MAY / JUNE 2023 # 111

the Analytical Scientist®

Future Shock

From next-generation battery development to improving our understanding of the brain, electrochemistry promises concrete solutions

SHIMADZU
Excellence in Science

Rookie of the year

Small size. Massive impact.

The MALDI-8020 is the newcomer in the Shimadzu family of MALDI products. This linear MALDI-TOF mass spectrometer combines talents and skills such as outstanding speed, accuracy and perform-ance. It targets researchers developing MALDI-based diagnostic methods as well as labs where quality control methods or rapid screening of intact samples are routine.

Small size
due to benchtop design with a compact footprint

Massive impact
through performance similar to larger, more expensive devices

Multi-talent system
for analysis of proteins, peptides, polymers and other analytes

Additional 'Rookie of the year' talents
such as TrueClean automated cleaning source, barcode reader and MALDI Solutions software for Pharma quality control labs

www.shimadzu.eu/rookie-of-the-year

A Solution to the Talent Gap?

Fix the world's most pressing problems – and then tell people

How can we attract budding young scientists to analytical science? At 15, when I was asked (forced) to choose just a handful of subjects in which to specialize, I remember considering my career options and wondering how I could maximize my impact on the world. Naturally, I set out to cure cancer. This, as you might have guessed, didn't quite work out. And despite an interest in chemistry (one of my chosen subjects), analytical science wasn't remotely on my radar.

Nowadays, there's an entire movement concerned with maximizing one's positive impact, especially at work – where most of us spend most of our time – namely, effective altruism. There's even an organization, called 80,000 hours (the average amount of time you'll spend working during your career), devoted to helping people choose high-impact careers. Interestingly, biomedical research features among their list of high-impact areas, but analytical science is nowhere to be found.

Does analytical science have a PR problem?

Last year, Lutgarde Buydens argued that young people today value more than ever the prospect of doing something to benefit society in their work, asking, "Why not analytical science – with the prospect of saving the planet?"

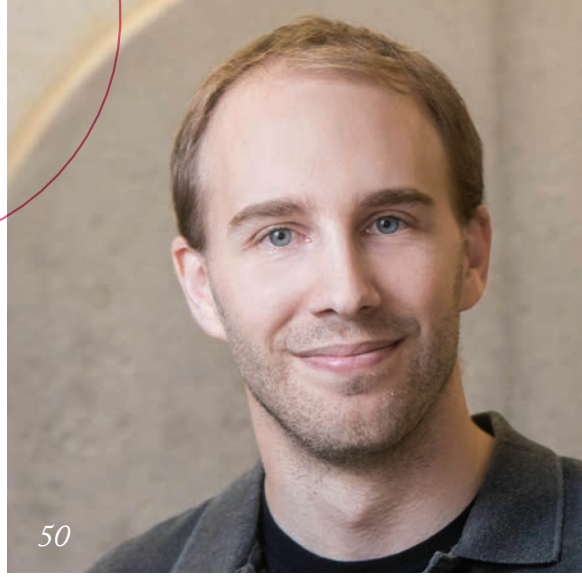
On our page 12-19 cover feature, Joaquín Rodríguez-López explains how electrochemistry holds the potential to expand renewable electricity with next-generation battery development, remove atmospheric carbon dioxide, and improve our understanding of the brain. Impactful enough?

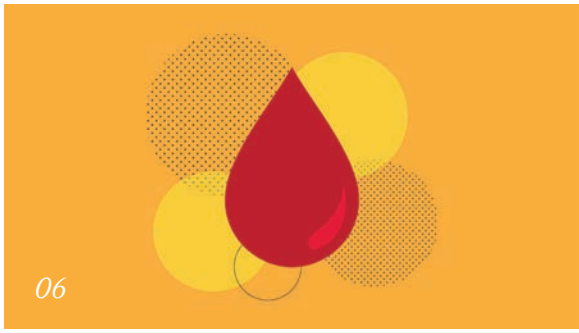
Rodríguez-López also highlights the importance of interdisciplinary working in the electrochemistry field, which involves materials scientists, physicists, engineers, and – crucially – analytical scientists. In fact, this was also a central concern for Buyden, who set up EuroFAST to reorient the field away from its silos to focus on common problems, such as access to good health and food, clean water and air, and sustainability. We've also taken this on board with our upcoming Power List, which will highlight the impact of "Connectors and Interdisciplinarians" (the List will be unveiled in August).

To get students in, the solution might be to get analytical scientists out – out into the wide world, working across disciplines, fixing important problems, and shouting about it! The more examples of impactful analytical scientists working on society's most pressing problems, the greater the chance of a young but aspiring individual thinking: "What about analytical science?"

James Strachan
Editor

Contents





On The Cover

Abstract artwork; concrete solutions. Electrochemistry emerges.

Core Topics

Sitting Down With

the Analytical Scientist

ISSUE 111 - MAY/JUNE 2023

Feel free to contact any one of us: first.lastname@texerepublishing.com

Content Team
Editor - James Strachan
Georgia Hulme (Associate Editor)
Jessica Allerton (Associate Editor)
Frank van Geel (Scientific Director)

Commercial Team
Publisher - Lee Noyes
Gaurav Avasthi (Associate Pubisher)
Chris Clark (Business Development Manager)

Design Team
Creative Director - Marc Bird
Hannah Ennis (Senior Designer - Commercial)
Téa Hewitt (Designer)

Digital Team
Head of Digital Operations – Brice Agamemnon
Digital Team Lead - David Roberts
Jody Fryett (Salesforce & Audience Sytems Manager)
Peter Bartley (Senior Digital Producer)
Jamie Hall (Audience Insights Analyst)
Shea Hennessey (Digital Producer)
Oliver Norbury (Digital Producer)
Seamus Stafford (Digital Producer)

CRM & Compliance
CRM & Compliance Manager - Tracey Nicholls

Commercial Services
Commercial Service and Social Media Manager - Matt Everett
Lindsey Vickers (Sales Support Manager)
Hayley Atiz (Sales Support Coordinator)
Julie Wheeler (Sales Support Coordinator)
Emily Scragg (Sales Support Coordinator)
Sophie Hall (Social Media Executive)
Emma Kaberry (Project Coordinator)
Bethany Loftus (Project Coordinator)

Marketing Team
Marketing Manager - Katy Pearson
Lauren Williams (Brand Marketing Executive)
Charlotte Shaw (Brand Marketing Executive)

Accounts Team
Kerri Benson (Assistant Accountant)
Vera Welch (Junior Accounts Assistant)

Human Resources
Human Resource Manager - Tara Higby

Management Team
Chief Executive Officer - Andy Davies
Chief Operating Officer - Tracey Peers
Senior Vice President (North America) - Fedra Pavlou
Financial Director - Phil Dale
Commercial Director - Richard Hodson
Content Director - Rich Whitworth
Creative Director - Marc Bird

Change of address info@theanalyticalscientist.com
Tracey Nicholls, The Analytical Scientist, Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK

General enquiries
www.texerepublishing.com | info@theanalyticalscientist.com
+44 (0) 1565 745 200 | sales@texerepublishing.com

Distribution: The Analytical Scientist (ISSN 2051-4077), is published bi monthly by Texere Publishing Limited, Booths Park 1, Chelford Road, Knutsford, Cheshire, WA16 8GS, UK. Single copy sales £15 (plus postage, cost available on request info@theanalyticalscientist.com). Non-qualified annual subscription cost is available on request.

One Drop Is All It Takes

Could a new multiomics microsampling platform "Amazonize" healthcare?

Looking to mine more health information from more people – and in an easier way – researchers from Stanford University have developed a strategy that combines multiomic profiling from a series of blood samples with physiological measurements from wearable sensors (1). Rather than using traditional blood draws, the potential of the approach relies on the notion that a microsampling device and 10 µl of blood is all it takes to measure thousands of metabolites, lipids, cytokines, and proteins. Sound familiar?

"It's Theranos that works," quipped Michael Snyder – Stanford W. Ascherman Professor of Genetics at Stanford and corresponding author of the paper – according to an article in USA Today (2). But how does it work? Well, it relies on lab-based mass spec.

"Mass spectrometry has gotten much faster and more convenient, and we can now measure thousands of analytes in a small blood droplet," says Snyder. "This analysis gives a much clearer picture of a patient's immune function, inflammation, metabolic markers, and overall health."

Indeed, the approach could allow scientists to ask interesting questions about the impact of lifestyle on health; for example, how the persistence of caffeine may correlate with sleep quality or how people respond to a nutrient shake. "Interestingly, some people had a proinflammatory response and others had an anti-inflammatory response to the exact same shake," says Snyder, reminding us of some healthcare buzzwords – precision and personalized medicine. Snyder believes that, in addition to answering very interesting research questions, the strategy will open the door to more large-scale biomarker discovery, monitoring, and health profiling. As for the Stanford team, further studies are underway, including research into chronic fatigue syndrome.

As with any new technology, there is scope to evolve the strategy: "There will be additional improvements in stability and sensitivity, allowing the device to follow many more molecules," says Snyder. But, while his team is busy working away, he wants diagnostic professionals to know this type of testing is coming. "It will be more powerful than what is measured in a physician's office," he says. "I believe that at-home testing will become common."

Snyder concludes with a thought-provoking prediction: "Healthcare will be 'Amazonized.'"

References

1. *X Shen et al., Nat Biomed (2023). DOI: 10.1038/s41551-022-00999-8.*
2. *USA Today News, "A geneticist studied one drop of his blood – and saw things he couldn't from a vial of blood" (2023). Available at: https://rb.gy/c4my2.*

TIMELINE

Four Years of LC Innovation

From Milan to Düsseldorf: the key liquid chromatography products launched since HPLC 2019

2019

Infinity Lab LC/MSD iQ

Agilent aimed to bring mass detection to a wider market with the LC/MSD iQ.

2019

Solvere Carbon Selective Detector for HPLC

Activated Research launched the world's first universal flame ionization detection (FID) for HPLC in 2019.

2020

Thermo Scientific Vanquish Core HPLC System

Thermo released their advanced LC system for optimizing routine testing workflows in 2020.

BUSINESS IN BRIEF

Oligo analysis, innovation research awards, digital acquisitions, and more

- **Waters** recently unveiled its new MaxPeak Premier Oligonucleotide Columns – now with ethylene bridged hybrid (BEH) particle technology in 300Å wide pore versions, which Waters says enables high resolution separation of long-mer oligonucleotides. Waters is targeting applications in cell and gene therapies, mRNA vaccines, and nucleic acid therapies.
- **Bruker** has announced the acquisition of ZONTAL: an integrated digital laboratory. ZONTAL products aim to streamline communication between IT systems and lab devices, preserve digital assets, and eliminate manual processes through regulatory automation. The acquisition builds on Bruker's BioSpin's Integrated Data Solutions (IDS) software division, which includes Mestrelab Research, Arxspan, and Optimal.
- **SomaLogic** recently announced their sponsorship of the Genomax Research Grant Award for researchers in Singapore – their first grant in the APAC region. Two winners will receive proteomic data from SomaLogic's 7,000-plex assay to be used at Singapore-based Molecular Genomics – the first site to offer the SomaScan Platform.
- **SCIEX** and **Waters** have collaborated to create the BioPhase 8800 driver for Empower software. The technology aims to give scientists direct control of system integration in standard workflows, minimizing method development, improving characterization, and shortening drug development timelines.
- Jiangbin Ye, Assistant Professor of Radiation Oncology, has been recognized for his work in cell biology and cancer research with the **Agilent** Solutions Innovation Research Award. This award aims to support advancements in drug discovery and cancer research – helping Ye with further work on the intricate connections between the Warburg effect, epigenetic remodeling, and cancer cell dedifferentiation.

Toxic Gear

The volatile organic chemicals responsible for the "new car smell" could increase incremental lifetime cancer risk

Could the well known and oft-desired "new car smell" pose health risks to drivers and their passengers? A team of researchers based out of China and the US set out to find out.

Used gas chromatography-mass spectrometry, the team sampled and analyzed the air in cars exposed to realistic environmental conditions over a 12-day period. The results revealed 20 chemicals present in the air that were linked to the car manufacturing process. Some were volatile organic compounds (VOCs), such as formaldehyde and acetaldehyde, which exceeded government safety standards by up to 61 percent (1). Unsurprisingly, VOC emission behavior depended on the temperature of material – which rose significantly when exposed to direct sunlight.

The authors concluded that exposure to the VOCs via inhalation, ingestion, and dermal uptake pose a "high health risk for drivers," by increasing incremental lifetime cancer risk.

Reference

1. *H Wang et al., Observation, prediction, and risk assessment of volatile organic compounds in a vehicle cabin environment (2023). DOI: 10.1016/j.xcrp.2023.101375.*

2021

BioAccord System with ACQUITY Premier

Waters aimed to enhance biotherapeutic monitoring through improved analyte recovery and increased assay-to-assay accuracy and precision.

2021

LC-Raman System

Shimadzu and Horiba joined forces to merge LC separation and Raman visualization.

2022

The Agilent 6475 triple quadrupole LC/MS system

Agilent launched the 6475 triple quad – with automated sample reinjection – at ASMS 2022.

2023

Alliance iS HPLC System

Most recently, Waters launched the Alliance iS – with touchscreen interface, in-built system health checks, and guided troubleshooting.

Tame the Flame

Paleoenvironmental analysis reveals indigenous burning has been present in Australia for over 10,000 years – and could help suppress the intensity of bushfires in the modern day

Researchers in Australia analyzed charcoal sediment from Lake Couridjah using infrared (FTIR) spectroscopy to learn more about indigenous cultural burning practices. By applying chemometrics and combining the results with local archaeological records, they found that cultural burning has been taking place in Australia for at least 10,000 years (1).

The research showed that fire intensity in the current interglacial period is significantly lower than it was in the one previous, despite having similar climate conditions and vegetation characteristics. The only difference was the presence of people during the present interglacial – suggesting that indigenous people were influencing the timing and frequency of bushfires. The researchers believe the findings about that past could help with developing mitigation strategies that could reduce the intensity of bushfires in the modern day too.

The researchers prepared the charcoal samples without using oxidants, such as hydrogen peroxide, after discovering that those commonly used methods removed a significant proportion of charcoal formed at lower temperatures (below about 450°C). "This is important when considering all fires – including so-called 'cool' fires, which are thought to have been used by Indigenous peoples to reduce large bushfires," says Mark Constantine, Paleoecologist and Researcher at the Earth and Sustainability Science Research Centre in Sydney Australia, and lead author of the study.

The samples were scanned with FTIR spectroscopy, and the spectra were compared with a lab-produced reference library using statistical modeling to identify and compare their chemical characteristics. Next, a charing intensity value is assigned to the data, which is a combination of maximum heat influx and time of exposure that formed the charcoal.

"The next step for the research would be to focus on improving and expanding the model by using charcoal that has been produced through more advanced methods to better approximate bushfire conditions."

Constantine ends with a tempting invitation: "I'd like to welcome other analytical scientists who are interested in extending this research further to collaborate!"

Reference

1. *M Constantine et al., "Exploration of the Burning Question: A Long History of Fire in Eastern Australia with and without People," Fire, 6, 4 (2023). DOI: 10.3390/fire6040152.*

RIME and a Reason

Why African Americans are more susceptible to aggressive prostate cancer at a younger age

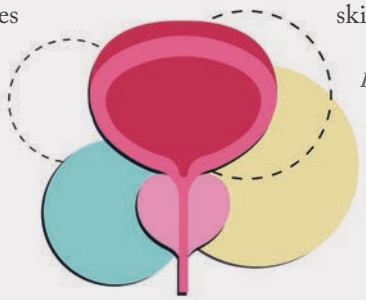
An association between African American prostate cancer and vitamin D_3 deficiency is well established – but why?

Researchers undertook proteogenomic analyses of vitamin D receptor (VDR) in European American and African American prostate cell lines to find out.

They used chromatin immunoprecipitation sequencing (ChIP-seq) to isolate VDR DNA and associated proteins, which they analyzed with rapid immunoprecipitation mass spectrometry of endogenous protein (RIME).

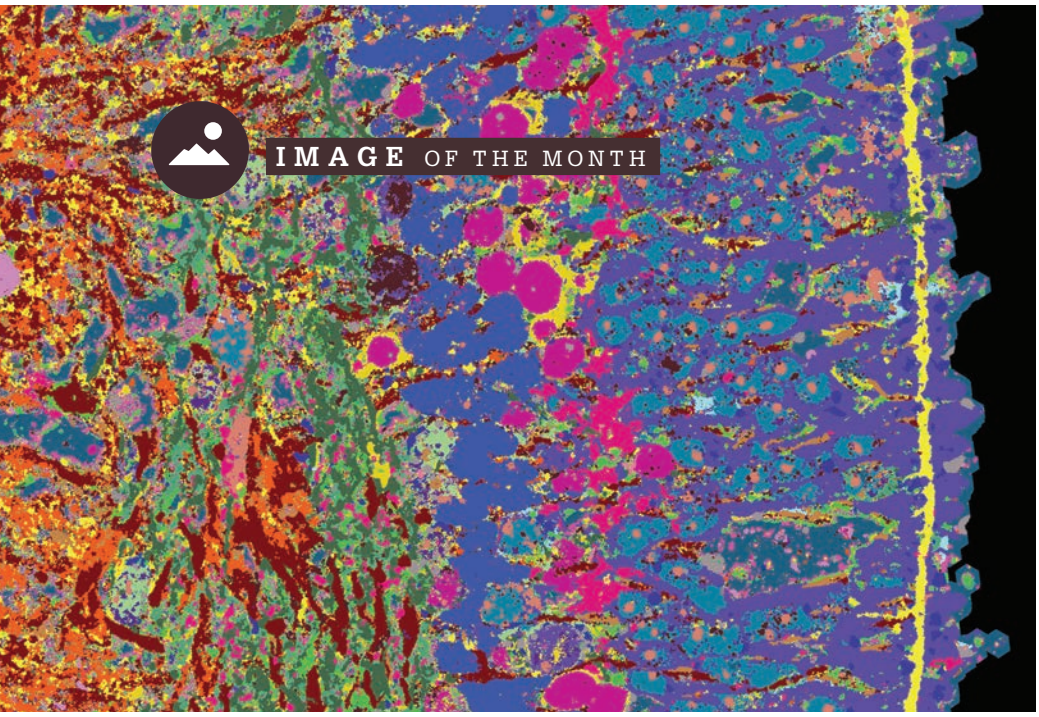
The results showed that African American and European American prostate cells have clear differences in VDR complex composition and transcriptional function that could explain the differences in prostate cancer susceptibility (1).

"Individuals with European ancestry may have adapted to environments with reduced sunlight exposure by having lower melanin content in their skin, which allows for more efficient vitamin D synthesis," says Moray Campbell, lead author of the study. "However, this adaptation may have resulted in other biological changes beyond skin pigmentation."



Reference

1. M Campbell et al., Cancer Research Communications (2023). DOI: 10.1158/2767-9764.CRC-22-0389.



Pollock's organoid

In order to understand more about how retinal organoids develop, researchers from Switzerland employed new immunofluorescence imaging technology – 4i – to visualize several dozen proteins in a thin tissue section at high resolution. The resulting Pollock-esque image isn't just for show – the colors represent the various tissue structures that make up the retina, and ultimately, the research may lead to new insights into ophthalmic diseases.

Credit: Wahle et al., Nature Biotechnology (2023).

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

QUOTE OF THE MONTH

"People hate to admit and talk about their mistakes (which is why errors are very seldom discussed in publications). People are also frustratingly resistant to changing their false convictions. I also learned to hope that this should change – after all, the human mind is both inherently brilliant and inherently fallible, and no real progress nor real innovation can happen without making a fair number of mistakes."

Csaba Szántay Jr. See page 26.

Snacking on Snails

Vibrational spectroscopy and scanning electron microscopy reveal cooking and consumption of giant land snails amongst early *Homo sapiens*

There is still a lot we don't know about our ancestors' behavior and diet – but it may have been somewhat slimier than you might expect. According to a recent study, African land snails may have been an integral part of human diets.

Credit: Marine Wojcieszak

The international team of researchers analyzed ancient snail shells using Raman and Fourier transform infrared (FTIR) spectroscopies, alongside scanning electron microscopy (SEM), which showed modifications related to rapid drying from heat.

The results suggested that vast numbers of snails were brought to Border Cave in South Africa between 170,000 and 70,000 years ago to be cooked and eaten (1). Additionally, the study showcased some important social implications: the transport of snails to a home base suggests food sharing among early *Homo sapiens*.

"It is interesting to see that altruism was just as fundamental in the early days for survival and well-being as it is today," says lead author Marine Wojcieszak.

Reference

1. M Wojcieszak et al., Quaternary Science Reviews (2023). DOI: 10.1016/j.quascirev.2023.108030.

In My View

Experts from across the world share a single strongly held opinion or key idea.

SFC Goes Large Scale

Supercritical fluid chromatography adoption is increasing, slowly but surely, across several industries – especially for large-scale applications. But more minds must change before SFC reaches its true potential.

By Isabelle François, Founder and Director, Chromisa Scientific, Belgium; and SFC Product Manager, Thar Process, USA

Supercritical fluid chromatography (SFC) is perceived as "green" in comparison to other chromatography methods: it makes use of available, non-toxic carbon dioxide, lowers energy consumption, and generates less waste than traditional chromatography approaches. Furthermore, separation speed is significantly higher in SFC, and the technology offers orthogonal selectivities, combined with high robustness and reproducibility. With increasing industry demands to enhance sustainability across all disciplines, I (and others in the field) expected the popularity of SFC to increase such that it would become standard in many analytical labs. Unfortunately, this hasn't happened.

My colleague Caroline West recently discussed the lack of development in SFC instrumentation over the past decade – despite our overall understanding of the technique having improved by quite some margin. She attributed the resistance primarily to people not wanting to learn a new technique or invest in a new instrument. This is a fair comment. But, in my position as a consultant working across various industries, I'm beginning to see some encouraging signs – especially where large-scale chromatography is concerned.

An increasing number of companies are looking for greener solutions, and there simply aren't many other practicable solutions on the market. By using carbon dioxide, we can access high pressures – even up to 800 bars – which opens up many different types of compounds for separation, and similarly for extraction. Furthermore, 60 cm (or even larger) columns are now available for large-scale SFC systems, which has opened up many new opportunities for purification. For example, KD Pharma uses multiple SFC systems in their facility to successfully purify omega 3 and omega 6 products.

As a highly regulated industry, pharma is reluctant to adopt SFC – as it is with any new system. But the COVID-19 pandemic has begun to change things, certainly for large scale purification – with companies involved in vaccine development realizing that

"I'm beginning to see some encouraging signs – especially where large-scale chromatography is concerned."

the large amounts of purified lipids required for vaccine encapsulation necessitate a rethink to reduce costs. Compared to large-scale normal-phase LC purification, the process mass intensity (PMI) of SFC is significantly lower because CO_2 recycling is easily obtained, and purified compounds are collected in smaller volumes of solvent, which reduces solvent evaporation as well as waste disposal. In addition, ATEX requirements for a facility utilizing pure CO_2 or CO_2 combined with a low co-solvent flow are more attractive from a cost saving perspective.

There is, however, a long way to go. Even pharmaceutical companies that have adopted SFC for discovery are often still mitigating to take SFC to the development stage and revert back to the synthesis route, but SFC can be profitable enough to balance this for intermediates production, or even finished products. But there are many other interesting extraction applications utilizing supercritical (or subcritical) CO_2 currently being considered at very large scale. Many revolve around recycling and valorizing waste streams but all by applying a sustainable footprint.

Polymer producing companies will soon be required by government regulations to use recycling sources for most of their products. With successful alternatives available, there is no need to waste huge quantities of water for dyeing clothes when the same results can be obtained by using carbon dioxide. Another advantage in SFE is the fact that the raffinate is dry when pure CO_2 is applied, which facilitates waste disposal or further raffinate upcycling.

One industry in particular that appears more open to adopting SFC and supercritical fluid extraction (SFE) is medical cannabis These are often start-up companies, and they start from scratch, with sizable budgets. I often talk to CEOs of start-up cannabis companies who aren't sure on what exactly they're required to do on the separation front. But they lack the preconceived notions about SFC and its rivals that you see in other industries, which means they're often more open to greener and cost-effective approaches.

"I would also say that a lack of knowledge is holding adoption back in some areas, so education is of utmost importance. There is a misconception that SFC cannot match the benefits of HPLC, for example."

Despite the improved sustainability of the approach, the clincher for most companies across these various industries is often capital cost. The systems cost more at initial purchase compared to liquid alternatives, but this is quickly recouped through reduced operational costs. This needs to be addressed in the business case, the total-cost-of-ownership is significantly more profitable at the large scale for SFE and SFC.

Over the past 5–6 years, I've found it increasingly easy to convince people that SFC is more cost effective, in many cases, than traditional chromatography approaches. At Thar Process, projects often start with R&D trials, where you can make calculations; it's black and white. This wasn't always the case for large-scale SFC – indeed, large scale SFC systems haven't always been available. As the message begins to filter down, I expect the number of companies adopting both SFE as well as SFC to increase.

I would also say that a lack of knowledge is holding adoption back in some areas, so education is of utmost importance. There is a misconception that SFC cannot match the benefits of HPLC, for example. And many simply aren't aware of the possibilities of using carbon dioxide; for example, as a recycled product, carbon dioxide can valorize waste products, which removes toxic and environmentally harmful chemicals from the process. Those more familiar with SFC may know that it eliminates the use of toxic solvents and reduces energy consumption and waste. But do they know that it can also simplify post-extraction or post-purification workflows? Do they know it can reduce operation costs in the long run?

As Caroline said, when considering analytical-scale SFC, we do need further developments on the instrument side. There were several new developments around a decade ago, but, since then, vendors have largely stopped improving SFC instruments. Today, I can only see one of those 2012–2014 SFC pioneers continuing in this area.

The recent increase in interest for large-scale chromatography applications is encouraging. But as a true SFC fan, I'd like to see many more users open their minds to SFC!

FUTURE SHOCK

Electrochemistry has almost limitless potential. Whether expanding renewable electricity with next-generation battery development, removing atmospheric carbon dioxide, or improving our understanding of the brain, electrochemistry is front and center – but collaboration between materials scientists, physicists, engineers, and – crucially – analytical scientists will be key.

By Joaquín Rodríguez-López

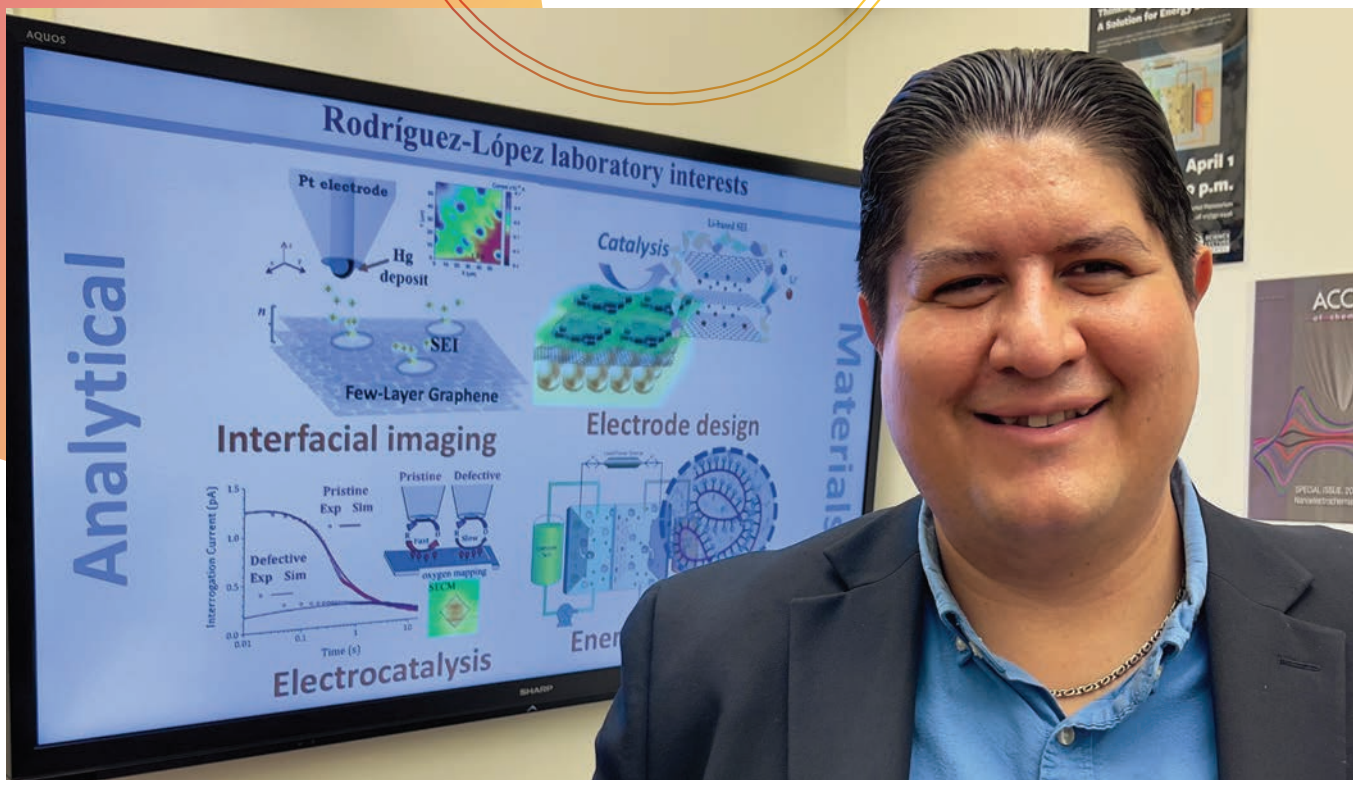


Image 1. Rodríguez-López and his team work at the interface of analytical and materials chemistry.

Most of the students interested in joining my lab do not come because they want to make measurements with small electrodes. They come because they want to solve big challenges – reducing the levels of carbon dioxide in the atmosphere, creating next-generation batteries that can store the electricity from growing solar and wind farms, putting renewable energies to good use in water remediation, or addressing the accumulation of polymers in the environment. However, understanding electrochemistry – a science at the interplay between chemistry and electricity – is central to tackling these challenges.

Electrochemistry is obviously not new – starting from Volta's and Galvani's experiments in the late 1700s, it continued developing alongside our ability to transduce chemical quantities into electric quantities, which is what we now do in most modern analysis. What is new and gives electrochemistry a new potential for change is the scale and the scope of its applications.

The scope? We have our growing appreciation for the potential of directly using electricity, primarily from emerging and rapidly growing renewable sources, to perform chemical reactions that were previously the domain of thermocatalysis or traditional synthesis. We now see emerging trends in using electrosynthesis for the production of hydrocarbon fuels, ammonia, hydrogen peroxide, and many specialty organic chemicals.

The scale? Well, that's largely driven by the rapidly dropping costs of renewable electricity, which couples to societal needs – that go beyond small batteries for your cell phone. Now, we are ambitiously targeting transportation and storage for the electrical grid, as well as the chemical industry, which also looks towards decarbonization to accomplish goals set by environmental policy makers. Direct air capture of carbon dioxide, for instance, has implications at the scale of our "globe-sized beaker!"

"MY TEAM WORKS AT THE INTERFACE OF ANALYTICAL AND MATERIALS CHEMISTRY."

The challenges described above are complex, but one thing is clear to us: It's a great time to do electrochemistry – and that forms the basis of our group's philosophy. My team works at the interface of analytical and materials chemistry (see image 1); in short, we develop new ways to look at electrochemical energy materials and interfaces. We also make use of materials concepts to develop new analytical methodologies. This

Image 2. Rodríguez-López and a student inspect an interdigitated array used in the detection of degradative events for redox flow battery electrolytes.

Image 3. Rodríguez-López and a student work at an electrochemical scanning probe microscope, which can use many different modes to look at the reactivity of samples in solution.

interplay gives us great versatility to both figure out what happens with a material and come up with strategies and solutions to immediately address materials issues as we measure them. In a way, this is not a new strategy – our colleagues in disciplines such as materials science and engineering have been doing this for a while, using advanced instrumentation, such as X-ray and electron microscopy methods. What distinguishes us? Our ability to conduct chemical and reactivity measurements in situ and operando (in other words, in the proper electrochemical environments or during operation of the electrochemical devices) with high versatility in the laboratory, using spatially resolved (electro) chemistry tools. In that sense, we are closer to what many analytical scientists do.

A great example in our own laboratory is the development of nonaqueous flow batteries – a main collaborative project that our lab contributes to as part of the larger Joint Center for Energy Storage Research (JCESR, an energy innovation hub funded by the US Department of Energy). We are looking into ways to enable the use of energy storage in the form of charged molecules in organic electrolytes, which may afford them greater energy densities and versatility in structures than those allowed by aqueous electrolytes. One development from our end was the introduction of size-exclusion flow batteries, which involves using highly soluble redox polymers as charging media. Sitting at the interface of analytical and materials chemistry, we've been able to conceptualize the design of these materials, collaborate hand-in-hand with materials chemist to create more optimal molecules and battery devices, measure the electrochemical attributes of these molecules in different contexts from solutions to single particles, and then operate the devices while we monitored the activity using our in situ methods (see image 2).

Big things in small packages

One interesting trend in the electrochemistry field is that, though the ambition of the field has grown in scale as previously discussed, progress in these areas is often driven by a reduction in the scale of the analyses themselves.

Certain inventions, such as the glass electrode used for measuring pH, and the development of popular techniques, such as cyclic voltammetry, were true game changers at the foundations of analytical chemistry in the 20th century. We now teach these concepts routinely at the undergraduate level. But when you combine these kinds of concepts with scanning probe microscopy, we're able to learn about smaller-scale applications, such as battery interfaces, catalysts in action, or living biological entities. This integration happens under the umbrella of "electrochemical imaging," and reminds me of the movie where the rat pleasantly combines the cheese and the strawberry (separately they are both good, but together they create a whole different concept). Similarly, we combine sensors with a scanning probe and advanced electrochemical techniques to evaluate the electrochemistry of small structures – whether cells, microorganisms, metal nanoparticles, individual polymer particles, or even features or adsorbates on a catalytic surface (see image 3).

> "WHAT GIVES ELECTROCHEMISTRY NEW POTENTIAL FOR CHANGE IS THE SCALE AND THE SCOPE OF ITS APPLICATIONS."

There are many questions at this frontier. How do you measure the activity of a single catalytic site? How do you understand the complex processes undergoing a single particle of a Li-ion battery? How do you map the activity of a single synapse or a single droplet of an aerosol? These types of measurements distinguish themselves from others in that they capture the action of processes – not solely the structure. Measuring nanoscale phenomena and individual entities is one area where the electroanalytical community is highly active.

This trend is driven by developments in scanned probe techniques, the availability of nano-resolved scanners, and some maturity from the communities engaged in associated techniques (for example, scanning tunneling microscopy and atomic force microscopy). But in my field of (electro)chemical

Image 4. Rodríguez-López discusses new experiments on CO_2 capture with students and postdocs.

measurement and imaging, the main realization was that one could produce probes in many different formats to address reactivity in solution – from tiny hollow pipettes to small electrodes made of various materials to probes that can pick up a single particle.

Looking to the future, machine learning looks like a distinct game changer. Scientists working on scanned probe microscopy have started using these computational tools to perform automated measurements of materials in vacuum setups. Recent publications have showcased exciting strategies using advanced algorithms that are capable of reducing the complexity of some electrochemical measurements, including electrochemical imaging ones. Using such methodology, we can perform a variety of tasks – repeating otherwise dull routines with high precision or using advanced pattern recognition to decouple sample morphology from activity, for example. It is only a matter of time before these types of developments are used in a significant portion of labs.

The big (and bigger) picture

I discussed the grand opportunities in using small probes to look at the action at electrodes for batteries or electrolyzers. But we also need to develop the ability to translate what we learn from these techniques to the actual devices – or to go from the in situ experiment to the operando experiment. Operando experiments, as outlined above, perform measurements in the actual device – for example, inside a battery, electrolyzer, or fuel cell. I think this is an area where analytical chemists – in partnership with many other disciplines including materials scientists, microscopists, physicists and most definitely engineers – have much to contribute. For example, analytical scientists could develop methods based on separations, spectroscopy, and diffractometry that speak to the needs of self-contained, closed systems that could be under pressure, electrochemical control, or in other difficult to probe environments.

These kinds of next-level measurements require two things: active collaboration/cooperation and education in electrochemistry. Collaboration implies a willingness to not only understand what the other is saying or doing, but also to have a proactive attitude to learn new things, strategies, and ways to shape working teams. An attitude of putting yourself

OUR PIECE OF THE PUZZLE

Our group develops strategies to look at chemical and electrochemical processes at the surface of electrodes using small electrochemical probes positioned near the action. We use a technique called scanning electrochemical microscopy (SECM) – although there are many variations of this concept that can be used. Some of the techniques we have introduced include:

- a Hg microprobe that can look at Li+ and other ions as they are inserted and released from an operating Li-ion battery electrode
- the use of a redox-nanotitrations that can measure tiny amounts of surface-bound species (oxides, hydrides, and so on) at electrocatalysts to understand surface reaction mechanisms
- the use of redox-active spin traps to identify otherwise elusive reactive oxygen species right next to the electrode
- the use of nano electrodes that can touch individual redox-active polymer micro and nano particles intended for redox flow batteries so we can perform charge/discharge experiments on these tiny entities.

We can also use these electrochemical techniques to probe non-electrochemical systems. All these techniques rely on a small probe that can engage in highly versatile measurement and imaging experiments with its subject – a Li-ion battery, a flow battery, an electrocatalyst. Energy sciences are a big focus of our group, but many of these techniques can be easily extrapolated to other systems such as electrolyzers for chemical production or even living cells.

Automation towards the self-driving electrochemical experiment is another aspect that our laboratory is exploring. We are big believers in the use of small electrodes, which offer a powerful array of electroanalytical techniques in small volumes. They can help us more efficiently explore many materials, such as those produced from high throughput combinatorial experiments or more modest wet laboratories. For example, we have recently developed automated routines by combining Python programs and third-party libraries with microfabricated electrode chips that allow us to perform otherwise complex redox-active lifetime measurements with simplicity and high reproducibility. In another work, we simplified the staple experiment for any aspiring electrochemist – measuring the diffusion coefficient of a molecule – using automated commands via an application programming interface that acts as a translator of high-level functions for potentially several commercial potentiostats. In fact, we trimmed the time it took for an exert user to perform the experiment from ~30 min to 1 min 40 s of actual work, while presenting a rigorous electrochemical analysis. We are now looking closely into the idea of a self-driving electrochemistry laboratory for energy materials discovery.

Whatever the ultimate applications, we are really excited about increasing the sophistication of electrochemical experiments using these new computational tools.

in your collaborator's shoes is also key for decreasing the "activation energy" for collaboration and for communication. I always tell my students that, regardless of the topic, my mission when attending any seminar/conference presentation is to actively answer a question: "How can my expertise and methods help this person in front of me?" I don't always express these views to the speaker (and, when I do, it is typically in private), but it is always a useful exercise to think outside of your own technical and disciplinary boundaries.

The second ingredient is education in electrochemistry. I don't like to assume that my collaborators understand what I am talking about, but I know that, if I give them the opportunity to learn more formally about my experiments, it could catalyze the collaboration process. In this context, my group has for the past 10 years been developing an experience that we call the "Electrochemistry Bootcamp." In its current form, this program combines laboratory and classroom instruction over three days. The course includes six training modules (basic electrochemical setup, electrocatalysis, simulations, ultramicroelectrodes, batteries and preparative electrochemistry, and scanning electrochemical microscopy), with each one divided into three round-robin demonstrations and activities in the laboratory or computer. As a result, students are introduced to 15 different experiments, six lectures, and one simulation session that enables them to learn the vocabulary and practice of electrochemistry. For novice students, the course helps decrease anxiety around setting up and running experiments; for advanced students, it challenges their knowledge and fills gaps. We are only able to run the course about once every year (the last one had 45 participants) – but imagine if we all did something similar, actively, and transparently helping others learn more about our own fields… I think we could dramatically accelerate collaborations to tackle big problems. And there are plenty of big problems.

Tackling climate change, understanding cancer, exploring the complexity of the brain, using versatile chemical sensors to make more efficient agriculture, optimizing water use and remediation, controlling pollution, designing the self-driving lab of the future, using renewable electricity whenever possible in industrial processes, removing plastics from the ocean, and addressing how redox species impact our body functions…

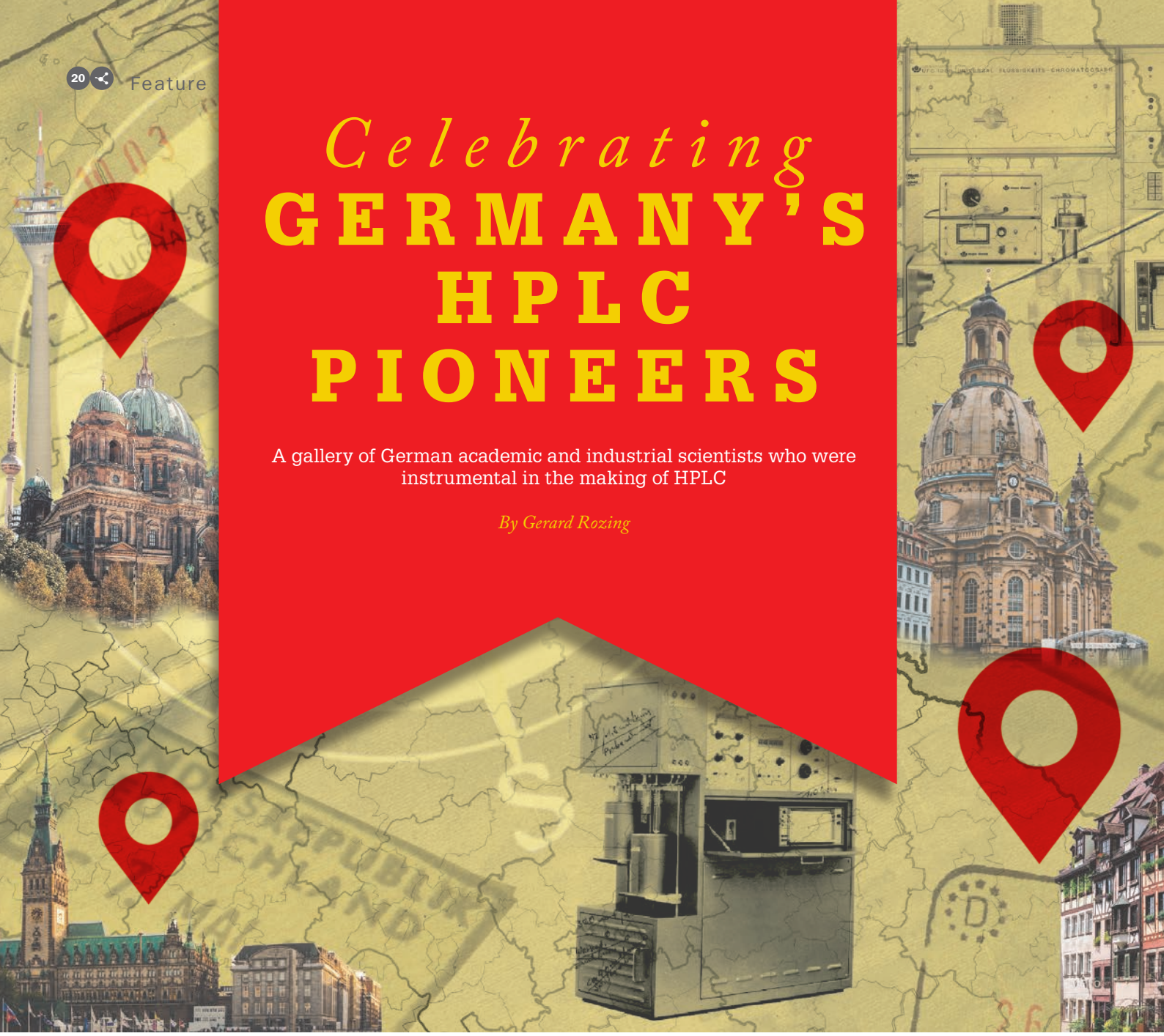
There are many complex and urgent gaps in human knowledge that we could help fill with better interdisciplinary electrochemistry. But, rather than being overwhelmed, I am excited and positive about the future of my field.

Joaquín Rodríguez-López is J. Andrew and Susan S. Langan Professorial Scholar, and Associate Professor of Chemistry, at the University of Illinois Urbana-Champaign, USA.

Celebrating GERMANY'S HPLC PIONEERS

A gallery of German academic and industrial scientists who were instrumental in the making of HPLC

By Gerard Rozing



Credit: Images sourced from Unsplash.com and Wikimedia Commons

The International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC) returns to Germany! After Baden-Baden in 1983, Hamburg in 1993, and Dresden in 2009, HPLC will be held for the fourth time at the Congress Center in Düsseldorf in 2023 (1) – its 51st incarnation!

HPLC has come a long way since the Symposium was founded in 1973 by Willy Simon, Jack J. Kirkland, Georges Guiochon, and Josef K. Huber – so far, in fact, that today's generation of (U)HPLC users may have forgotten about the pioneering scientists who helped build the foundations for today's LC innovation.

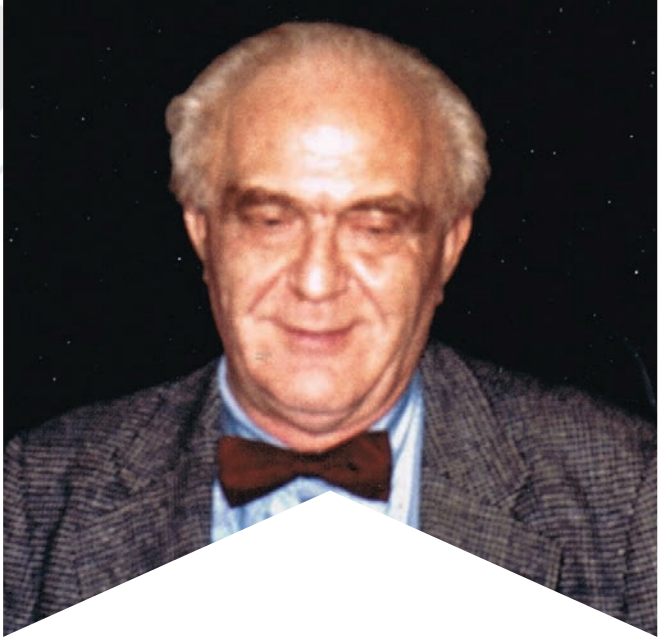
Over the years, many have broken new ground on the Symposium stage – from the fundamentals of liquid phase separation, HPLC column preparation, and stationary phase synthesis and characterization, to the latest equipment advances in high-pressure solvent delivery, sample injection, and coupling with mass spectrometry. It all happened at HPLC.

With that in mind – and to celebrate HPLC's return to Deutschland – we present a selection of German scientists and entrepreneurs who participated in the evolution and maturation of HPLC.

Welcome to HPLC's Gallery of Honor: German Edition.

István Halász (1922–88) – Ultimate Limits and Practice of HPLC

By Heinz Engelhardt, Retired



István Halász is an internationally renowned scientist in the theory and practice of chromatographic separation techniques.

Halász was born in Hungary and served in the Hungarian army during WWII. He moved to Germany in 1956 and started working at the Institute of Physical Chemistry at the University of Frankfurt/Main. Halász became a lecturer in 1961 and was promoted to Professor in 1964. Besides his academic work at Frankfurt University, he was the head of the "Gas Laboratory" at Scholven-Chemie AG at Gelsenkirchen (1957-1960).

Halász's work was focused on the fundamentals of optimizing chromatographic column performance. Initially, his improvements to capillary columns in gas chromatography led to the development of PLOT-Columns.

As early as 1967, Halász switched to liquid mobile phases. His practical and theoretical work in HPLC aimed to improve column efficiency via packing technologies, reduction of peak broadening in connecting tubes, the influence of column dimensions, particle diameter, and more. With his "pragmatical theory" of chromatography, he developed simple rules to evaluate the "limits" of HPLC.

Reproducibility, accuracy, and practical applicability of chromatography were at the center of his work – as well as transferring these values to his more than 50 students.

Halász died in 1988. In 1990, by his last will, the István-Halász-Foundation was established at the University of the Saarland, Germany, to support young analytical chemists.

The Hungarian Society of Separation Science (HSSS) has awarded the Halász-Medal to renowned scientists in separation science since 1997.

Heinz Engelhardt – *The* Test for Characterizing HPLC Stationary Phases

By Frank Steiner, Scientific Advisor, Thermo Fisher Scientific, Germany; and Markus Martin, Senior Staff Product Manager, Strategic Projects, Thermo Fisher Scientific, Germany

Heinz Engelhardt's main field of work has always been HPLC. His focus has ranged from pragmatic approaches to complex theoretical descriptions of LC, through stationary phase characterization, to developing stationary phase binding technologies for HPLC. His systematic studies of gradient elution in the 1970s and 1980s made the theoretical models of Giddings, Snyder, and others generally digestible. They led to practical user concepts for method development and scaling, laid down in a widely acclaimed monograph.

Another area in which Engelhardt made a significant contribution was the characterization of HPLC stationary phases with simple test compounds representative of hydrophobic, polar, or ionic molecular interactions. This work resulted in the well-known Engelhardt Test as one of the most prominent HPLC stationary phase classification methods (3).

Other research areas included synthesizing stationary phases for new selectivity in reversed-phase chromatography, ion-exchange chromatography, and enantiomeric separations.

After HPLC, Engelhardt's second main area of interest was capillary electrophoresis. In the 1990s, he investigated various surface modification technologies to actively modify and control the electroosmotic properties inside fused silica capillaries and suppress detrimental analyte-wall interactions.

His involvement in CE applications ranged from analyzing inorganic ions with indirect detection to enantiomeric separations and the characterization of polyelectrolytes.

While at Saarland University, he supervised 97 doctoral students who continue to pass on his knowledge and work ethic in industrial and academic research facilities worldwide. More than 250 research publications to date testify to his extensive and multifaceted scientific creativity in liquid phase separation science.

In 1990, after German reunification, he and Werner Engewald founded the first series of meetings for young German separation scientists, bringing together the generations of a divided country to learn from each other and bridge the gaps left by 40 years of separation.

In addition to publishing in peer-reviewed journals, Heinz Engelhardt has always aimed to communicate scientific knowledge to the everyday user of liquid phase separations, focusing on making it accessible to the German-speaking community. He published the first convenient guide to HPLC in the German language. Since its first publication in 1975, the book "Hochdruck-Flüssigkeits-Chromatographie" has gone through several editions and translations into several languages, including English, Russian, and Chinese. Among his many activities as a reviewer and consultant, he was editor of the journal Chromatographia for 13 years and a member of the Permanent Scientific Committee of the HPLC Symposium Series.

Klaus K. Unger (1936–2020) – the Master of Porous Silica

By Michael M. Schulte, Senior Director R&D, Merck Group, Darmstadt, Germany

From the beginning of his career, Klaus K. Unger aimed to increase the separation power of chromatographic systems. For example, he optimized the A-term of the van Deemter equation by using smaller and smaller particles (down to submicron particles), monodisperse instead of polydisperse particles, and spherical instead of irregularly shaped particles. He also worked on reducing the C-term in the van Deemter equation by using smaller particles (with shorter diffusion paths) down to the extreme of non-porous particles. The construction of uniform pores in an adsorbent, thus decreasing inhomogeneities in the pore structure.

In 1973, Klaus Unger spent six months as a visiting scientist in Barry Karger's laboratory at the Northeastern University in Boston. He, together with Istvan Halasz, Bengt-Arne Persson, Johan Kraak, Heinz Engelhardt, Peter Schoenmakers, and Wolfgang Lindner, formed an outstanding group of postdoctoral fellows who in later years became a network of pioneers in the field of HPLC. In Boston, Klaus Unger packed 1–3 µm silica into HPLC columns for the first time in his search for highly efficient chromatographic separation systems.

Unger's first publications in the 1960s demonstrated the need for highly efficient chromatographic systems. Unger packed glass columns of 1 cm diameter and 195 (!) cm length with different silica gel materials varying in their pore diameter from 20–25 Å up to 500–700 Å.

The Unger group worked on the synthesis of porous materials, mainly silica, that are used as adsorbents and catalysts. In addition, he worked on the functionalization and characterization of the surfaces of porous materials and especially on their application in chromatography.

In a landmark monograph published in 1979, Unger condensed all his knowledge on the structure of porous silica, the surface chemistry, the measurement of porosity, specific surface area, particle size, and size distribution, packing methods, surface modification and applications to ion exchange, and size exclusion chromatography. This book has become the bible of liquid chromatography on silica.

Unger also worked with E. Merck, in Darmstadt. The first two commercial products, Perisorb A and B, were based on the process

developed by Unger in his laboratory to produce porous silica from tetraethoxysilane via poly(ethoxysiloxane). These were followed by the well-known LiChrosorb and LiChrosphere.

After his (first!) retirement, Unger led a research group at Merck KGaA in bioseparation science in Darmstadt for another eight years until his final retirement in 2009.

Klaus Unger has been the author of over 400 publications, an author/co-author on 15 monographs, and co-editor of 17 Symposium Proceedings, the inventor of 55 patents on chromatographic materials and inorganic catalysts, and the supervisor of 125 PhD students.

The work of Klaus Unger has shaped the world of HPLC from the 1970 until today's modern UHPLC. He was truly a German giant and pioneer of chromatography.

Klaus died in 2020 at the age of 84 years.

Klaus-Peter Hupe – a True Scientist and Entrepreneur

By Gerard Rozing, Emeritus Agilent Research Fellow, and technical consultant at Rozing.com Consulting

As a scientist, Klaus-Peter Hupe brought HPLC from its infancy to maturity; as an entrepreneur, he shaped and established the industry. Hupe is considered the seed of Agilent's high-tech campus in Germany.

Hupe was born in 1931 in northern Germany. He studied mechanical and process engineering at the Technical Universities of Braunschweig and Karlsruhe, and received his PhD from the Institute of Thermodynamics at the Technical University of Karlsruhe in 1959 on "Heat and Mass Transport in Trickle Sheath Flow."

While working at the Technical University of Karlsruhe (which he did until 1962), Hupe was approached by Ernst Bayer, who asked him to build a preparative gas chromatograph (2). This instrument was received with great enthusiasm by users and created the demand for more systems.

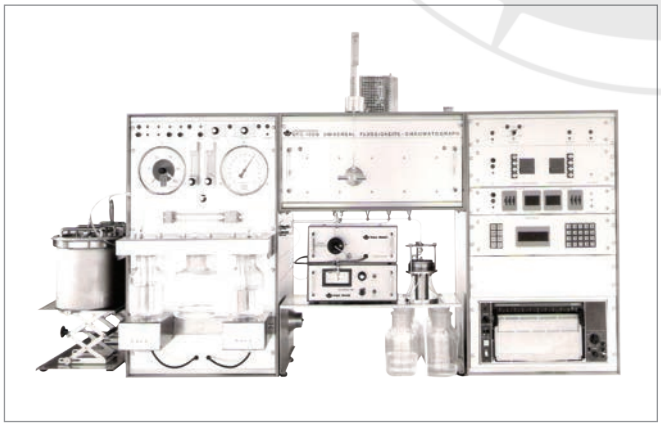
This was the basis for the foundation of the company "Dr. Hupe Apparaten Bau" in Karlsruhe, which was dedicated to the design, manufacture, and marketing of preparative gas chromatography systems. In this instrument, a column packed with polyethylene glycol particles of 40 mm i.d. and 2 m length was used to collect fractions in mL volume fully automatically.

With the emergence of the new field of liquid chromatography in the late 1960s, Hupe partnered with Ulrich Busch and founded the "Hupe and Busch" company, which entered the LC field in 1972 with the UFC 1000 Universal Liquid Chromatography System. This was the first fully integrated HPLC system on the market and the technology leader.

With this system, Hupe & Busch company was acquired by Hewlett-Packard in 1973, and the system was marketed under the HP brand name 1010.

Hupe became general manager of the HP division and oversaw the development of the next generation of integrated HPLC systems – the 1080 series. With the company's relocation from Karlsruhe to the new site in Waldbronn, Hupe stepped down as General Manager to concentrate on separation science until his retirement in 1995. During that time, he continued to guide Hewlett-Packard's HPLC instrumentation as a scientific advisor to the Waldbronn division, remaining very influential in product development, culminating in the prestigious HP 1090 series, which introduced narrow-bore HPLC to the market and made HP the technology leader in liquid chromatography systems into the late 1990s.

Hupe has been a member of the scientific committee of the HPLC Symposium for many years and was the chairman of HPLC 1983 in Baden-Baden.



The Universal Liquid Chromatograph Model UFC 1000

Herbert Knauer – a Passion for Scientific Instruments (Especially HPLC Systems)

By Oliver Gültzow, Senior Manager Marketing, KNAUER, Berlin, Germany

As a freshly graduated doctor of engineering working as a scientific assistant at the Technical University of Berlin in the early 1960s, Herbert Knauer spent his afternoons and weekends in the kitchen at home - not as a hobby cook, but instead working on new analytical solutions. Here, Knauer developed a precision instrument for measuring the smallest temperature changes of 1/1000 degree Celsius, which was sensational at the time.

With the university's permission, Herbert Knauer and his wife Roswitha founded the company "Wissenschaftliche Gerätebau Dr. Ing. Herbert Knauer Gesellschaft mbH" in Berlin-Schmargendorf on October 1, 1962. After further development, KNAUER made a name for itself as the first company in Europe to manufacture osmometers for chemistry and medicine.

Inspired by a visit to a trade show in Bratislava in the early 1970s, Knauer had the idea of entering the HPLC instrumentation market. As a chemist, he recognized the great potential of this analytical technology, which was still in its infancy. The only competitor at the time was Waters, and in 1974 Herbert Knauer and his company became the first German manufacturer of HPLC systems.

The first generations of HPLC systems were bulky and difficult to use. Their components, such as pumps and detectors, were all in the same housing, making repairs or replacements difficult. Knauer saw this as a problem that needed to be solved. After discussions with chemists and researchers at

Credit: KNAUER.

To view extended versions of the HPLC's Gallery of Honor, visit the HPLC website: *https://bit.ly/41F33R6*

References available online

Angela Merkel examining a production system for lipid nanoparticles.

various institutes in Berlin, he developed the solution: the first modular HPLC system. The stackable components were easily interchangeable, allowing different pump and detector combinations to be easily configured and repairs to be easily performed. This design would soon become the standard for HPLC systems around the world.

Herbert Knauer was always interested in further developing the instrumentation and columns. By 1987, KNAUER had dozens of stationary phases in its product line and manufactured separation columns with internal diameters ranging from 2 to 32 millimeters. Developments in this area also included column-filling equipment and capillary connection systems.

Early on, KNAUER also developed and produced instruments for competitors. The OEM business is an important pillar of the company. "The competition has always forced us to improve – we are very grateful for that," Herbert Knauer once said at an anniversary celebration.

On September 10, 2021, German Chancellor Angela Merkel visited the company and congratulated Herbert Knauer on his 90th birthday. She described the company as a "jewel in the German SME sector."

Although Herbert and Roswitha Knauer handed over the company's management to their daughter Alexandra Knauer in 2000, Herbert's passion for science and technology remains unbroken; he remains a familiar face at the KNAUER headquarters, where he still tinkers with components for HPLC systems.

Credit: Images sourced from Unsplash.com and Pixabay.com

WHO ARE WE?!

An analytical scientist's constructively fallibilist reflection on the meaning of "analytical scientist" – and how to avoid mental traps

By Csaba Szántay Jr.

In terms of taxonomical ranking, I am a member of the species *NMR spectroscopist* within the genus analytical scientist belonging to the family scientist. More than that, I am a rather rare kind of specimen that belongs to both the subspecies applied *NMR spectroscopist* and the subspecies theoretical *NMR spectroscopist*. During my career, I solved thousands of molecular structural problems and fiddled around quite a bit with the physical/mathematical theory of NMR. I learned from personal experience that there is a huge difference between the meaning of "scientific truth" depending on whether it relates to structure elucidation or theory. I learned that the "science" of structure determination is a completely different mental universe from the "science" of theory. I made mistakes, I uncovered mistakes made by others, and witnessed others uncovering mistakes both in the world of structure determination and in the world of theory.

My personal experiences, along with several studies published by others, have shown that there are shockingly many erroneous structures published in the scientific literature (see, for example, reference 1) – very often not because of a lack of good experimental data or a lack of technical expertise, but because of psychological reasons – *or mental traps*. Similarly, despite NMR theory being a robust intellectual construct, it is interwoven with a surprising number of misconceptions, even regarding the very fundamentals of that theory, which have become widely accepted by the scientific community – as has been pointed out by several NMR theorists (see, for example, references: 2,3,4,5,6,7). Again, the reasons for this can be traced back to various mental traps.

I developed a keen interest in – and explored – the nature of these mental traps quite thoroughly (8). In doing so, I also learned that people hate to admit and talk about their mistakes (which is why errors are very seldom discussed in publications). People are also frustratingly resistant to changing their false convictions. I also learned to hope that this should change – after all, the

human mind is both inherently brilliant *and* inherently fallible, and no real progress nor real innovation can happen without making a fair number of mistakes. I steadfastly believe that, besides learning how to avoid mistakes, one of the essences of good scientific thinking is self-revision – the ability to overrule our own convictions. But I recognize that self-revision is an incredibly difficult feat from a psychological point of view because our convictions are an integral part of our identity; changing our identity can be a painful inner metamorphosis.

But who else should lead the way in embracing our fallibility and channeling this understanding into a constructive direction if not scientists themselves – principally (self-)endowed with their quest to search for *truth?* It is in this spirit – and it is through this perspective – that I wish to put forth a few ideas below regarding the concept of *analytical science*.

Difficult familiarity

In his book, *Introduction to Mathematical Philosophy*, the famed mathematician and philosopher Bertrand Russell expressed the following piece of penetrating wisdom: "Just as the easiest bodies to see are those that are neither very near nor very far, neither very small nor very great, so the easiest conceptions to grasp are those that are neither very complex nor very simple (using 'simple' in a logical sense)" (9). Let me rephrase this idea to convey an even more generic notion: *Just as it is more difficult to see objects that are either very far or very near, so it is more difficult to understand concepts that are either very unfamiliar or very familiar.*

Albeit "difficulty of understanding" is a common denominator in these two scenarios, there is a fundamental difference between them; namely, we are typically *aware* of not understanding a very unfamiliar concept, but we are also typically *unaware* of *not* understanding a very familiar one (8). For us, analytical scientists, our own taxonomical label "analytical scientist" can all too easily fall into the latter category. After all, by *doing* analytical science day in, day out, and by seeing a representative member (the *same* member) of the species each time we look in the mirror, the very gist of *being* an analytical scientist becomes *so* familiar to us that we tend to develop a sense of *obviousness* about the *apparent* meaning of the term, thereby rarely pondering upon its *true* meaning.

Well, what does it mean? At the *obvious* level, the analytical scientist is a person whose job is to perform the task of physical/chemical analysis – may it be any segment of this broad field. But at a deeper and less obvious level, both words "analytical" and "scientist" carry further important layers of meaning, as I will point out briefly below, starting first with "science," and following with "analytical."

Science

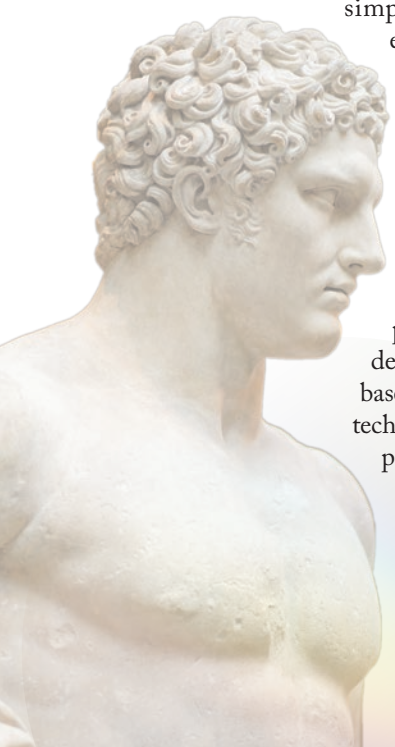
What does "science" mean in general? And what does it mean in the context of analytical science in particular? Defining science is not an easy undertaking. If you look up the term in, say, Wikipedia, you will find the following statement: "science is a systematic endeavor that builds and organizes knowledge in the form of testable explanations and predictions about the universe." True enough! But, as with most attempts aimed at condensing a complex and even somewhat elusive concept into an overly concise description, although true, it falls short of grasping many crucial aspects of science – perhaps even to the point of becoming misleading. For example, does this sentence indicate what type of knowledge constitutes *scientific* knowledge – or more basically, *scientific truth?* Does it convey the idea that there is a *scientific way* of arriving at that knowledge? By the same token, does it tell us *who*, of all the different kinds of "knowledge-builders," are truly *scientists?* Also, given the fact that there are significant differences in how people – including the knowledge-builders themselves – interpret the meaning of "science" and "scientist" both in practice and in principle, does this sentence provide sufficient academic guidance for resolving these different opinions? Hardly!

Let me have a go at a single-sentence portrayal of science that drills somewhat deeper into the matter – at the cost of becoming outrageously knotty, while still suffering from many shortcomings:

"As opposed to our apprehension of the natural world resting on non-evidence-based faith, science is a mode of investigation – the 'scientific method' – that aims to expand our knowledge of the universe by providing valid descriptions of the laws and facts of nature (in the form of verbally and/or graphically and/or mathematically formulated theories, models, or statements based on certain premises) that are applicable within certain boundaries, are created with a view to having both explanatory and predictive power, and are developed or validated through hypothesis testing involving sufficiently reproducible, precise, accurate, and unambiguous experimental data together with data-interpretation using deductive and/or inductive reasoning, with the process and the conclusions being thoroughly documented and shared with the global scientific community so as to be open for further verification, falsification, correction, modification, or advancement."

Well, that's admittedly quite a mouthful. But the reason I dwell on this issue (with such outlandish philological consequences) is not because I have some self-serving fixation on semantics, but because I firmly believe that a proper and collectively accepted (as much as the vision of such acceptance is not some impossibly idealistic ideal) understanding of the crux of science has a direct bearing on our identity as a scientist. And that identity truly matters!

For many people, *anything* that has been published in a peer-reviewed *scientific* journal carries *scientific* content by definition (otherwise how could it have possibly been *published?*). Also, for many people, the concept of science is associated primarily and simply with special technical expertise, precision of thought, highly developed and reliable methodology, and elaborate instrumentation, rather than with the quest of generating new knowledge. Though this broader interpretation of "science" seems to prevail, the truth is that the vast majority of papers published in scientific journals are derivative (evolutionary) research based on known ideas, methods and technologies, and only a much smaller portion of the papers exhibits really innovative, seminal (revolutionary) ideas (10). For me, it is the latter that constitutes genuine science in its purest sense. One of the essential identity-defining aspects of science is that it strives – via the *scientific* method – to stretch the limits of our knowledge with a view to attaining *new universal wisdom* through *new ideas*. By "new universal wisdom" I mean new insights and new theories about the way the universe works, new facts about nature that are of universal relevance, or new methodologies that can help us gain a wider and deeper understanding of nature's workings and provide better control over influencing nature-related systems (such as designing drugs). Consequently, a true scientist is not only a bearer of special expertise and high-level cognitive skills that they use to solve problems within the realm of our existing understanding of the universe, but someone who is constantly on a quest to expand that understanding. The difference can be huge in terms of mentality, attitude, and risk taking!



Of all the sciences, analytical science carries a special duality that is of particular relevance regarding the above considerations: analytical science is both the science of the *analyte* and the science of the analytical *method*. The vast majority of all analytical scientific activities are directed towards uncovering some information about the analyte via known analytical methods. A much smaller proportion of such activities focuses on advancing the theory and methodology of the analytical method itself. Published papers involving analytical science dominate in analyte-focused results, while method-focused discussions are far rarer. Of course, the two themes can be intertwined, since often it is a challenging analyte that calls for novel method development. Nevertheless, the difference between "analyte science" and "method science" can be sharp. Gaining knowledge about the analyte may or may not fall in the category of new universal wisdom, but, either way, it is the information about the analyte itself that is new, separately from the road taken to access that information. Often the analytical methods used and needed to extract that information are well established, and no innovative thinking nor new scientific insight is required on the part of the analyst beyond their technical expertise. Nonetheless, such cases – when published – are widely regarded as "scientific" due to the novelty of the information gained on the analyte. The quest for advancing the theory and practice of analytical methodology is an entirely different story. Here, new universal wisdom truly emerges based on innovative scientific thinking. That is where analytical science gains a broader significance than that associated with the investigation of a particular analyte. Again, it is this aspect of analytical science that I regard as *science* in its truest form.

The implications of these ideas go beyond being mere philosophical musings on the meaning of analytical science, and even beyond the issue of defining our identity on the basis of that meaning. In fact, there is a very real and very big difference between "analyte science" and "method science." One crucial aspect of this difference lies in the concept of

proof. Sure enough, in "analyte science" one can make faulty deductions. But if data is available in sufficient amount and quality, and if the problem is handled with sufficient expertise, the deduction can be considered "proven" for all practical purposes. The analyst can sleep well, with a smile on their face. Not so in the world of theories. This is the intellectual realm of inductive thinking, of constructing models, of simplifications, of gray zones of interpretations, of complicated (hard-to-see) mistakes. Nothing can be proven here with absolute certainty.

By venturing into the world of theories, the analyst must face nightmares revolving around whether they have made the right kind of simplifications, used the right kind of mathematics, and not overlooked something of fundamental importance. No matter how good the theory looks at the time of publication, there is always a chance that someone will point at the analyst publicly, saying: "Hey, this is where you did not take this-and-this into account!" This is the world of taking perpetual risks intellectually as well as in terms of self-esteem. Also, this is the world of true science, in all of its magnificence and painfulness. And understanding this aspect of analytical science places even more importance on becoming well versed in mental traps!

Analytical

In the context of the above considerations, the word "analytical" has a dual meaning: a technical and a mental. An analytical scientist is technically "analytical" because they perform analytical tasks. An analytical scientist is mentally "analytical" because they *think* analytically. Analytical scientists are very good at that – principally they are the epitomes of analytical thought. By the very nature of their job, an analytical scientist approaches and surrounds every statement with a healthy dose of analytical "lore" (involving such terms as accuracy, precision, reproducibility, systematic error, random error, limit of detection, limit of quantification and so on) that reflects this way of thinking. For an analytical thinker, a statement such as "the current outside temperature is 23°C" makes no sense. The analytical thinker would regard the statement as correct only if it is properly embedded in the necessary analytical rhetorical framework: "The current temperature is measured to be 23°C by such-and-such a thermometer that works with such-and-such accuracy, precision, systematic error, random error, and so on."

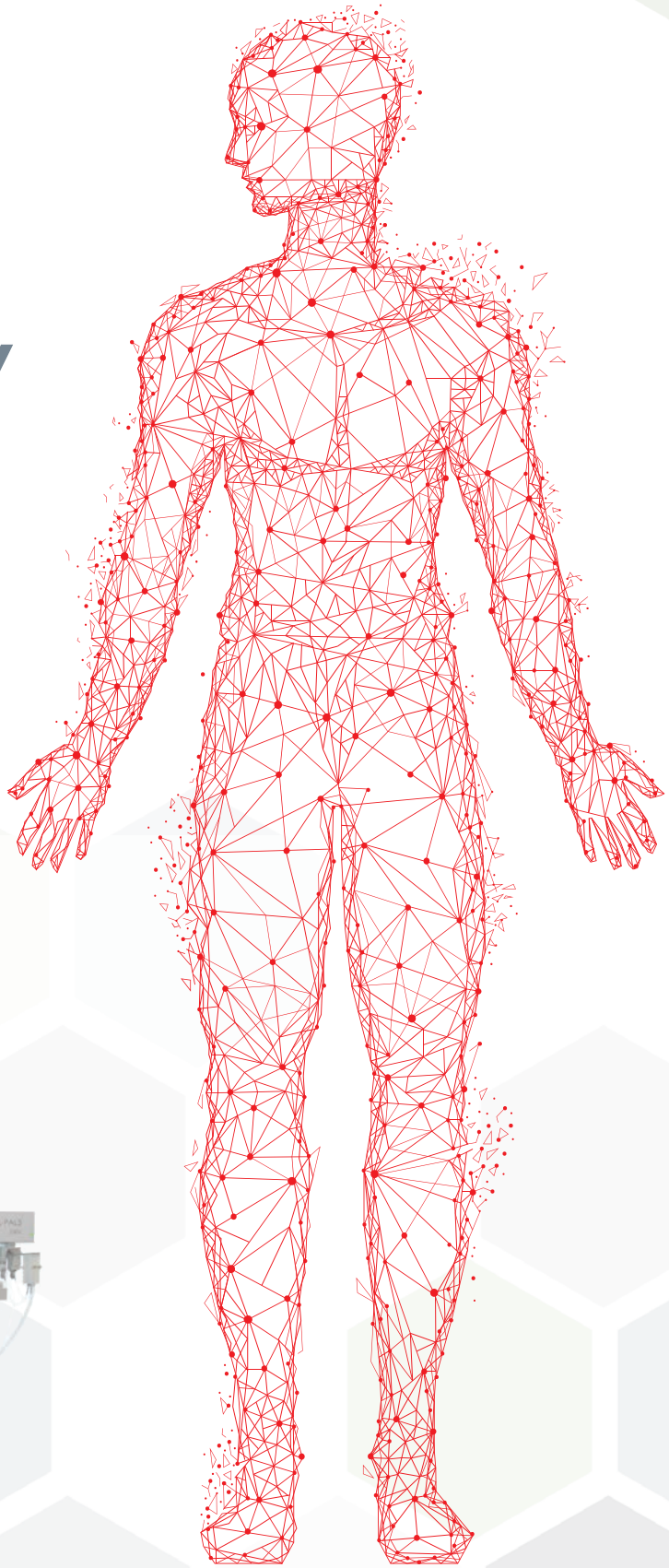
As for structure determination, a statement about the structure can range from "truly proven" to "the proposed structure is consistent with the available spectral data." Any good analytical scientist working within the realm of "analyte science" learns to think this way. Still, mistakes can happen.

For example, *believed* to be true can be all too easily confused with *proven* to be true (11,12). In fact, one of the main reasons that lead to erroneously assigned structures is the confusion of belief with fact. And that is why it is crucial to take analytical thought to a higher level, sensitizing oneself to mental traps. Things are much more subtle in the world of theory, where the whole concept of "a mistake" can become much less tangible than with facts. It is in this mental realm where analytical thinking is truly challenged, further raising the need to increase our acuity regarding mental traps. I truly believe that to preserve and advance the essential values of scientific thinking – and for this kind of analytical thought to have a ripple effect onto everyday thinking (which the world seems to need more than ever) – we not only need to understand and avoid our mental traps, but we need to embrace our fallibility in a constructive, open, and honest manner.

Based on the above ideas, I would invite all analytical scientists to share their experiences regarding the mistakes that they have made and the misconceptions that they have harbored. Let us learn from each other both technically and attitude-wise! Let us become better in being "analytical," as well as in being "scientific!"

References available online: https://bit.ly/3OLon4t.

Studying the Small Has Never Been a Bigger Priority



Multiple chromatographic approaches are needed to accurately study the metabolome. Fortunately, LECO has a complete GC metabolomics solution to take your samples from start to finish.

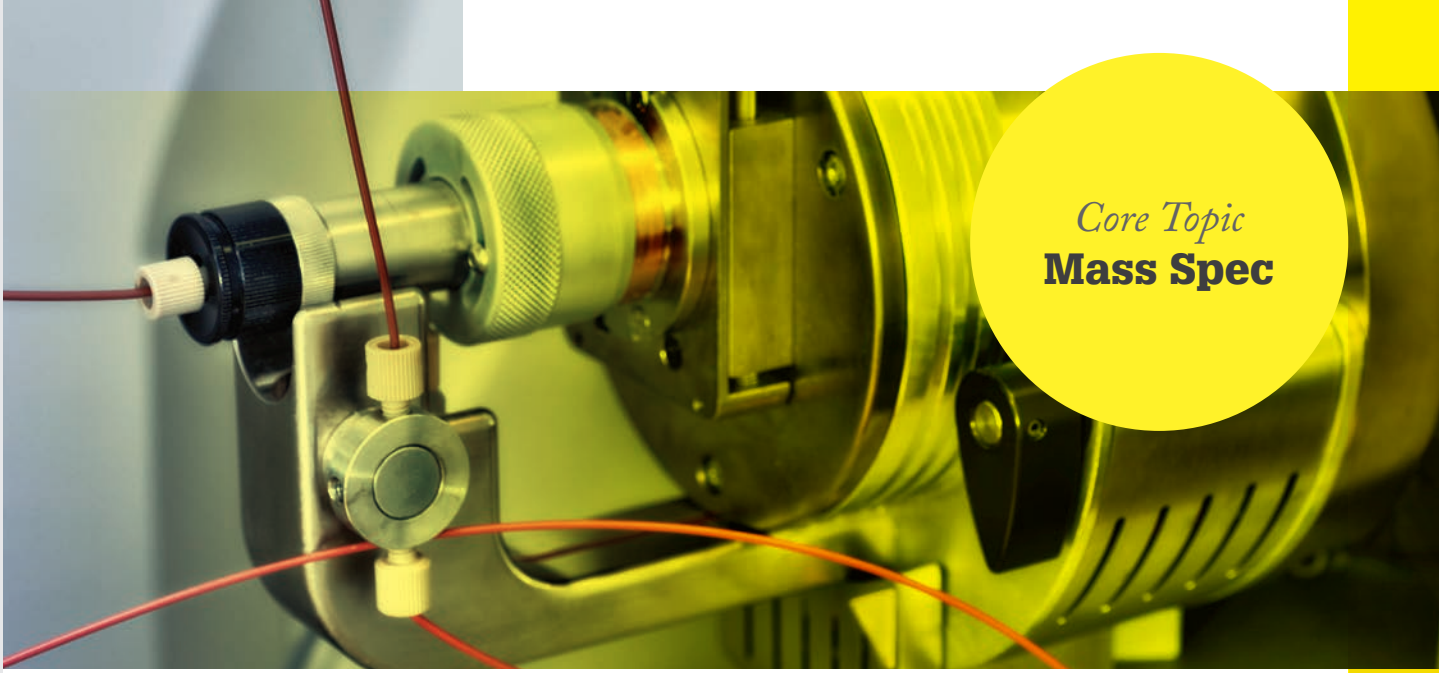
- New L-PAL3 with Derivatizer offers automated, just-in-time derivatization
- Seamless integration with GC-TOFMS technology
- Unparalleled speed, sensitivity, and ruggedness
- Innovative and user-friendly ChromaTOF brand software

Turn your data into discovery with LECO's turn-key solution to metabolomics.

Learn more at: https://info.leco.com/metabolomics

Phone: 1-800-292-6141 | info@leco.com
www.leco.com | © 2023 LECO Corporation

LECO
EMPOWERING RESULTS



Core Topic
Mass Spec

Could mass spec identify cognitive dysfunction? With millions suffering from dementia worldwide – and with the number only predicted to rise – early diagnosis and intervention for individuals with cognitive dysfunction (CD) are vital, meaning that a brief and effective screening tool could be life-changing. And that's why researchers have recently explored the potential of volatile organic compound (VOC) detection. Using high-pressure photon ionization time-of-flight mass spectrometry (HPPI-TOFMS), they were able to distinguish VOC patterns between CD and healthy groups – with 10 VOC ions showing significant differences.

The test of time. The ability to determine exactly when a bloodstain was deposited at the scene of a crime is invaluable information. And that's why researchers at the Zurich Institute of Forensic Medicine, Switzerland, are using untargeted LC-MS-based metabolomics to estimate the time since deposition (TsD) of bloodstains. The bloodstain metabolome changed significantly with increasing TsD, with phenylalanyl alanine appearing to be a promising candidate biomarker.

The metabolomics of ADHD. There is an increasing amount of evidence suggesting that there may be a link between gut microbiota and symptoms of ADHD. To explore this evidence further, researchers used using NMR spectroscopy and LC-MS to perform unbiased metabolomic profiling of urine and fecal samples collected from a Swedish twin cohort enriched for ADHD. Results revealed sex-specific patterns in the metabolic phenotype of individuals with ADHD: the urine profile of males with ADHD was characterized by greater excretion of hippurate, a product of microbial-host co-metabolism that can cross the blood-brain-barrier with bioactivity of potential relevance to ADHD. According to the authors, as we increase our knowledge of the effect of the microbiota-gut-brain axis in neurological disorders, microbiota will become an increasingly attractive target for neuropharmacological treatments.

Detecting SARS-CoV-2. Although RT-PCR tests are typically regarded as the gold standard for SARS-CoV-2 diagnosis, they do have limitations – namely, their inability to target multiple genes, limiting identification of SARS-CoV-2 variants. As such, in a recent study, researchers combined multiplex PCR with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for disease detection.

References available online

IN OTHER NEWS

Extraction protocol for RNA-seq and metabolomic analysis promises more complete picture of cellular activity than either technique alone.

New machine learning tool dubbed CRANK-MS – aka Classification and Ranking Analysis using Neural network generates Knowledge from Mass Spectrometry – shows promise in detecting Parkinson's disease years before first symptoms.

Zooarchaeology by mass spectrometry (ZooMS) reveals that domestic chickens have been successively bred since the fourth and third century BCE in Japanese archipelago.

Using MC-ICP-MS, researchers may have identified new biomarker for early diagnosis and screening of autism spectrum disorder – noting significant alterations in blood metallome.

Mass Spectrometrists Versus Mass Spec Users

Is the demand for analytical scientists with extensive training in advanced MS techniques exceeding the supply of such individuals?

By Rick Yost

When I attended my first ASMS Annual Conference as a new grad student way back in 1976 in San Diego, there were perhaps 600 attendees, and it was clear that all (or nearly all) of them were "mass spectrometrists" – that is, individuals who understood, or at least wanted to understand, what was happening inside the mass spectrometer. And that was indeed necessary to be able to use a mass spectrometer properly, because at that time it was operated with an array of knobs and gauges, producing very raw data, typically on roll of paper from a stripchart recorder. That was quite an insight for a new grad student (not yet a mass spectrometrist) who was thinking about the role of computers in the design of next-generation mass spectrometers.

When I started my faculty career at the University of Florida in 1979, one of my concerns was that we would never be able to produce enough "mass spectrometrists" to know how to use all those mass spectrometers that were becoming much more common in analytical labs. And I was concerned that the lack of trained mass spectrometrists would hold back our field.

Over the next decade, however, computers to control the instruments and aid in interpreting the data made it possible for "non-mass spectrometrists" – or perhaps "mass spec users" – to perform quality mass spectrometry. And that was reflected in a dramatic increase in attendance at ASMS Annual Conference – with 1800 attendees at the Tucson meeting in 1990. One of my colleagues pointed out the dramatic increase in the number of mass spectrometrists at the conference, but I argued that the number of mass spectrometrists – again individuals who understood or wanted to understand what was happening inside the mass spectrometer – had increased only modestly. The majority of the attendees were mass spec users. And that was good for our field.

I believe that one of the success stories of the ASMS Annual Conference – with close to 7000 attendees these days – is that it successfully integrates the mass spec users who are most concerned with how to solve analytical problems with the mass spectrometrists who are most concerned with fundamentals and instrumentation. That crossover – with a poster describing a new way to ionize biomolecules right next to a poster describing a new LC-MS/MS method for quantifying a drug in urine – and both of them across from a booth of a vendor who designs mass spectrometers using those advances to better address such applications – has helped propel mass spectrometry to the forefront of modern analytical science. Even those who want to use mass spectrometers to solve an analytical problem benefit from learning more about fundamentals and instrumentation. And we design better mass spectrometers and do more important fundamental studies when we learn more about how mass spectrometers are used to solve important problems.

Nevertheless, I agree with Daniel DeBord that the demand for analytical scientists with experience and training in advanced MS techniques exceeds the supply of such individuals. That makes it a challenge for any organization – whether an instrument company, a pharmaceutical company, or a university – to find scientists with the necessary education and experience to succeed at developing new mass spec instruments or using them to solve important problems. I don't think this is in any way a new problem, but it does warrant a conversation about ways to address the problem, including those of us who educate young mass spectrometrists – and mass spec users – and those who recruit them. I look forward to that conversation!

"I believe that one of the success stories of the ASMS Annual Conference is that it successfully integrates the mass spec users who are most concerned with how to solve analytical problems with the mass spectrometrists who are most concerned with fundamentals and instrumentation."

Rick Yost is Professor Emeritus, Analytical Chemistry at University of Florida, USA

A Mass-Perspective

Renã Robinson discusses health disparities in Alzheimer's disease, new mass spec tech, and whether we're moving in the right direction with representation

What has been the most significant development in analytical science and mass spectrometry in the past decade?

The incorporation of automation protocols in sample preparation is propelling the analytical science field forward, affecting the entire pipeline, including automated sample injections on mass spec instruments and automated data analysis for informatic platforms.

Particularly in mass spec, instrumentation continues to get better, faster, and more sensitive. There's been an attentiveness from companies and vendors to deliver information to consumers that are using their mass spectrometers in a timely fashion. Because of this, we've seen technologies that have evolved to accommodate faster analyses and more research overall, which has been crucial for my work and for other scientists in the field.

What are you currently working on?

There is a big push for greater knowledge of health disparities in Alzheimer's disease. My current project includes a focus on the link between hypertension and Alzheimer's disease in the African American community. We've been funded with a grant from NIA to learn what protein pathways define hypertension in this population, and how this creates a higher risk of Alzheimer's disease.

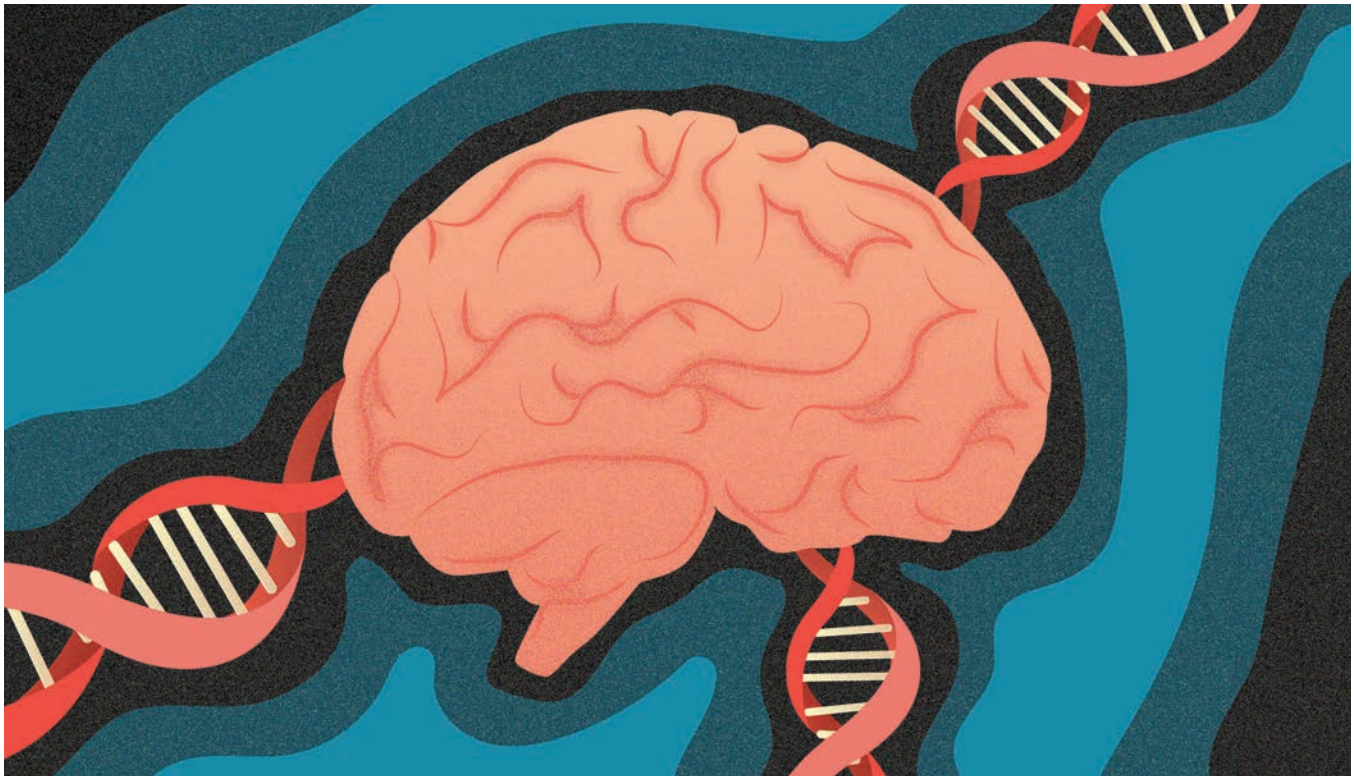
We're also looking into the heterogeneity within this community, both in plasma and brain tissue. There are a number of collaborators involved in this project – sharing samples from longitudinal cohorts of African American individuals.

Alongside this, we have other projects looking at different health disparities. One particular angle we're currently exploring is the ABCA7 gene with its great effect size in terms of risk for Alzheimer's disease in the African American population. Through collaboration with colleagues at Meharry Medical College, we are looking at the mechanics of ABCA7 and the downstream impacts on proteins and lipids. Similar projects in my lab take this ideology and apply this to other disease areas such as sepsis.

"With inflation and potential for recession, it's difficult to fund equipment and instrumentation when regular project expenses are taking up all project funds."

Do you think things are moving in the right direction for diversity and representation in science?

In the RASR Lab, we've been working alongside other groups across the US to make sure that different groups are represented in the research they're doing. This is a good signal that people are talking about representation and diversity in the field. Even across social media, I can see an increased awareness about these issues, which is a good start in getting people to understand the importance of diversity, equity, and inclusion in analytical science.



Could we be doing more to push this mission?

Absolutely. There's always room for improvement. For example, we can be more transparent about the populations featured in our scientific research. Additionally, we can encourage other researchers to ensure there's diversity in the participants of their study.

What's most exciting within the world of analytical science right now?

I'm really excited about the new technologies and advancements coming out for mass spectrometers. There are also interesting developments in liquid chromatography systems that are helping with throughput issues, standardizing chromatography, and allowing use of short gradients.

Proteomics has been exciting – different mass spec platforms have really taken advantage of data independent acquisition, allowing samples of thousands of proteins within a short timescale. There's a lot to be excited about moving forward.

Back in 2019, you said your priorities were affordability and accessibility for mass spec developments – do you think this is being fulfilled today?

No, I don't think they are. Since then, the pandemic has changed a lot for industries across the globe, including analytical science. With inflation and potential for recession, it's difficult to fund equipment and instrumentation when regular project expenses are taking up all project funds. We still have a long way to go.

What have you learnt during your career in analytical science – what advice would you give to recent graduates?

The most important thing to note is rigor: ensuring that all analytical practices within the lab are completed at a high standard. On a more relaxed note, I've realized how fun science can be if you think outside the box – expanding on problems with mass spec instruments and analytical platforms to learn about the importance of protocols and being robust. It's also important to make strategies to keep up with fast advancements in technology – which has been a fun personal challenge.

Overall, new analytical scientists should act like a sponge and soak up as much knowledge as possible. Regardless of the technique you're using, integrating analytical science across other disciplines could open up plenty of opportunities. Keeping an open mind and not being afraid to step outside your comfort zone is key.

Renã Robinson is Professor and Dorothy J. Wingfield Phillips Chair in the Department of Chemistry at Vanderbilt University, USA.

TOSOH

HPLC 2023
DÜSSELDORF
18–22 June 2023
Germany

SEC-MALS/MS FOR ACCURATE SAMPLE CHARACTERIZATION

A column optimized for MALS/MS and biotherapeutic analysis
The **TSKgel® UP-SW3000-LS** U/HPLC SEC column offers unique noise suppression, resulting in increased sensitivity of advanced detection.

A MALS detector with revolutionary technology
The **LenS3® MALS** detector's design eliminates noise from stray light, thereby maximizing S/N. This results in incredibly sensitive and accurate biomolecular MW measurements.

A team of experts to support your work
Our team of chromatography experts provides our biopharma partners with solutions to develop safe and efficient therapies.

Join us at our Booth G1 at HPLC, June 18-22 in Düsseldorf, Germany!

+ 49 6155 7043700
info.tbg@tosoh.com
www.tosohbioscience.de

TOSOH BIOSCIENCE

Core Topic
Chromatography

The gene therapy analytical toolbox grows. Researchers from StrideBio in North Carolina, USA, have developed an optimized method using size exclusion chromatography (SEC) with UV and multi-angle light scattering (MALS) to measure empty capsids in the gene therapy manufacturing process. The SEC-MALS method outperformed droplet digital PCR (ddPCR) with capsid enzyme-linked immunosorbent assay (ELISA) and cryogenic electron microscopy, and correlated well with sedimentation velocity analytical ultracentrifugation (SV-AUC) values of full-to-empty particles. "This work indicates that SEC-MALS is a valuable analytical tool in the analytical development and QC testing of AAV," wrote the authors.

Beds of beads. A study from researchers in Belgium and the Netherlands has shown that the placement of spherical particles in pockets forming microgrooves can act as perfectly aligned chromatographic columns. Whether stacked in a multi-layer column or positioned individually in a single-layer column, the design gives the microgrooves a mechanism for correcting velocity differences. Further research will i) explore removal of occasional particles that remain on the sides of the micro-pockets, sealing the column, and ii) perform actual separations using the columns.

Rats on drugs. The application of liquid chromatography-mass spectrometry (LC-MS) to three different types of rat brain tissue samples has been the first study of its kind to delve into the neurological effects of cocaine and ethanol consumption. The researchers measured the levels of endogenous compounds present in different brain regions (prefrontal cortex, striatum and hippocampus) in male and female rats, and found that metabolic purine and pyrimidine pathways and their functions were altered with self-administration. Female rats were found to be more sensitive to metabolic changes.

Bronze Age party. Researchers at the Valladolid University in Spain have discovered human hair dating back to the Late Bronze Age found in archeological excavations in Menorca, Spain. Hoping to learn more about our West Mediterranean ancestry, they analyzed the hair strands with ultra-high-performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS). The analysis showed direct evidence of ancient communities in the Western Mediterranean consuming alkaloid-bearing plants that contained multiple psychoactive species – as early as 3,000 years ago.

References available online

IN OTHER NEWS

Liquid chromatography–tandem mass spectrometry analysis reveals that the acrylamides and protein-bound ceramides in mice are vital in forming their oral barrier – protecting them from infection.

Ion chromatography provides researchers with answers about the degradation of plasticizer in plastic explosives – concluding that temperature control quickens the process.

Gas chromatography–mass spectrometry/flame ionization method identifies compounds in a traditional medicine plant that could be used to combat malaria.

A new adaptable end-column platform reduces pressure and improves efficiency and sensitivity – making it a versatile tool to improve high-performance liquid chromatography (HPLC) separations.

HPLC Is Back!

It's been four long years since the last HPLC in Milan. So we spoke with the minds behind this year's scientific program – Michael Laemmerhofer and Oliver J. Schmitz – to find out what key developments we've missed over the past four years, how the conference is shaping up, and what they're looking forward to most.

What key trends have emerged since HPLC 2019 in Milan?

Michael Laemmerhofer: Four years is a long time in analytical science!

One topic that has significantly grown in importance in recent years is nucleic acids, such as mRNA, oligonucleotide, and gene regulation therapeutics, and in vaccines, which has been greatly accelerated by COVID-19 – there's a great deal of research going on around those new drug modalities.

Digital transformation is another important topic, especially in industry; companies are increasingly concerned with making themselves fit for the future. Green and sustainable chemistry, which is connected, continues to grow in significance.

Oliver Schmitz: I'd also mention polymer analysis – another hot topic. REACH requires every chemical introduced into the market to be registered, but polymers must be characterized with additional data. By 2030, around 200,000 polymers will have to be characterized and registered – a big challenge for the field.

What about developments on the instrument side?

Laemmerhofer: There are many different varieties of new columns on the market, as different applications require different selectivities for stationary phases. The new columns with surface coatings that deactivate biomolecule interactions with surfaces are leading to improved performance, for example. I'd also like to mention the pillar array columns for the analysis of complex samples. They are manufactured using photomasks, which helps with reproducibility in manufacturing and run-to-run repeatability – an important benefit for proteomics applications in particular. Overall, the trend seems to be towards developing columns with higher capacity and more robust batch-to-batch reproducibility.

Schmitz: From my point of view, the increasing number of ion mobility mass spectrometers by more and more companies offering them is very exciting. By combining chromatography with ion mobility, two orthogonal separation techniques are coupled with each other and thus enable, without much optimization effort, an outstanding separation that is otherwise only achieved with comprehensive two-dimensional chromatography. The latter shows a strongly growing interest, also in industry, and will therefore be covered in three sessions at HPLC 2023.

What are you most looking forward to at HPLC 2023?

Laemmerhofer: I'm looking forward to the panel discussion featuring green technologies in LC and various applications related to the topic of HPLC in the chemical industry. I believe there are many parts of the conference that are interesting to industry, and we want to

involve industry more – both the chemical and pharmaceutical industry.

Schmitz: We will also be addressing some of the emerging topics highlighted previously. For instance, we'll have a tutorial on LC separations of oligonucleotide and RNA-based therapeutics, which will address the challenges in dealing with impurity profiles and characterizing these drugs, as well as addressing issues with their bioavailability. In addition, Joachim Richert, Vice President Analytical Science at BASF, will give a plenary lecture on digitalization and the lab of the future.

Does the program reflect ongoing changes in the (bio)pharma industry?

Laemmerhofer: We have a couple of talks about biopharmaceuticals. Analysis, of course, relates on the one hand to the characterization of the process and on the other hand to the characterization of the product. Of 47 recent drug approvals in Germany, only 40 percent were small molecules – the others were complex biopharmaceuticals, including antibodies, antibody-drug conjugates, bispecific antibodies, peptide conjugates, oligonucleotide drugs, chemotherapy, and even cell therapies.

Schmitz: Yes, biopharmaceutical structures are complex, which makes characterization difficult and demands more analytical technology. Usually, one method is not sufficient; instead, you need an array of analytical methods to fully characterize these products. In addition, the new paradigm in pharma is to systematically investigate the product during the entire process – not just characterizing the product at the end. We have a couple of talks on this, as well as an interesting plenary on using ion mobility MS to infer biopolymer folding and interactions.

Are there any new focus areas for this year's scientific program?

Schmitz: There will be sessions dedicated to the needs of the chemical industry – which is a new focus for us. These sessions will include a full-day track, tutorials on SFC, reaction monitoring, and on-line LC hyphenation.

"Another new area covered is molecular phenomics. This is essentially what analytical chemists are driven to do: comprehensively and precisely identify and compare the molecular content of a sample."

Laemmerhofer: Another new area covered is molecular phenomics. This is essentially what analytical chemists are driven to do: comprehensively and precisely identify and compare the molecular content of a sample. This need goes hand in hand with new technologies, especially in LC and ion mobility mass spectrometry, that are allowing us to capture an increasing number of molecular features. This trend is especially important in areas such as systems biology, but also in environmental science and in the food industry, where you want to see all the contaminants in a sample. Focusing on one specific analyte is usually not enough anymore. Two renowned speakers, John McLean (Chair of the Department of Chemistry and Director of the Center for Innovative Technologies at Vanderbilt University, USA) and Jeremy Nicholson (Pro-Vice-Chancellor for Health Sciences at Murdoch University and Director of Australian National Phenome Centre in Perth, Australia), will explore the strategies available and the benefits of these approaches.

Anything else attendees should look out for?

Schmitz: We offer a total of 13 short courses with different topics. Participants can talk to the experts and learn about the latest developments. It's an excellent opportunity for PhD students and industry professionals who are interested in new technologies.

One unique aspect of HPLC 2023 is short course 1 about "Understanding how to perform good practical RPLC". The morning session will be about fundamentals in HPLC method development, and, in the afternoon, there will be hands-on training on modern instrumentation. Most conferences simply offer lectures and seminars, so this benefit should not be ignored – especially by the younger generation. After all, not every PhD student has the opportunity to work on every technology, and these short courses provide a quick introduction to the state-of-the-art in a very condensed format.

Laemmerhofer: Last but not least, I'd like to highlight the return of the Separation Science Slam, supported by The Analytical Scientist and KNAUER. Here, we invite younger scientists to take to the HPLC stage and, in 5 minutes, impress the audience with a presentation, slideshow, poem, rap, cabaret – or whatever they can think of – for the chance to win €1,500. This session is sure to be good fun!

Overall, I'm sure I speak for both of us when I say we're excited to welcome everyone back to HPLC 2023 in Dusseldorf on June 18–22!

The GC-MS Application Challenge

We catch up with 2023's GC-MS Application Challenge winners

The Analytical Scientist, in collaboration with LECO Corporation, Restek, Axel Semrau and GL Sciences, invited those working in the GC-MS space to submit their most impressive application notes for the chance to win some exciting prizes – all expenses paid facility or conference trips, consumables worth $3,000, and instrument discounts, to name but a few.

Our expert panel of judges – including Robert K. Nelson, James Harynuk, Susan Richardson, Hans-Gerd Janssen, Giorgia Purcaro, Erich Leitner, Jaap de Zeeuw, and Robert Trengove – have considered the entries and the results are in! Our winners across the following categories are:

- Dmitry Koluntaev for Best Novel Application
- Anika Lokker for Excellence in Chromatography
- Flavio A. Franchina for Creative Use of Application Workflows, Sample Prep & Automation
- Katelynn Perrault – Special Recognition

We caught up with the winners to find out more about their work, the lessons learned, and thoughts on the future of GC-MS.

Who's Afraid of Picasso?

Best Novel Application: "Analytical approach to GC-MS determination of museum varnish compositions"

With Dmitry Koluntaev, Application Specialist, Q-Tek, Montenegro

Please introduce yourself…

As an application specialist, I work for a small private company that manufactures GC-MS systems. Searching for new applications for GC-MS is one of our main focuses.

I am a biologist by education, but I have been working with mass spectrometry since university – it's become a great hobby! I really enjoy discovering new possibilities for GC-MS; for example, in museum object analysis.

What was your main inspiration?

I always wanted to work with samples filled with history – and around a year ago, we were asked for assistance by restorers to analyze samples of varnish taken from two museum items – book covers and chest lids. It was very important to determine exactly what type of varnish had been originally used by the artist so that it could be properly removed – without damaging the drawings underneath. We agreed! It was a great opportunity to try our hand at such a field of research and use real historical samples for analysis.

I spent several days analyzing the literature to find out what types of varnishes had been used back then, how they had been prepared by masters, and what recipes had been used. Having studied the objects of research, I started looking for various samples of varnish in specialized art stores. Developing a specialized library of markers for each type of varnish became the optimal solution for discovering the steps of sample preparation.

The aspiration to find the answer to the question posed and the desire to touch historical subjects – that's what really fascinated me in this work.

Any challenges?

To prevent harm to the picture, historical research usually involves incredibly small samples. And for this study, all stages of sample preparation had to be efficient and universal – meaning that one extraction method was used across a variety of target compounds; there was no possibility of re-analysis. To uphold these requirements, we implemented a unified method of sample preparation that would allow determination of resin markers and oils in which the artist melted the resin and prepared the varnish.

Another challenge of our research was that the obtained chromatographic profiles of the resin extracts are usually complex chromatograms, which are difficult to interpret without using resins of known botanical origin. Therefore, it was necessary to develop a list of characteristic markers for each type of resin.

Any lessons learned?

Having immersed myself in the literature, I noticed the broad potential of using GC-MS in the study of museum objects. For example, characterization of the binding oils used by artists in painting is in high demand not only in the process of restoration of an object of art, but also in answering the question about the date of painting to confirm its authenticity.

"You must go beyond the framework to grow and develop as an analytical scientist – this is where creativity and discovery lies."

Another main takeaway from this study was that a large extent of any task can be solved and there is nothing to be afraid of when approaching your research. A thorough study of the objects of research at the initial stage allows you to choose the direction of the study. Working with intermediate results also allows you to assess their correlation with expected results.

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?

By combining the exploration of new GC-MS trends, tracking applications from major manufacturers, and reading scientific reviews, we can find answers to questions that may otherwise have been unsolved. You must go beyond the framework to grow and develop as an analytical scientist – this is where creativity and discovery lies.

What applications do you hope to explore in the future?

I remain deeply immersed in exploring the possibilities that GC-MS offers in targeted and non-targeted metabolomics. Today, we have many opportunities to use open-source software for processing mass spectrometry data (for example, MzMine, GNPS, XCMS, MetaboAnalyst), which allow us to use GC-MS systems in new ways, access harmonized data, and visualize interpretation of results.

THE KILLER GC-MS APPLICATION?

Dmitry Koluntaev: It's very difficult to imagine. I suppose such an application should be very simple in terms of sample preparation (maybe even fully automated), and should provide a comprehensive analysis of any object – whether it be a museum object or the analysis of biological or environmental objects.

For example, there are interesting studies with exhaled breath taking place in the world. I try to actively follow the publications and reports in this area.

Flavio A. Franchina: The multivariate information received by hyphenating gas chromatography and mass spectrometry make the combination the most suitable technique for broad non-targeted analyses, in my view. For example, the quest for novel energies and materials, where it is important to know, in depth, the composition of the new feedstock in order to finely tune the process. The high-selectivity and sensitivity of the GC-MS also make it also suitable for targeted analysis.

Lastly, when considering the evolution of GC and MS into multidimensional and high-resolution coupled together – i.e., GC×GC-HR MS – then it's possible to combine either (multi)targeted, non-targeted, and post-targeted (retrospective) analyses into a single experimental analytical pipeline, reducing cost and time. Such a high-resolution technique can untangle the complex information contained in biological samples.

However, it's important to doubly underline that such powerful high-resolution couplings aren't magic and require broad understanding and/or training. For example, for the best results, the separation step must also be in harmony with proper sample preparation techniques and experimental designs.

Anika Lokker: I'm interested in the development of non-destructive GC-MS analysis in cultural heritage research. HS-GC-MS analysis is already gaining more popularity in the field and l am curious what kind of applications might be coming in the future.

Katelynn Perrault: I am really interested in the application of GC×GC-MS to non-targeted profiling in forensic investigations. There are several areas where GC×GC could be used on a more regular and routine basis. We hope to investigate some of these applications in the future to improve complex chemical separations that can be applied in legal investigations.

Getting to Grips with Prehistoric Adhesives

Excellence in Chromatography: "Breaking the secret of prehistoric stone tools design using multidimensional chromatography"

With Anika Lokker, PhD student, University of Liège, Belgium

Please introduce yourself…

I am an analytical chemistry PhD student with a heart for archaeological research. From a young age, I was interested in history (archaeology, in particular) and science. Combining both of these areas for my PhD project has been very stimulating.

My PhD project focuses on non-destructive identification of prehistoric adhesives, which could have been used to attach a handle to the tool or as a protective wrapping. Resins, waxes, gums, animal glues, tars, and dry distillation of birch bark might have been used as adhesives in these times. Since the adhesives tend to remain present on stone tools, whereas the organic handle is often gone, the analysis of adhesives is important; it can tell a lot about how the tool was used.

We are currently developing a non-destructive analysis method, using the volatile organic compounds (VOCs) released by the adhesives. VOCs are trapped with dynamic headspace (DHS) before being separated and detected with comprehensive two-dimensional GC-TOFMS (GC×GC-TOFMS). The technique is fully automated with a multipurpose sampler (MPS). The main focus of the project is on artifacts from several excavation sites in South Africa from the Middle to the Late Stone Age (150,000–20,000 years ago).

This project is interdisciplinary, with two groups from different faculties working closely together – the analytical chemistry group of Jef Focant (OBiAChem, Faculty of Sciences, ULiège) and the prehistoric research group of Veerle Rots (Traceolab, Faculty of Human Sciences, ULiège).

Another PhD researcher and a postdoc researcher are working alongside me with a focus on functional analysis of stone tools, which entails technological use-wear and residue analysis. This process involves several non-destructive microscopic and spectroscopic techniques. Together, we are trying to understand the life cycle of stone tools.

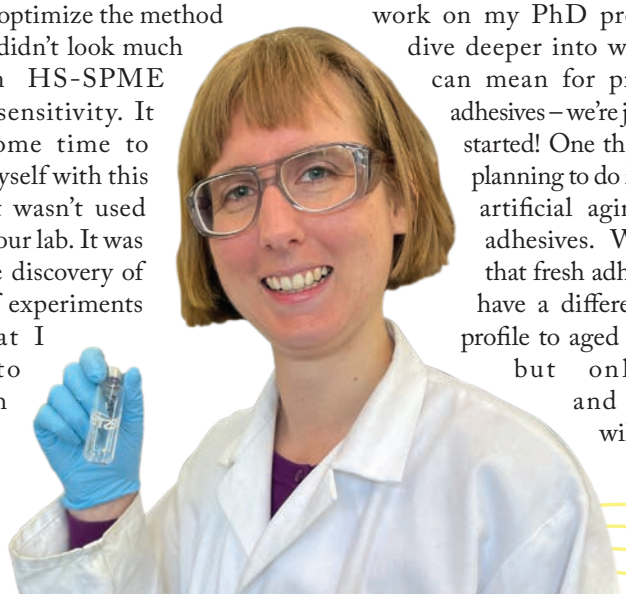
How did you become involved with this project?

A few years ago, a proof-of-concept study was conducted in my lab using HS-SPME-GC×GC-TOFMS to identify prehistoric adhesives. I started my PhD project with this technique, too.

However, it soon became clear that HS-SPME is not sensitive enough to detect the VOCs of very old archaeological artifacts. Therefore, I started to look further into different HS techniques options, finding articles in which they compared HS-SPME with DHS. I discovered that DHS has a higher response and sensitivity. With this knowledge, we changed our system to use the potential of DHS and optimized the extraction. This change showcased the measurement of a real archeological artifact and highlighted the working benefits of DHS.

What were the main challenges?

The results were not very promising at the beginning of the study. I was struggling to optimize the method of DHS – it didn't look much better than HS-SPME in terms of sensitivity. It also took some time to familiarize myself with this method as it wasn't used much within our lab. It was only after the discovery of the design of experiments concept that I managed to break through this barrier.



What were your main findings?

Using DHS allowed us to detect VOCs from a very old artifact with more success than HS-SPME. We're only at the beginning of the project, but the results with DHS look very promising in being able to identify the adhesives used on ancient artifacts.

Any lessons learned?

In this line of research, I am constantly learning new things, especially because I just started working with this kind of instrumentation. One thing that I can take forward from this project is to have patience – things will work out eventually if you are determined; troubleshooting (a lot) is part of the job!

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?

It might be obvious, but keep expanding your search for interesting applications – go broader than the intentional field of interest. If you face a problem with the current applications in your research field, try to look for other methods applied to another research field with similar molecules of interest; I took a lot of inspiration from DHS applied to food and beverages research and applied it to archaeology.

What comes next?

I'm currently planning to continue work on my PhD project and dive deeper into what DHS can mean for prehistoric adhesives – we're just getting started! One thing we are planning to do is look into artificial aging of the adhesives. We expect that fresh adhesives will have a different VOCs profile to aged adhesives, but only time and research will tell.

Creatively Controlling Pesticides

Creative Use of Application Workflows, Sample Prep & Automation: "Determination of phytosanitary products in surface waters and groundwaters by GC×GC-TOFMS"

With Flavio A. Franchina, Assistant Professor of Analytical Chemistry at the University of Ferrara, Italy

Please introduce yourself…
My research is focused on the implementing and integrating into analytical workflows effective sample preparation, separation, detection, and data elaboration techniques for targeted and non-targeted analysis of small molecules. We apply these strategies and finely tailor them to tackle challenges in food, biomedical, and environmental applications.

Here, we described the development and validation of a method for the determination of phytosanitary compounds in environmental waters. For this, we relied on a solid-phase extraction (according to the EPA method 3535A), followed by injection into a comprehensive two-dimensional gas chromatography coupled to mass spectrometry (GC×GC-MS) system.

What was your main inspiration?
The idea popped into my mind when tutoring one of my students, who was working on his dissertation. At the time, he was working part time for one of the national environmental agency's local labs, so I had the chance to hear about some of the analytical challenges they faced. I thought that developing a GC×GC-MS methodology for phytosanitary compounds could really be helpful, and that comparing it with its GC counterpart would be interesting – and a good learning exercise for the team, both in terms of theoretical and technical skill development.

What were your main findings?
We analyzed some real-world water samples in collaboration with the national environmental agency, and we found that – fortunately – the total amount of phytosanitary compounds was below the legal limit for most of them. The occurrence of contamination was also more evident among superficial water samples, compared to groundwaters. Curiously, the most common compound detected over the limit (0.1 µg/L) was caffeine.

Any challenges?
As shown in some of the figures of the application, 1D GC separation was insufficient to resolve some target peaks, which co-eluted with other species. Depending on the case, some of them were spectrally resolved by the MS, thus a single separation would successfully do the job; others instead greatly benefitted from the additional separation into the second dimension with the GC×GC method. We also quickly realized the need to develop quality control procedures because the extracts and standards were not very stable during storage.

"Contaminations from other fundamental disciplines might let you find different angles."

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?
I think it's important to acquire a solid and deep knowledge of specific topics or techniques; then, contaminations from other fundamental disciplines might let you find different angles for the topic you master.

Looking at something that you know well from a different perspective is a way to exercise your creativity and make a difference.

What applications do you hope to explore in the future?
I would like to develop and transfer more informative and effective analytical strategies to companies' R&D and QC laboratories.

Regarding the environmental monitoring, we are more recently using a programmable-temperature vaporizer for the GC×GC-MS system. I believe this can greatly help to reduce solvent consumption during the extraction process.

We're also working on the development of robust methodologies of chemical analysis for the investigation of metabolites in clinical settings... Stay tuned!

Sandalwood in the Second Dimension

Special Recognition: "Comprehensive two-dimensional gas chromatography analysis of commercial essential oils from different sandalwood species"

With Katelynn A. Perrault, Associate Professor of Forensic Sciences and Chemistry and joined Chaminade University of Honolulu, USA

Please introduce yourself…
Our research involves the non-targeted profiling of complex samples by comprehensive two-dimensional gas chromatography (GC×GC). Specifically, we focus on volatile matrices that comprise complex odors related to forensic science, biomedical, and natural product applications.

What was your main inspiration?
Recently, we worked on several complex samples of Pacific Island origin due to our location in the Pacific and our interest in complex plant products in Hawaii. These included samples of kava (a beverage made from Piper methysticum) and poi (food product from Colocasia esculenta).

Many personal care and aromatherapy products in Hawaii contain Royal Hawaiian Sandalwood within them, and we were curious to see if our non-targeted methods could differentiate the entirety of the volatile profile of other sandalwood species from other regions in the world. This was particularly interesting because some ISO methods are developed to look at quality indicators within sandalwood essential oils, but they are based on targeted analysis of only a few compounds.

Our non-targeted GC×GC method allows us to see a wealth of information within these complex plant products. With this knowledge, we were interested to see if the method would provide further value in differentiating the oils.

Any challenges?
We had to optimize our method to improve our separation because the samples were quite different from other samples we analyze. The samples were injected using our one-dimensional GC instrument to see what the samples contained. I tasked my Instrumental Analysis class (in groups) to come up with different ways of improving the 1D GC separation by playing with parameters, such as oven ramp, split flow, and carrier gas flow rate.

Taking the optimized 1D GC method, we translated it to our GC×GC system for further analysis. The students had a lot of fun learning about the GC technique using this "optimization challenge" and it brought up some great points of discussion of chromatographic theory in class after the results were obtained.

What were your main findings?
GC×GC was a powerful tool to elucidate subtle but important changes in the complex chemical sandalwood essential oil profiles. Non-targeted analysis was used to investigate compounds that are quality indicators – according to standard methods – simultaneously with other product components that could be relevant to monitoring these products. Monitoring additional compounds within the profile may assist in understanding their different therapeutic benefits by tracking a component to its known pharmacology.

Any lessons learned?
During the study, we were contacted by representatives of an essential oil company who asked how GC×GC might help them with product monitoring. Having data to share with them and explain the benefits of GC×GC was incredibly valuable.

Do you have any tips for scientists hoping to bring a touch of creative flair to their application workflows or method development?
Crowdsourcing ideas for method development can be valuable. For our study, a group of undergraduate students came up with ideas for optimizing our first dimension separation – a fun exercise to tackle together. I could also see this group approach being a valuable tool in R&D laboratories when meeting challenges in method development.

What's on the application horizon?
There is always a long wishlist of new applications that we want to tackle; you will have to wait and see what we do next!

Winning application notes available online

Thank you to our supporting partners:

Continuous Evolution

For the right task, SMB chromatography will result in higher yields, higher recovery, and higher purity than traditional batch approaches – all while using less solvent. The key hurdles are method development and process optimization, but that's exactly where KNAUER's SMB systems come into play.

With Yannick Krauke, Senior Application Scientist Purification, KNAUER

In a nutshell, what is SMB?

Simulated moving bed (SMB) chromatography is an HPLC technique for the separation and purification of binary mixtures with high productivity and purity. SMB chromatography relies on a multi (typically eight)-column set-up (as opposed to the classic single column format for batch processes), which results in a continuous process in isocratic mode with constant feed and solvent input and output of two fractions. It is applied in process chromatography and can be found in many industries, for example, in pharmaceutical manufacture and fine chemical production. The production range can vary enormously – from a few grams per day to tonnes per day!

What are the advantages of SMB over batch chromatography?

In a single column batch process, only a portion of the column is used. But during the SMB process, the whole column bed is used for the separation, which leads to higher resolution and better separation of pairs that are difficult to resolve. And because SMB is a continuous process, there is a constant out-stream of the product, which is significantly less diluted than in batch processes. Often, the product can be recovered with high purity and yield.

Which kinds of substance mixtures is SMB best suited for?

As noted above, SMB technology is applied in many different industries such as refinery, food and pharmaceutical industry, which showcases the wide variety of starting mixtures that can be separated. The main criteria: i) the separation must be performed in isocratic mode, and ii) the target substance is either the first or last eluting fraction. Furthermore, the sample should be free of as many impurities as possible.

Are there any major challenges with SMB?

Because SMB chromatography is a continuous process feed, solvent consumption can increase quickly during method development – which, overall, is a key challenge. Process monitoring is another challenge, primarily because no classic separation chromatograms are received at the fraction outlets.

How do KNAUER's SMB systems address those challenges?

KNAUER offers two systems, the AZURA Lab and the Pilot SMB system. The Lab SMB is the ideal system for SMB method development using small columns (ID 8 mm), whereas the Pilot SMB system is used for production up to several kilos per day. Another advantage of SMB is the ease of scale up; once the parameters are set with a small system, they can be easily transferred to a Pilot SMB. The SMB parameter calculator within the delivered software allows the determination of starting parameters for the SMB process. KNAUER offers different possibilities to monitor the process: flow meters that measure the flow at the pumps or the outlets, a sample extraction valve to obtain a concentration profile, and UV or refractive index (RI) detectors.

What common questions do customers ask?

Customers often have separation and purification tasks and want to know if SMB can be used. KNAUER offers direct support with the SMB experts from the Product Management and Application department to discuss the potential with customers.

Recently, many requests have come from the cannabis industry. Depending on the sample, SMB can be applied in this field – mainly to increase Delta-9 THC purity from treated cannabinoid samples. KNAUER is working with academic and industrial partners to integrate SMB in the cannabis field.

Have there been any recent developments in SMB technology?

The core principle of the SMB process has not changed significantly over the years, but there are many system setups. The classic setup consists of eight columns arranged in four zones with different flow rates and valve(s) that enable the rotation of the column. But column and zone numbers – and their distribution – vary by system. Most interestingly, there are now several approaches to integrate SMB into existing downstream processes and/or to replace batch chromatography steps.

Are there any wider trends that SMB technology is addressing?

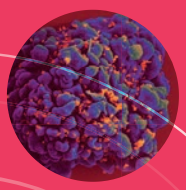
When it comes to sustainability – a big topic on many people's mind, SMB processes have the potential to make chromatographic processes greener. In comparison to batch chromatography, SMB requires less solvent – because of the recycling that takes place as part of the process – and it uses less energy given the use of higher concentrated products. Plus, the system size and columns are smaller compared to similar preparative systems, thus using less material.

ID Transmission

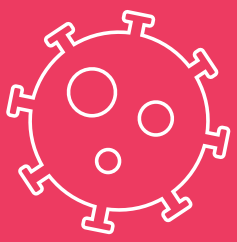
Covering the front lines of the fight against infectious diseases for researchers, clinicians, and policymakers

ID Transmission brings you the latest research and innovations in the field, whilst also tackling hard-hitting and thorny topics that many others shy away from. We keep you informed, start conversations, and connect all disciplines and specialities within the field of infectious diseases.

Visit our website now to find out more
idtransmission.com



Receive updates direct to your inbox by signing up to our weekly newsletter



Join the conversation today

@IDTransmission
ID Transmission
@IDTransmission
ID Transmission

Core Topic
Spectroscopy

Aqua space lasers. NASA has awarded the Planetary Science and Technology from Analog Research (PSTAR) grant to the In-situ Vent Analysis Divebot for Exobiology Research (InVADER) team. The project will study underwater hydrothermal systems using the Laser Divebot, which combines laser Raman and laser fluorescence spectroscopy. In a press release, grant recipients Pablo Sobron said, "Our project offers unprecedented opportunities to bridge studies of Earth's oceans and mission concepts to explore oceans in our Solar System."

Hot, hot Raman. A team of researchers at Universidade Federal do Maranhão in São Luís, Brazil, have used Raman spectroscopy to study monoclinic silver dimolybdate microrods at high temperatures. The study revealed that Raman spectra provide important insights into high-temperature behavior of the microrods, which have practical applications across material science, chemistry, and physics fields.

Geological progression. A new technique for accurately defining the aging of geological systems has been developed by scientists at the Chinese Academy of Sciences in Beijing. They used laser ablation inductively coupled plasma-tandem mass spectrometry (LA-ICP-MS/MS) analysis to date Paleozoic-Precambrian xenotime, apatite, and garnet more accurately than ever before.

A nuclear discovery. A study conducted by Yuji Ikeda from i-Lab and Japan Atomic Energy Agency (JAEA) shows that microwave-enhanced laser-induced breakdown spectroscopy (MWE-LIBS) can effectively analyze nuclear fuel debris. This technique provides a non-destructive and rapid method for zirconium metals and oxides – improving the understanding of nuclear fuel debris in extreme environments and providing a basis for further analyses.

A breath of fresh air. COVID-19 can now be detected via breath analysis – using cavity-enhanced direct frequency comb spectroscopy (CE-DFCS). This discovery bypasses the need for invasive COVID-19 testing – with further potential for diagnosing a range of diseases.

References available online

IN OTHER NEWS

Magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (MRS) reveals that an excitatory–inhibitory imbalance increases the risk of psychotic symptoms in 22q11.2 Deletion Syndrome.

Combination of two X-ray spectroscopy techniques captures the chemical reaction of ferricyanide for the first time; the approach could help map other complex chemical reactions, like oxygen transportation in blood cells.

Handheld Fourier transform near-infrared (FT-NIR) spectrometer determines total petroleum hydrocarbon content within soils.

Atomic emission spectroscopy and mass spectrometry analysis of archeological stone artifacts reveals evidence of long-distance voyaging across the Pacific Islands going back at least 1,000 years.

DKD Detection

ATR-FTIR spectroscopy combined with machine learning can help detect early stage diabetic kidney disease – and it could be implemented into point-of-care settings

Albumin excretion in urine is a common biomarker of diabetic kidney disease (DKD). Current routine screening methods not only have point of care limitations, but only identify patients in the late disease stages. Recently, a group of Australian researchers have combined ATR-FTIR spectroscopy with machine learning to rapidly profile proteins – such as albumin – in urine to detect DKD in its early stages (1). To gain more perspective, we asked the lead authors of the study – David Pérez-Guaita, Bayden Wood, and Karin Jandeleit-Dahm – a few questions.

Could you please introduce your work and its importance?

Our work focuses on the development of an infrared-based spectroscopic method that combines machine learning for the quantification and characterization of proteins in urine. The presence of specific proteins in urine is indicative of a wide range of diseases – including DKD and chronic kidney disease. And so, analytical tools that can quantify proteins at low levels are fundamental for the early detection of DKD. These techniques should be simple, fast, and portable enough to be used in close proximity to the patient in pharmacies or community clinics. Our instrument is the size of a shoebox, and provides accurate results in a few minutes at a reasonable per test cost.

Could you please share some details of your research?

We measured the urine protein extract – obtained by ultrafiltration – from 22 controls and 155 diabetic patients with normo-, micro-, and macro-albuminuria. Visual inspection of the spectra indicated a strong correlation between the amide bands from proteins and the total protein content of the samples. We applied support vector machine classification and regression models to extract the diagnostic information contained within the infrared spectra. The model was independently tested and successfully identified micro- and macro albuminuria with sensitivity of >91 percent and specificity of >99 percent. Furthermore, the regression methods predicted the amount of albumin with error values of 17 and 44 mg/L for normo- and micro albuminuric patients, respectively.

What are the advantages of using ATR-FTIR spectroscopy over conventional diagnostic techniques?

The current diagnostic test for DKD uses dipsticks for screening and can only detect albuminuria at medium levels with low sensitivity. More sophisticated techniques used in pathology labs have appropriate limits of detection (1 mg/L), but they are expensive and difficult to translate into point-of-care (POC) settings. There is therefore an urgent need to increase screening for early kidney disease – not only in the diabetic context. Early detection and treatment means less patients with end stage kidney disease that require costly dialysis and transplantation.

The main advantage of our technique is that it combines appropriate sensitivity, specificity, and POC capability for screening urine samples. Furthermore, ATR-FTIR is portable, so it may be taken to more remote, indigenous communities where DKD is far too common. We believe that – with automatization and cleaning of the spectral window – the technology can be improved and decrease the measurement time to a few minutes. The use of stable filters could also put the cost at the $1 level. Even so, commercialization of a technology is a long and expensive process.

What do you want diagnostic professionals to know about this type of testing?

When the term "machine learning" is used, professionals often assume that analysis is complex and can only be used by experts. We want people to know that the methodology is simple and can be performed in user-friendly software, cloud-based systems, or even interfaced through a mobile phone! Such apps have already proved successful for other IR-based clinical applications – including malaria diagnosis. These methods simply require the press of a button to input the spectra and, in a few seconds, test results are returned.

What about applications in other disease areas?

We would like to test the potential of the technique for diagnosing other diseases that modify the composition of proteins in urine (for example, hemoglobinuria). But, before tackling other diseases, we want to work on automatizing the protein extraction and cleaning process to improve the techniques POC capabilities. We are also studying the robustness of our method and plan to perform multicenter studies with different hospitals in Europe and Australia so that our method can hopefully – in the future – be implemented in a real-world setting.

Reference

1. *Z. Richardson et al., Analysis & Sensing (2022). DOI: 10.1002/anse.202200094*

HILIC Analysis of Oligonucleotides Using Bioinert Columns

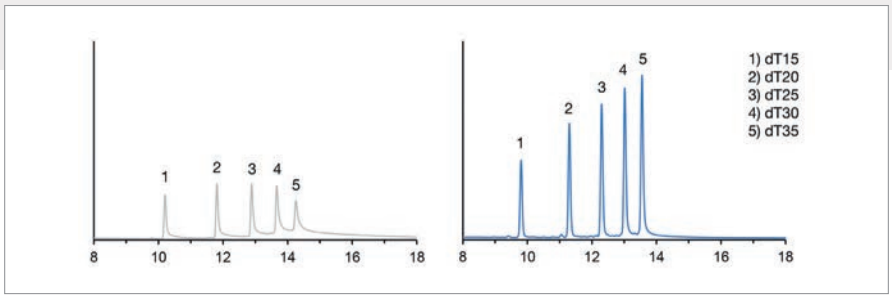


Figure 1. Analysis of dT15-35 using a regular YMC-Triart Diol-HILIC (left) and a bioinert coated YMC-Accura Triart Diol-HILIC column (right) and a bioinert system.

Due to the highly polar nature of oligonucleotides, hydrophilic interaction liquid chromatography (HILIC) can provide an alternative approach to the standard methods: ion pair reversed phase liquid chromatography (IP-RP) and anion exchange chromatography (AEX).

Oligonucleotides can be irreversibly adsorbed due to ionic interactions with metal parts of conventional column hardware, which leads to decreased recovery and bad peak shapes. A bioinert column body and frits will bring a distinct improvement in performance because it represents more than 70 percent of the surface that the analytes are in touch with. Therefore, YMC provides the bioinert YMC-Accura Triart series of columns, which have a bioinert coating on the column body and frits.

In this application, two DNA oligonucleotide mixtures, dT15-35 and dT40-100, and an RNA oligonucleotide mixture, rU15-30, were analysed using a YMC-Accura Triart Diol-HILIC column and the corresponding stainless-steel column after pre-conditioning. A gradient of 75-45 percent acetonitrile in 30 minutes, with an ammonium acetate buffer at pH 6.9 as aqueous phase, was applied. Higher sensitivities, peak areas and less tailing were achieved using the bioinert YMC-Accura Triart Diol-HILIC column. Non-specific adsorption did not vary according to length, even though the adsorption is usually higher for longer oligonucleotides in IP-RP.

Full method details can be accessed here: https://ymc.eu/d/brDoG

Making a Point of Caring

Sitting Down With… Russ Algar, Associate Professor, Department of Chemistry, University of British Columbia, Vancouver, British Columbia, Canada

Did you always want to be a scientist?

It was a gradual evolution. Growing up, I didn't know anyone in science and I don't recall any specific media personalities or newsmakers who drew me to the field. A childhood memory book indicates varied career aspirations: graphic design, law enforcement, and aviation. "Scientist" did show up at least once, though! A sustained interest in science – specifically chemistry – began in high school and was catalyzed by a great teacher. By the time university came around, I had decided to double-major in physics and chemistry and eventually zeroed in on becoming a researcher in bioanalytical science.

What is your greatest scientific achievement so far?

Whenever I'm asked which of my scientific papers is my favorite, the answer is, "The one being written." This is because our research is always presenting new challenges, teaching us new things, and reaching new milestones. Assessing some sustained themes of my research: the development of concentric Förster resonance energy transfer is perhaps the most innovative, the work toward smartphone-based molecular diagnostic devices is most likely to impact society, and the studies of how nanoparticle surface chemistry affects enzyme activity are interesting fundamental science. So perhaps my greatest scientific achievement is successfully leading a productive research group.

You've previously mentioned single-molecule detection as an exciting research area – why?

From an analytical perspective, detection limits become less about the smallest reliably measurable quantity of analyte and more about the statistical significance and contextual implications of whatever quantity is measured. Moreover, analytes can be quantitated by counting rather than by calibration curves. This benefit is quite important. There's a lot of value in assaying molecules in industrial, health, and environmental sectors; however, these measurements typically require a lab facility and training as an analytical scientist. Even with the great progress in easy-to-use portable devices, calibration is still a potential barrier for routine quantitative analyses by industrial, health or environmental workers without formal training in analytical science. Single-molecule detection is becoming increasingly accessible for R&D labs thanks to advances in photonic technology, luminescent materials, and methods with microwells and molecular amplification. For example, digital PCR assays are now commercially available, but still involve substantial costs, multiple user steps, non-robust reagents, and long incubation times. The next challenge is addressing these limitations to enable low-cost, portable, robust, fast, and easy-to-operate devices and methods for calibration-free detection by counting analyte molecules.

What is the single biggest challenge facing your field?

I think translation is one of the biggest challenges. The gap between the research lab and societal application has always been one of the largest to bridge. For example, the selective detection of single molecules and single cells are great achievements for analytical science, but technologies, workflows, and data interpretation are nontrivial. When and how will a physician or a grocer be able to harness these advancements to check the health of patients or ensure that produce is safe to eat? Analytical scientists develop cutting-edge technology to provide a solution to a problem, but we also need to devise ways of making that solution accessible, simple, and reliable for non-expert users.

Do you have any predictions for your field over the next five to 10 years?

In light of the COVID-19 pandemic, it will be interesting to see the extent to which new biosecurity and biosurveillance technologies will be devised. Will rapid checkpoint-based molecular screening for infectious disease eventually become as common as X-ray machines, metal detectors, and millimeter wave scanners? Indeed, ion mobility spectrometers already offer some molecular screening capability at security and safety checkpoints. And what would prospective biosurveillance technologies aim to detect? Would the technology be limited to known infectious agents or will we have ways to detect unknown agents that could cause the next pandemic?

Field-deployable diagnostics for managing infectious disease outbreaks have been of longstanding interest for public health, but these contexts are somewhat different from an airport implementation, for example. I expect that there will be renewed and expanded interest in biosurveillance – particularly in asking how to screen for undefined viral threats.

What do you credit most for your success in the early part of your career?

Hard work, persistence, and pride in our work. My team and I have built a culture in which we aspire to make each research project its best – creative, rigorous science with both breadth and detail, clearly and professionally presented. Although some research takes multiple rounds of failure to achieve results, we always gain more knowledge and satisfaction when we eventually succeed.

If you weren't an analytical scientist, what would you be?

I would say computer science or engineering were alternative career paths. Whatever the career, it would need to have substantial creative components, because I receive the most satisfaction from being curious and constructive. Maybe some versions of me end up in fields that combine visual art, design, and creative writing. In any case, I don't think about a career change – analytical science allows me to combine chemistry, physics, biology, engineering, and computer science on a regular basis.

Syft™

Syft Tracer™

Faster. More Sensitive Detection of Volatile Product Contaminants

- The Most scalable solution for detecting product & environmental contaminants
- No sample prep or pre-concentration required
- Trace-level sensitivity
- Highly selective & quantitative
- Easy to operate & interpret data

Debuting Worldwide in 2023

Schedule Your Live Demo