FFF has its place in the analytical techniques toolbox for macromolecular and particle characterization. However, it is far from being a routine technique as it's still at the pioneering stage with a relatively small user base of enthusiasts. These people produce high quality research and publications, which are relevant for other researchers in the same field. This is why I am confident that FFF will grow and become more widespread. Many others will accept the technique as a standard tool for a variety of applications.

How are people using FFF?

I would like to highlight the separation of virus like particles, which is achievable by flow FFF coupled with a MALS detector. As VLPs are becoming more important as vaccines, we are witnessing a growing interest for FFF instrumentation in the pharmaceutical industry and in research. The advantage of FFF is that its resolution is superior to SEC. Recoveries are typically very good and it is possible to separate VLP populations, which maintain their biological activity throughout the separation process. This allows further evaluation of fractions after separation with respect to their efficacy of stimulating an immune response.

Where does FFF stand within analytical science?

FFF is similar to liquid chromatography (LC) although it does not have a stationary phase. Therefore, it is possible to use it in high-performance liquid chromatography (HPLC) laboratories, because it needs a similar infrastructure. But most FFF applications, such as particle characterization, are not performed with HPLC. This is an interesting problem; the scientists from the particle characterization labs have a harder time operating an FFF system than their HPLC colleagues would have. However, the HPLC group does not look at particles, so a reorientation of perspectives, know-how and organizational structure is necessary in order to use FFF successfully. This is especially true of industry, which makes it difficult to implement FFF.

Are people missing opportunities to utilize FFF?

Yes, I think so. FFF is still considered as a replacement to SEC.

FFF Through the Years

By Thorsten Klein

1966 – J. Calvin Giddings invents FFF
1972 – The first FFF instrument is built for thermal FFF
1983 – FFF is coupled with Low Angle Light Scattering using a Sofica LALS detector
1986 – Giddings founds FFFractionation in Salt Lake City, Utah, USA
1986 – FFFractionation launches the S101 colloid separator for sedimentation FFF
1987 – The company introduces the world's first commercial thermal FFF instrument; the T100 polymer separator
1988 – World's first commercial flow FFF (F1000 universal separator) is introduced
1988 – Giddings and Wahl present the idea of asymmetric flow FFF (AF4) for the first time
1990 – Launch of the SF1000, the world's first commercial split thin flow cell fractionation (SPLITT)

Moreover, the general expectation is that FFF should produce similar results to column separations. This is not the case because FFF has very different characteristics. For example, the sample is concentrated at the accumulation wall and shear forces are absent during separation. The species eluting from FFF can be different from that from an SEC column. This does not mean one technique is right and the other wrong or that there are artefacts – the fractogram will reflect the properties of the sample under such conditions. Therefore, FFF can be an excellent tool to study a sample's behavior under high concentration conditions and this gives an opportunity to retrieve information about stability or aggregation behavior of proteins and other macromolecules.

What do you think are the most important recent developments?

Hollow-fiber-flow FFF (HF5) is an important new development. This technique employs a disposable channel that can be replaced in a few minutes. The system has a very low channel volume (below 100 microliters) which leads to less sample dilution and a 33 percent higher retention at the same cross-flow density. Applications are particularly interesting for nanoparticles, and HF5 has distinct advantages when coupled to ICP-MS.

Where do you think FFF will be in five years?

I believe that FFF will get closer to becoming a routine method within the next five years. There will be several applications, mainly for nanoparticles, required by EU legislation to classify a product as being 'nano' or not. Some of the new vaccine candidates in development today – that are characterized using flow FFF methods – will be on the market by then. FFF will be used for QC of some of these drugs. The instrumentation and software will be markedly improved and automated. Performing an FFF analysis will be almost as simple as using an automated coffee maker. The software will constantly monitor the system status and actively prompt the user to take service action or it will give instructions how to maintain perfect status and functionality of the FFF instrument.

Taking FFF to the Next Dimension

Way back in the mid-1960s, somewhere in the North American Midwest, J. Calvin Giddings spent a sleepless night in a noisy motel room. Even the room's rattling air-conditioning unit gave him a hard time. Giving up on the idea of sleep, he turned his thoughts to the separation principle of field-flow fractionation (FFF).

By Thorsten Klein, founder and CEO of Postnova Analytics, Landsberg, Germany.

Following his restless but inspirational evening, Giddings embarked on his FFF pursuits, first building a thermal FFF channel about two metres in length for his proof of principle. A primitive device, the top and bottom of the channel were held together using screw clamps (see photo on page 40). The elution of the polystyrene polymers in tetrahydrofuran (THF) took many hours to complete and the peaks the instrument produced were huge.

Giddings' initial work was on thermal FFF, with sedimentation and flow FFF coming much later on. Then in 1986, he founded FFFractionation, the world's first FFF company to commercialize his ideas. That company eventually merged and led to the formation of Postnova Analytics.

Revolutionary technique

The history of FFF is remarkable because for two decades after Giddings first began focusing on the theory and science, there weren't any professional standard commercial instruments available. It was the 1990s before any real commercialization effort emerged with FFFractionation/Postnova leading the way, followed later by Wyatt Technology.

1994 – The F1000 instrument for flow FFF is the company's most popular instrument
1999 – FFFractionation invents "FOCUS-Technology", which provides first-time focusing without the need for switching valves
2000 – Invention of "Slot-Outlet Technology", giving five times more sensitivity without the need to change detector hardware
2001 – FFFractionation merges with Postnova, resulting in Postnova Analytics USA
2006 – First commercial asymmetric flow FFFs (AF4) instrument is launched
2011 – First commercial hollow fiber FFF instrument launched (the approach was first published in the 1980s and then patented by Pharmacia Biotech)
2012 – First centrifugal FFF capable of 4900 RPM is launched (previously instruments spun at 2000 RPM) – a significant breakthrough as it enabled nano applications.

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Top left: J. Calvin Giddings' colleague Marcus Myers with an original prototype FFF instrument. Bottom left: an early AF4 instrument dating back to 1996. Above: one of the first Pittcon 1991 shows for FFFractionation; (left to right: Calvin Giddings, Marcia Hansen (USA CEO in the 1990s), Ron Beckett (Monash University, Australia), Frank Yang (later CEO of Microtech).

FFF was first recognized as being ideal for large molecules and submicron particles; and, it remains so today. Then in the 1980s and 1990s, the technique began to make waves in protein and nanoparticle research. So FFF really was invented ahead of its time, some might say it was "too early", but now it fits perfectly with other technological scientific tools used in modern nano, bio and material laboratories.

More FFF education needed

Compared with chromatography, FFF is a niche technology. However, it is essential for certain applications in nano, biopharma and polymer science. Unfortunately, due to its lack of use in education, many scientists remain unaware of the technique; indeed only a few professors include FFF on their courses, whereas all chromatography methods are addressed in great length. Therefore, we need to see the educational sector changing its stance to provide students with up-to-date education that is more suitable for their future career paths. Clearly, they ought to be at the very least aware of FFF so that they can use it to solve their analytical questions. For a similar tale regarding education, see Gertrud Morlock's article on thin-layer chromatography on page 42.

Whenever larger molecules or particles are under investigation, FFF can provide significant insights – simple particle size analyzers or size exclusion chromatography (SEC) methods overlook hidden information in the sample. For those researchers who really want to get to know their samples, FFF becomes an obvious addition to the tool box – in fact, there is no real alternative.

FFF future is bright

Symmetrical FFF (SF3) and later asymmetrical flow FFF (AF4) have proven their worth in resolving a number of application problems. Commercial flow FFF systems have been available for some time and scientists have happily taken to using and adapting SF3 and AF4 for their applications. In addition, we now have access to reliable commercial instruments for thermal and centrifugal FFF. Such advances mean that there are great opportunities for the future use of other FFF separation forces (centrifugal for density separation and thermal for chemical composition) to achieve two and three-dimensional FFF separations. We have been working hard on this and a growing number of scientists have opened their eyes to the potential advantages.

I believe the future is bright for FFF and that over the next five years it will join other established chromatographic techniques used for larger molecules separations. I also think we will see the emergence of subtechniques that will make it even more useful for nano and macro characterization. And finally, I predict that we'll see more utilization of multidimensional FFF – both two and three-dimensional techniques.

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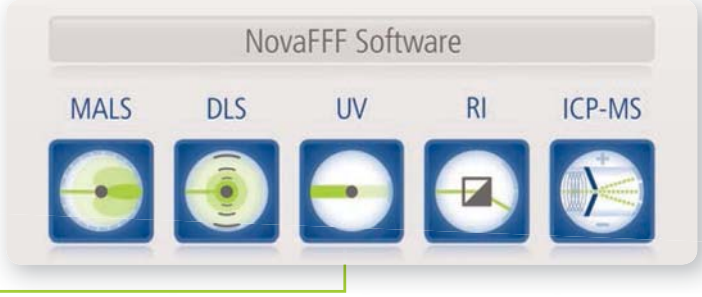

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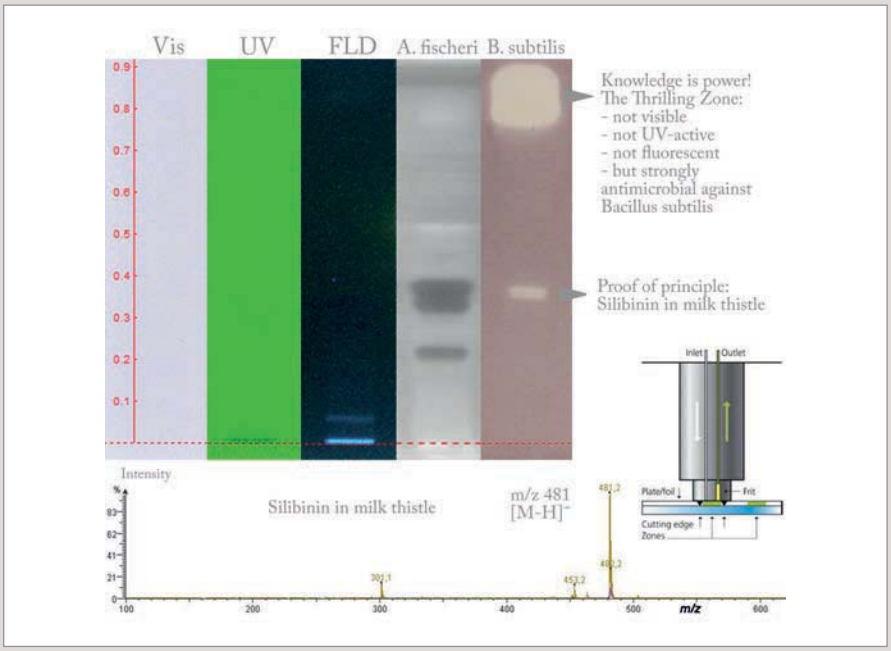
Finding TLC's Missing Link: MS

For years, thin layer chromatography has been working hard in the background, the choice technique in certain applications. More recently, hyphenation with mass spectrometry has been a relatively quiet – but very real – game-changer. What have you been missing?

By Gertrud Morlock, Chair of Food Science, Justus Liebig University of Giessen, Germany.



Effect-directed analysis of a milk thistle extract using high-performance thin-layer chromatography coupled with mass spectrometry (based on work from reference 3).

Though I hold the food science chair at JLU Giessen, I consider myself "free" from a research perspective, working regularly in pharmaceutical and environmental analysis.. I believe this freedom puts me in an excellent position to share advances in thin-layer chromatography (TLC) that have opened up new vistas in analytical capability.

I was quite fortunate that I started my planar chromatography journey early under the supervision of "the pope" of quantitative high-performance TLC (HPTLC) – Helmut Jork at Saarland University, Saarbrücken. I was fond of HPTLC even then, but wanted to move into industry and, subsequently joined CAMAG – a leading HPTLC instrumentation company – for three years. It was some years later, during my time at the University of Hohenheim in Stuttgart in the group of Wolfgang Schwack (food chemistry), that I started working on planar chromatography and its hyphenation with mass spectrometry (MS).

Today, every analytical scientist needs MS – it's the universally accepted detector. I was frustrated by the lack of developments in planar chromatography – and I was not alone. Coupling TLC with mass spectrometry was absolutely essential in gaining as much information from the zone on the plate as possible. Why should we not replicate the crystal clear success seen in coupling MS with HPLC and GC?

Mass spectrometry needs some TLC – or vice versa...

I've always loved the visual nature of TLC, which offers the unusual ability to run parallel separations and compare and contrast directly. Moreover, you can answer questions very rapidly. But seeing the samples separated in front of me made me even more aware of the missing link: MS. Coupling such a useful and fast separation technique with mass spectrometry became both my passion and my ultimate goal.

Ambient mass spectrometry was being explored at around the same time, and I could see synergistic benefits emerging between such ambient techniques and planar chromatography. On the elution head-based side of things, a colleague of mine – Heinrich Luftmann (University of Münster) – had attracted my attention with his paper, but the reliability was not proven and the technique was not yet convincing, especially as it only worked on flexible aluminum foils.

However, I saw great potential. I visited his lab and explained that his approach made excellent sense; that it was an extremely practical solution to the problem; and that I wanted to develop it further. He had two prototypes of the ChromeXtractor and promptly gave me the older one. I started work and published a paper that described three important modifications that allowed coupling to glass plates (1). Over the course of three years, I published about 13 research papers on the optimization and application of the elution head-based interface, which was finally commercialized.

Not satisfied with one coupling, I also published a paper on direct analysis in real time (DART) mass spectrometry (2). The DART paper won a "Highly Cited Author Award," clearly showing how interested the community was in HPTLC-MS.

Indeed, HPTLC – and TLC – had

rather suddenly been dragged into the 21st century, and the profile of the technique was raised considerably.

Modern (HP)TLC is born

Coupling of (HP)TLC to mass spectrometry opened up a new world of applications. After those papers, I received many requests from companies who were interested in impurity analysis, particularly with respect to breakdown products in the pharmaceutical field. Previously in such situations, a particular zone would be scraped off the plate and then subjected to mass spectrometry – a laborious and error prone process. And in food analysis, the same benefits applied to contaminants or simple analyte confirmation.

But the addition of mass spectrometry also made effect-directed analysis with HPTLC a reality. By using enzymatic assays or bioassays directly on the plate, we can quickly identify the zones of bioactive interest that can be further analyzed with mass spectrometry. For example, the cosmetics industry is very interested in plant extracts, and they want to understand more about bioactivity – the addition of mass spectrometry is essential in identifying the compounds of interest – and the use of HPTLC simplifies the process.

On page 24 in this magazine, multidimensional chromatography is a focus – complex separation techniques for complex samples and complex problems. And it sure is thrilling to get 4000 peaks from a sample. But sometimes that's not enough – there may still be coelution – so where do we stop? Or perhaps we can identify 300 compounds – but what about the other 3700? Are they important?

The use of HPTLC offers greatly simplified separation. It's certainly no match for HPLC or GC in terms of resolution and separation number – but it can separate a complex mixture with little to no sample preparation. For sure, there is coelution – but when we are interested in bioactivity, the addition of a bioassay could reduce 4000 peaks in a complex sample to five bioactive zones for mass spectrometric analysis. It's a beautifully streamlined approach that can give rapid answers to complex problems. And to address zone coelution, we can, for example in the case of a normal phase HPTLC, integrate a short C18 monolithic column between the plate and the MS, thus offering orthogonal separation – the time to result is just the same.

In short, there is always more than one way to solve a problem.

Understanding why

Given its utility, why aren't we all using advanced TLC-based approaches? Well, the reality is that the problem starts very early – in education. I've surveyed the problem for years, and in my experience the worldwide mean average teaching time for TLC is three minutes. A quick cursory introduction, a quick manual application to a plate, followed by development and the education is finished. It's no surprise that the field has been very slow to develop. Consider HPLC and GC – teaching absolutely requires instrumentation (unlike TLC) and students assume that those techniques are more quantitative, more reliable or even more profound.

I advise my teaching colleagues to buy HPTLC instrumentation, but they say, "We have limited budget. I can teach the principles with no instrumentation." But that's discrimination from the outset. In Germany we say "a tree must be bent while it is young"; the backwards-facing equivalent is "you can't teach an old dog new tricks"... What does this mean for HPTLC? Well, we simply don't have enough people working on HPTLC theory despite the potential.

Teaching old dogs new tricks

So, you're not a fan of sample preparation or the potential bias it introduces? Take a raw extract of your complex sample (feed or food, traditional medicine, supplement, nutraceutical, ...), subject it to HPTLC, couple it to a bio or enzymatic assay, and just see what happens. After all, HPTLC, especially when using area application on HPTLC plates, copes well with high matrix loads – we don't reuse plates – and it can essentially do sample preparation and separation in the same step. And don't forget, we see everything in thin-layer chromatography . If something is fixed at the starting zone, we don't miss it, we just know we need to develop the same plate with a solvent mixture of a higher elution power. If something gets stuck in a HPLC column or a GC liner, you may never know – it never hits the detector.

I worked with a huge pharmaceutical company that had spent half a year trying to figure out the difference between a good and bad batch using both HPLC and GC – they could not find differing peaks between the two samples. They came to me as a last resort and within half a day, we discovered that the difference was in the start zone. We re-analyzed the plate with a new solvent mixture and solved the problem.

Unfortunately, the lack of education and training means that many people who finally try HPTLC may fail and say, "What a stupid method!". But I assure you, it could well be an essential, highly complementary modern technique alongside those complex multidimensional chromatography methods already sitting in your toolbox.

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Solutions

Real analytical problems
Collaborative expertise
Novel applications

The Softly-Softly Approach

In 2014, Select-eV took a top spot in The Analytical Scientist Innovation Awards (TASIAs). Here, the technology is teamed up with (GC×GC)-MS in an unusual international environmental monitoring project.

By Laura McGregor, Anthony Gravell, Ian Allan, Graham Mills, David Barden, Nick Bukowski, and Steve Smith

The Problem

Continuous advancements in analytical chemistry raise the bar ever higher for monitoring contaminants in the environment. Conventional gas chromatography-mass spectrometry (GC-MS) instruments are beginning to struggle to identify emerging pollutants reliably at trace concentrations in highly complex biological and environmental matrices.

There are three main challenges associated with full chemical characterization of environmental samples:

- minimizing sample preparation
- efficient separation of complex components
- confident identification of trace-level contaminants.

Can recent advances in comprehensive two-dimensional GC (GC×GC), coupled with new methods of soft electron ionization present a solution?

Background

Human biomonitoring can very often identify new emerging contaminants at concentrations below observable health effects. However, identifying these emerging contaminants is analytically challenging, as the matrices are highly complex and require costly and labor-intensive methods.

Figure 1. Passive sampling device in a protective deployment cage (left) and its counterpart, explanted silicone breast implants (right).

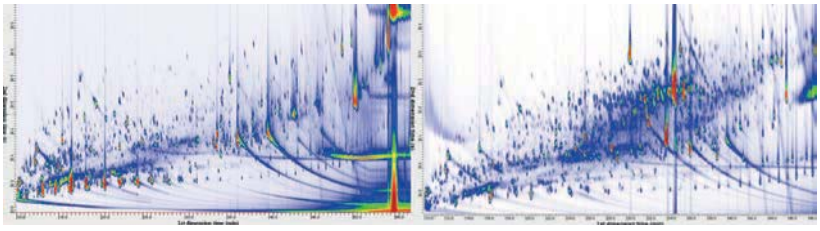

Figure 2. GC×GC-TOF MS color plots of extracts representing both a "used" (left) and new (right) silicone breast implant.

Environmental contaminants may enter the body through a variety of pathways, for example, inhalation, consumption, or dermal contact; hydrophobic and persistent chemicals can bioaccumulate over time in fats and lipids (1). As such, there are strict regulations controlling the release of many harmful substances into the environment.

Our recent collaborative project focused on monitoring pollutants in river water using a passive sampling device and advanced analytical techniques (2). Our absorption-based, passive sampling devices (Figure 1, left) were composed of a thin polyethylene film containing the lipid triolein (which concentrates contaminants over long periods) mounted inside a metal

cage. After deployment, the film with triolein was removed from the device and the sequestered pollutants were recovered using gel-permeation and solvent extraction, followed by instrumental analysis.

The work got us thinking. Is there anything that could potentially mimic the observed absorption process when humans are exposed to environmental pollutants?

The answer was "yes" – silicone breast implants (3)! (See Figure 1, right).

Researchers from the Norwegian Institute of Water Research (NIVA) teamed up with plastic surgeon Helge Roald (Colosseum Clinic, Oslo) to procure a number of "used" silicone prostheses from patients in Norway together with new, unused implants. Initial studies used gas chromatography with quadrupole mass spectrometry to identify compounds in the extracts. Unfortunately, the complex extracts resulted in significant co-elution, making full chemical fingerprinting a challenge.

NIVA consulted with scientists at Natural Resources Wales, the University of Portsmouth and Markes International for the analysis of the extracts from the implants using a novel analytical system that had been successfully applied to the analysis of the complex extracts obtained from the passive samplers.

The system used comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOF MS) for the improved separation and ultra-trace identification of contaminants.

Figure 2 shows the GC×GC-TOF MS chromatograms of a new breast implant (our control sample - taken fresh out of the bag), and an explanted prosthesis. Such complex samples would be extremely challenging for conventional GC-MS without further sample preparation (for example, chemical fractionation to split the sample into numerous sub-extracts).

Indeed, the use of GC×GC-TOF MS solved the problem of being able to adequately separate the complex samples for improved screening of the entire sample in a single analytical run.

Nevertheless, the third challenge remained. Extreme fragmentation and similar spectra when using conventional electron ionization (EI) settings (70 eV) can make confident chemical identification problematic and cause difficulties in detecting compounds within matrices giving a high background signal. For example, in the extract from

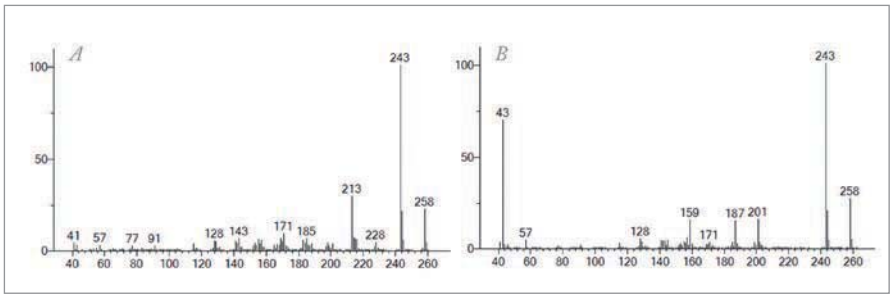

Figure 3. Mass spectra obtained at 70 eV for two polycyclic musks (a) Galaxolide and (b) Tonalide found in the extract from the used silicone implant

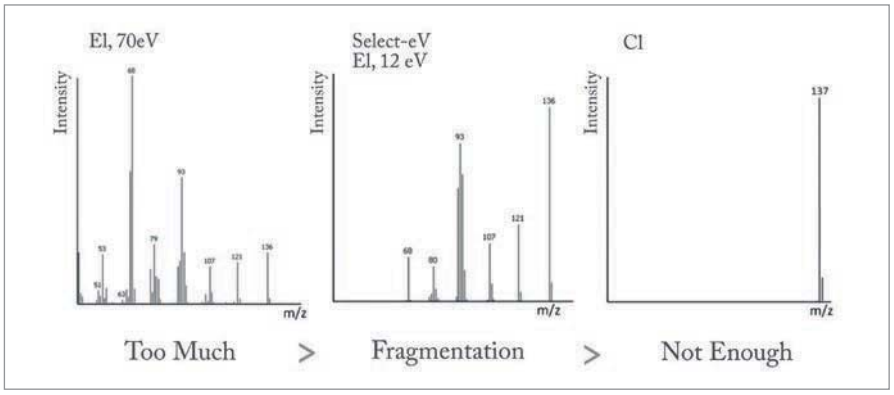

Figure 4. "Goldilocks and the three spectra." Fragmentation by various ionization techniques: electron ionization at 70 eV (too much), chemical ionization (too little) and Select-eV at 12 eV (just right).

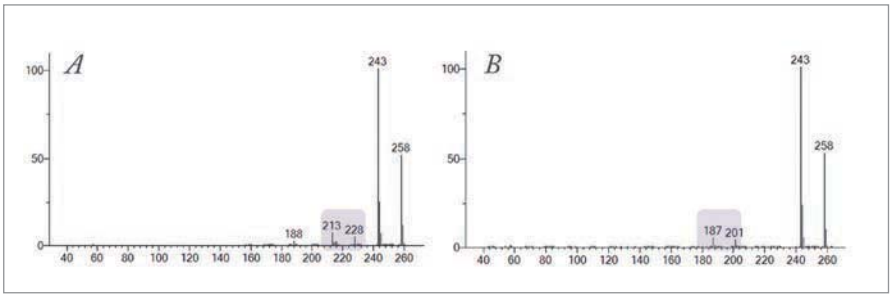

Figure 5. Select-eV soft EI spectra (14 eV) of the polycyclic musks (a) Galaxolide and (b) Tonalide (also shown in Figure 3). In both cases, molecular ions are enhanced, while retaining structurally-significant ions important for compound identification (see highlighted ions for examples).

the used implant, a number of emerging contaminants were detected, including the polycyclic musks, Galaxolide and Tonalide (see Figure 3). These fragrance compounds are found in many personal care products and trace levels can be difficult to identify within complex environmental matrices.

To overcome the problem, we turned to soft ionization – a term used to describe lower energy forms of ionization. Soft ionization results in a limited degree of analyte fragmentation, meaning that a higher proportion of the original ionized analyte molecules reach the detector. The ability to provide information about the unfragmented molecule makes soft ionization of great value to analysts, but for many laboratories, the approach is seldom used. From source-switching to reagent gas selection and source pressurization, soft ionization has a reputation for being difficult and time-consuming to set up, with an undesirable loss in sensitivity.

The Solution

When it comes to ionization, it's often a case of "Goldilocks and the three spectra", with 70 eV ionization providing too much fragmentation and soft ionization by conventional methods resulting in too little, with only the molecular ion being generated (see Figure 4).

In a conventional EI ion source, an electron beam is produced by the filament (or e-gun) and the bombardment of molecules by high-energy (70 eV) electrons results in characteristic ion fragments and consistent spectra, which can be compared against commercial libraries, such as NIST or Wiley.

When a stronger signal from the molecular ion (and other diagnostic ions) is required, it seems logical to simply lower the electron energy to reduce fragmentation, yet retain enough information on the compound structure. However, when using a conventional EI ion source with low electron energies, a catastrophic loss in sensitivity is observed, due to poor channeling of electrons and inefficient ionization. Such issues have prevented the implementation of low eV in routine analysis.

Technical experts at Markes International designed a novel ion source, known as Select-eV, that overcomes these issues and enables soft electron ionization down to 10 eV. The Select-eV source can be tuned to retain sensitivity at low eV, with full software control enabling comprehensive sample characterization in a single sequence.

And so, the polycyclic musks shown in Figure 3 were also investigated at 14 eV (see Figure 5). The 14 eV spectra are simplified; molecular ions are enhanced but key diagnostic ions are retained, providing increased confidence in compound identification. The absolute intensity of the molecular ion (m/z 258) for Galaxolide and Tonalide was increased by factors of three and two respectively, providing enhanced sensitivity for these (often trace-level) contaminants. In conventional soft ionization, the production of spectra containing solely the molecular ion would have been of minimal benefit in this particular case.

Extracts obtained from the used breast implants were also analyzed by GC×GC-

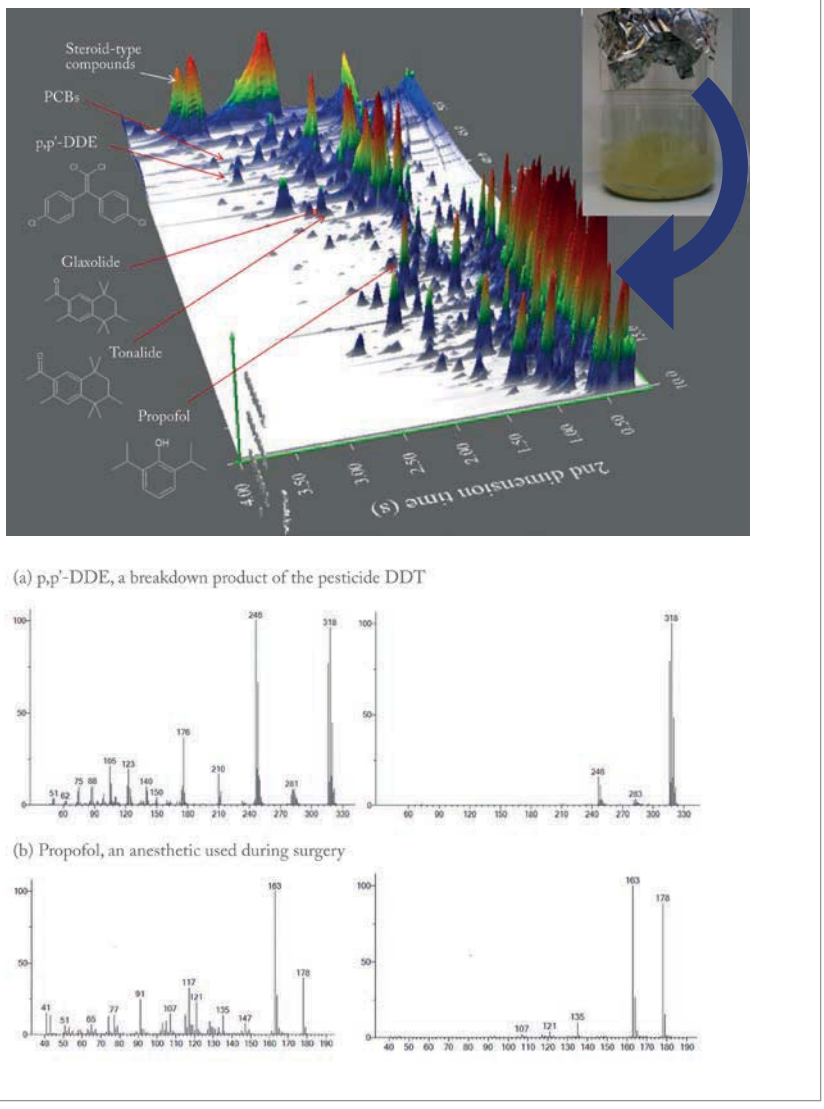

Figure 6. GC×GC-TOF MS surface plot representing compounds found in an extract from a used silicone breast implant (top), with spectral comparisons at 70 eV and 14 eV for two compounds of interest (bottom).

TOF MS with Select-eV ionization, resulting in the detection of a wide range of components, including priority pollutants, emerging contaminants and pharmaceutical compounds.

For the spectra shown in Figure 6, an increase in the absolute abundance of molecular ion was observed in each case, as well as reduced fragmentation – resulting in increased sensitivity and selectivity.

The results demonstrate that the simplified low eV spectra provide a reduction in "common" ions and enhancement of "diagnostic" and molecular ions, thereby retaining enough fragmentation for structural elucidation and library searching.

Beyond the Solution

There are many other complex matrices amenable to GC (or GC×GC)-TOF MS that could benefit from the addition of Select-eV. For example, the technique is having an impact in the analysis of crude oil. Select-eV results in a degree of fragmentation, a feature that is particularly useful for challenging petrochemical applications that require confident distinction between similar species, such as hydrocarbon isomers.

The simplified spectra produced by low eV analysis can also be helpful in the detection and identification of individual compounds belonging to chemical classes that share many common ions in their fragmentation patterns. For example, in the analysis of crude oil, there is a higher analytical peak capacity for biomarkers due to a reduced demand on the m/z domain. While in flavor and fragrance analysis, important allergens, such as the isomers of farnesol, can be identified more confidently through enhanced differences in their spectra at low eV.

Going back to our breast implant study, it is important to note that none of the environmental contaminants (for example, *p,p'*-DDE) were detected in the control (new implant) or blank (solvent) samples, meaning it is likely that they were absorbed from the human body after the patient was exposed to these chemicals. Certainly, this initial study has delivered a promising start to the project, but there are still many unanswered questions. Are the compounds in equilibrium between the bloodstream and the implant? What compounds will be absorbed by the implant? But those questions will have to wait for a future article.

Laura McGregor, David Barden, Nick Bukowski, and Steve Smith are from Markes International, Llantrisant, RCT, Wales, UK; Anthony Gravell is from Natural Resources Wales (NRW), Llanelli, Wales, UK; Ian Allan is from the Norwegian Institute for Water Research (NIVA), Oslo, Norway; and Graham Mills is from the University of Portsmouth, UK.

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2D Analysis of Thermoplastic Elastomers TPE

Copolymer analysis can be complex. The 2D approach is the only way to determine molar mass and chemical composition distributions simultaneously.

By Peter Kilz

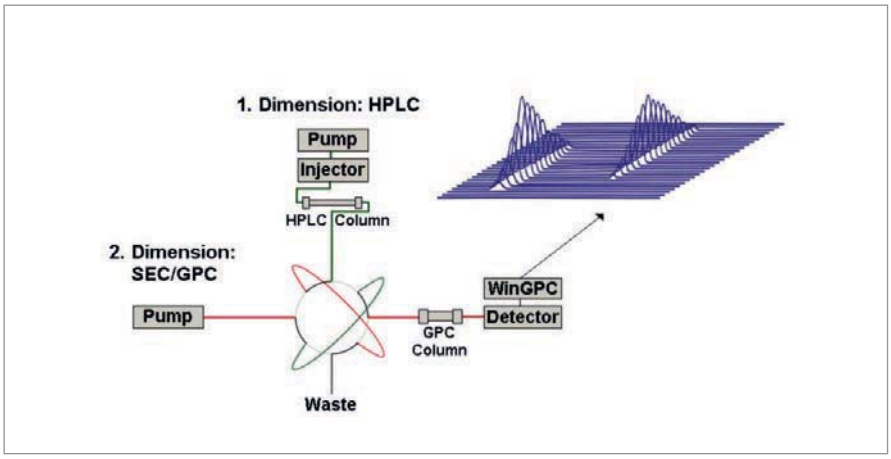


Fig. 1: Schematic 2D separation

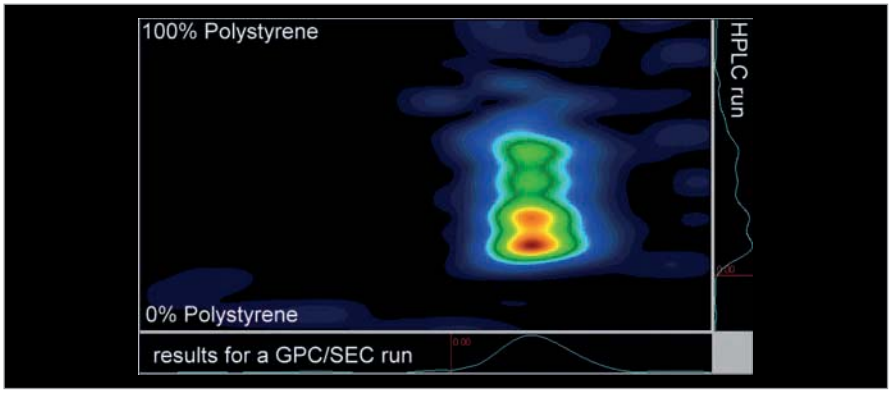


Fig.2: Contour plot of a thermoplastic elastomer

Introduction

Blockcopolymers such as SBS (Styrene-Butadiene-Styrene) are an important product class and a typical example for complex polymers. Special for copolymers is that in addition to the molar mass distribution also a chemical composition distribution may be present. While GPC/SEC is the established method for the determination of molar mass averages and distribution, gradient HPLC can be applied to separate based on chemical composition.

Gradient HPLC can be hyphenated with GPC/SEC in a fully automated setup to measure both distribution simultaneously with a high peak capacity and to detect differences in batches (cf. Fig 1).

Experimental

All experiments were performed on PSS SECcurity 2D polymer analysis equipment using the following conditions:

Eluent 1st dim.: n-Hexane/ THF p.a. gradient
Column 1st dim.: PSS Si-60 5 µm
Flow-rate 1st dim.: 0.1 mL/min
Injection volume: 20 µL

Transfer: 2D tandem transfer valve with two 100µL loops
Eluent 2nd dim.: THF p.a.
Column 2nd dim.: PSS HighSpeed SDV 5 µm, 10 000 Å
Flow-rate 2nd dim.: 6.25 mL/min
Detection: SECcurity VWD 1260 UV @ 254 nm
Calibration: PSS Polystyrene ReadyCal Standards, PSS Polybutadiene standards
Data System: PSS WinGPC UniChrom

Results

The online combination of gradient HPLC and GPC/SEC increases the peak capacity of the separations and allows to look behind peaks which cannot be separated by either method alone. The HPLC conditions are selected to separate according to Polybutadiene content.

Fig. 2 shows the contour plot for a thermoplastic elastomer that shows one narrow main peak in GPC/SEC. However, 2D separation reveals that 4 different compositions are present that co-elute in the GPC/SEC experiment. The species differ in composition and Polybutadiene content. The color code indicates the concentration of each peak. Simultaneous molar mass results and composition results can be measured using the calibrated GPC/SEC and HPLC axis.

http://www.pss-polymer.com/products/lc-instruments-and-detectors/2dpolymeranalyzer/

On-line Quality Control Measurements in Varying Conditions

By Yvette Mattley, Ph.D.

Background

Even as advances in engineering technologies and manufacturing processes have lowered the cost to make and distribute products, the demand for continued improvement is as strong as ever. In an environment where small improvements in characterization of raw materials or subtle changes in process parameters can result in significant production savings, the ability to design faster, smarter and more robust instrumentation is paramount.

When the emergence of miniature spectrometers coincided with development of modular fiber optics, spectroscopy was no longer limited to the lab. Now you can bring the instrument to the sample, which allows industrial users to integrate the measurement into the process. Small-footprint modular systems can be rapidly configured for a variety of absorbance, reflectance and emission measurements, with a number of potential applications.

The Flame spectrometer addresses some of the limitations associated with miniature spectroscopy systems in dynamic process environments.

Measurement Conditions

To evaluate the effectiveness of the Flame spectrometer at different temperatures, we measured transmission of several concentration levels of food dye mixtures on a simulated process line with simulated conditions encountered in a process environment. Then we isolated each Flame spectrometer in a different temperature environment – cool (using a chiller), ambient and hot (using a lab heater). Several sample mixtures were prepared for testing, using the Z-type flow cell to move each sample through the system. Water moving through the flow cell was measured as a reference (Figure 1).

Results

Although the Flame spectrometers measured the transmission of the mixtures flowing through the system at different temperature conditions, the resulting spectra – and sample composition information derived from the spectra – were nearly identical (Figures 2, 3). This result is significant for process line applications, where temperatures can vary from zone to zone within the stream. For quality control professionals, getting the correct answers under all sorts of conditions - including temperature extremes - is critical.

Conclusions

Process environments can be harsh, with extremes in temperature and humidity, and the harmful effects of dust and vibration. That's why process-ready spectroscopic instrumentation such as Flame is designed with few moving parts, is thermally stable, and is easily adapted for different setups. The availability of such robust, repeatable, thermally stable instrumentation allows manufacturers to assess sample quality online at multiple points in processes, helping to improve yields, eliminate waste and reduce costs.

Tel: +1 727 733 2447
Email: Info@OceanOptics.com
http://oceanoptics.com/product/flame-spectrometer/

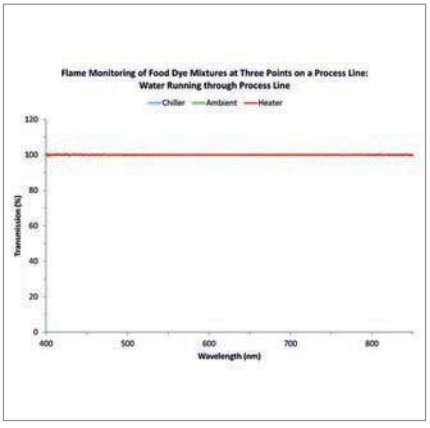


Fig. 1:

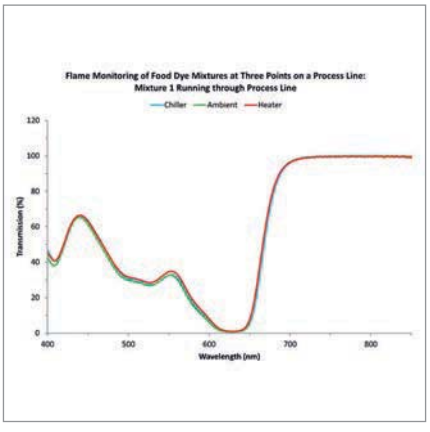


Fig. 2

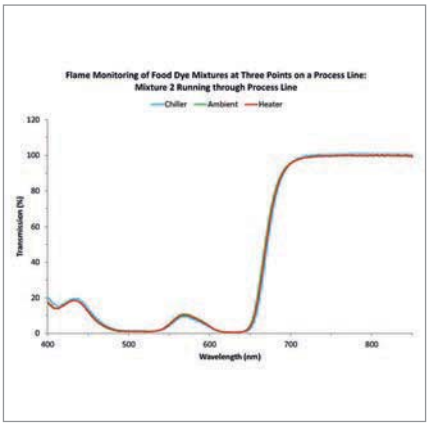
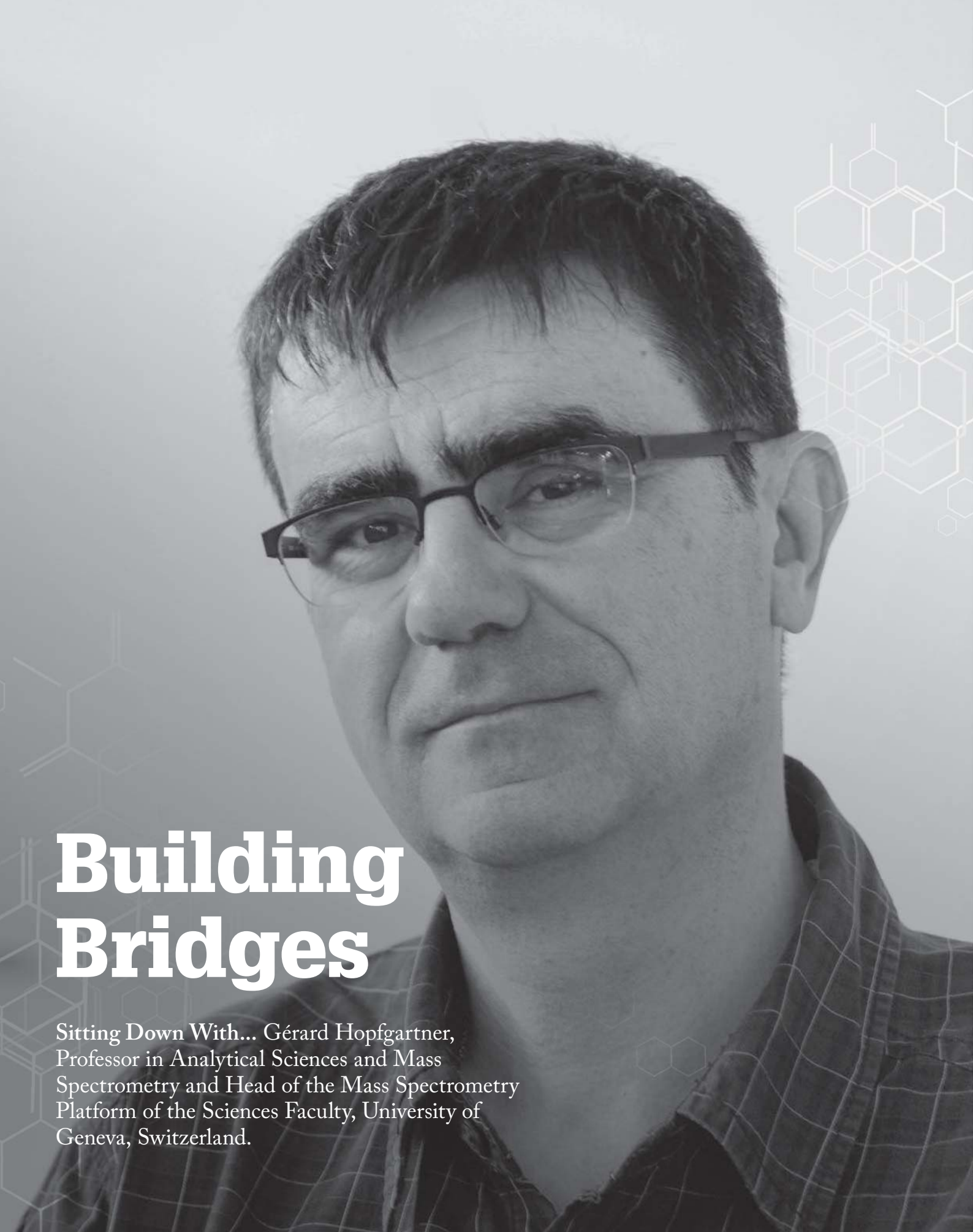


Fig. 3



Building Bridges

Sitting Down With... Gérard Hopfgartner, Professor in Analytical Sciences and Mass Spectrometry and Head of the Mass Spectrometry Platform of the Sciences Faculty, University of Geneva, Switzerland.

You're the chair of HPLC 2015 in Geneva – what's the plan?

The European series of HPLC events have been successful in the past, so we won't stray too far from the tried and tested. We are running four parallel sessions, three of those are dedicated to scientific themes: fundamentals (core separation technology); hyphenated techniques (mass spectrometry will play a large role here); and high impact applications (here we have placed an emphasis on sample preparation). The fourth parallel session is for tutorials – and we're introducing a rapid "five minutes, five slides" system, which gives more young scientists a chance to present their work.

And that's important?

Absolutely. I think exposure is very important for young scientists. The best poster award winners will also get "five minutes, five slides," so that everyone can understand why their work stood out for the judges – something that's often missing in other conferences.

Do you see any trends in the work submitted so far?

There are some definite trends – multidimensional separations seem hot this year (see page 24). Omics applications are also prevalent. And there is still a huge interest in the fundamentals, which is reassuring. Finally, as I've already said, mass spectrometry is ever present.

What's the motivation for organizing a major conference like HPLC?

Well, you certainly can't do something like this if you're forced into it! I've always been a fan of the analytical sciences, and I believe we must promote the field whenever we can – chairing such a conference is my way of contributing. Despite our interconnected world, I am still convinced that face-to-face scientific meetings are essential to keep a field moving forward – it's about building bridges. So I guess my passion for progress and community are strong motivators.

Has "Francogeddon" (the abandonment of the Swiss Franc ceiling) affected the organization of HPLC 2015 at all?

Things have settled down now I think. It does not seem to have affected anything on the sponsor side. People should not be afraid to come to Switzerland! Actually, the 30 percent increase on the Euro reported in the news only occurred for a few minutes – that's a banker's story, not real life. Plus, you get a lot for your registration fees – so you certainly won't starve!

Can you share your current research focus?

My laboratory is focused on pushing the very limits of mass spectrometry. In particular, we are developing innovative analytical workflows and tools to approach the whole gamut of analytes, from elements up to proteins, to support a systems biology approach. Because our field (from an analyte point of view) is very wide, it allows a great deal of cross-fertilization – something that I strongly believe in, which actually brings me back to the importance of conferences.

We currently have three main teams covering different facets of systems biology. The first team is involved in proteomics studies where we try to get a better understanding of proteins expression in dendritic cells in relation with early HIV infection. The "metabolomics" team is developing assays for metabolites in blood and tissue infected with malaria to measure the effects of drugs on these systems. The third team is working on surface analysis (MS imaging), particularly with forensics and plant materials. We are also spending a lot of effort in the data validation and in the automation of data processing workflows.

What drives your research interests?

I worked in the pharmaceutical industry before my time in academia, so I usually have an eye on medicine and biological processes. We need analytical technology and expertise to solve the complex problems found in these fields. Essentially, I want to be able to accurately measure any compound in any system.

Where is analytical science heading?

I am a big believer in the hyphenation of capabilities. We consider mass spectrometry as a separation technique and so we are very interested in the hyphenation of LC, ion mobility and fast high resolution MS. Another increasingly important area is the integration of automated sample preparation, where there are quite a number of new and interesting developments; for example, using magnetic beads, digital microfluidics and so on. Miniaturization is also key – however, I'm not talking lab-on-a-chip, but rather integrated small benchtop systems.

How did you find yourself so involved in analytical science?

I was educated as a chemist, did my masters work in a hospital as a toxicologist and my PhD thesis in organic mass spectrometry searching for biomarkers in sediments. I've always been interested in technology, whether mechanical, chemical or informatics-based. And analytical science combines technologies nicely. I want to follow technological advances but apply them in a very pragmatic way – and for me, anything related to health fits that ambition. I am convinced that analytical technology will have a major impact on personalized medicine.

HPLC 2015 Geneva takes place June 21–25. For more information, visit: www.hplc2015-geneva.org

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