JANUARY 2015 # 24

the Analytical Scientist

Through the Looking-Glass

Four gurus explore the world of stereoisomerism, discuss chiral separations, and assess the broad significance of chirality.

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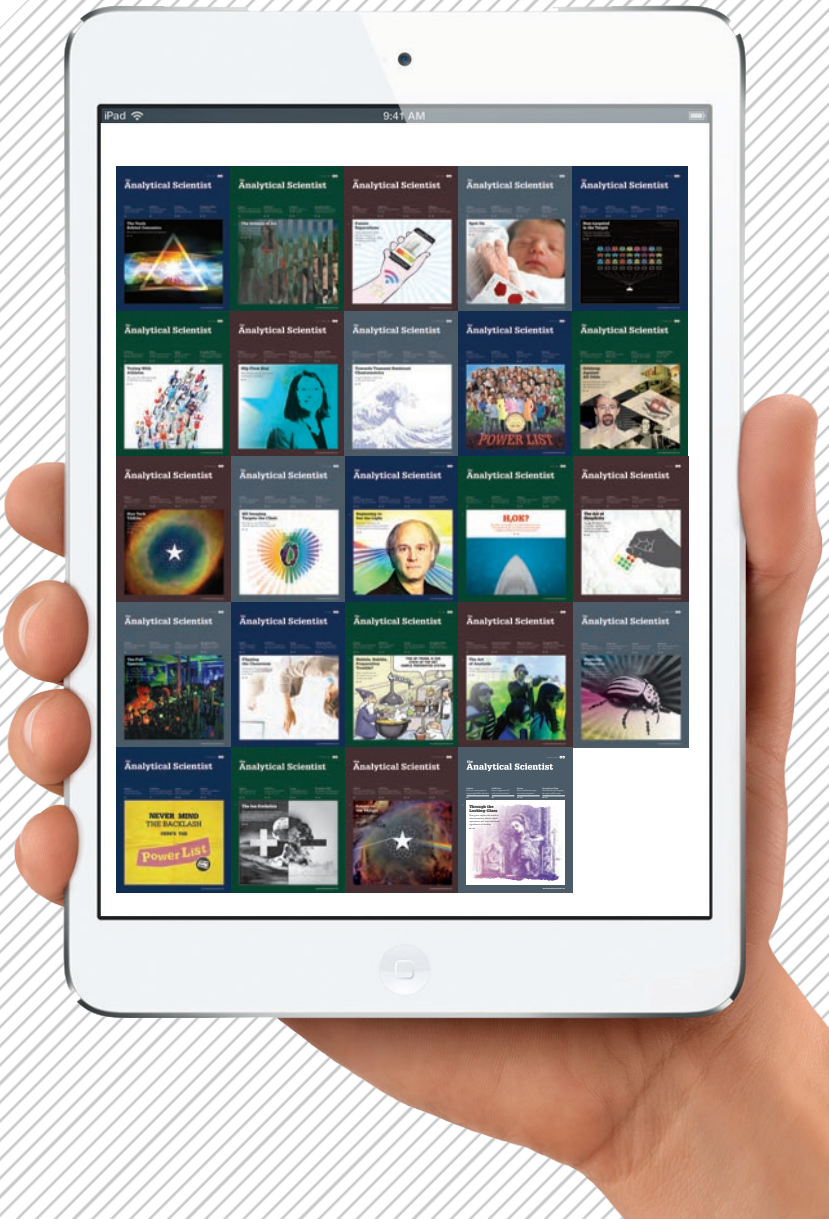

Celebrating Our Second Anniversary

It's hard to believe that two years have passed since the first issue of The Analytical Scientist rolled off the printing press and populated the iTunes Newsstand in January 2013.

We've published 24 issues (including this one) and over 350 articles. We've produced two Power Lists, featuring 138 analytical scientists, and we launched our TASIAs, resulting in 30 innovative winners.

Most importantly, we've made many friends.

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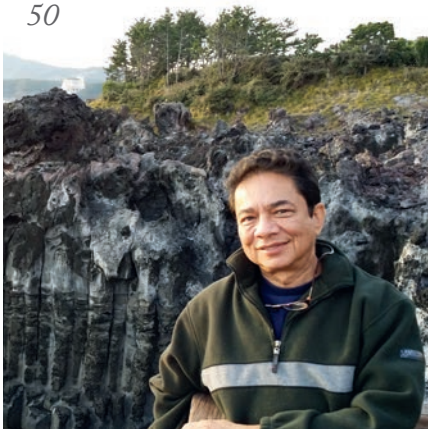

On The Cover

Alice steps through the looking-glass (mirror) and into the world of chirality

the Analytical Scientist

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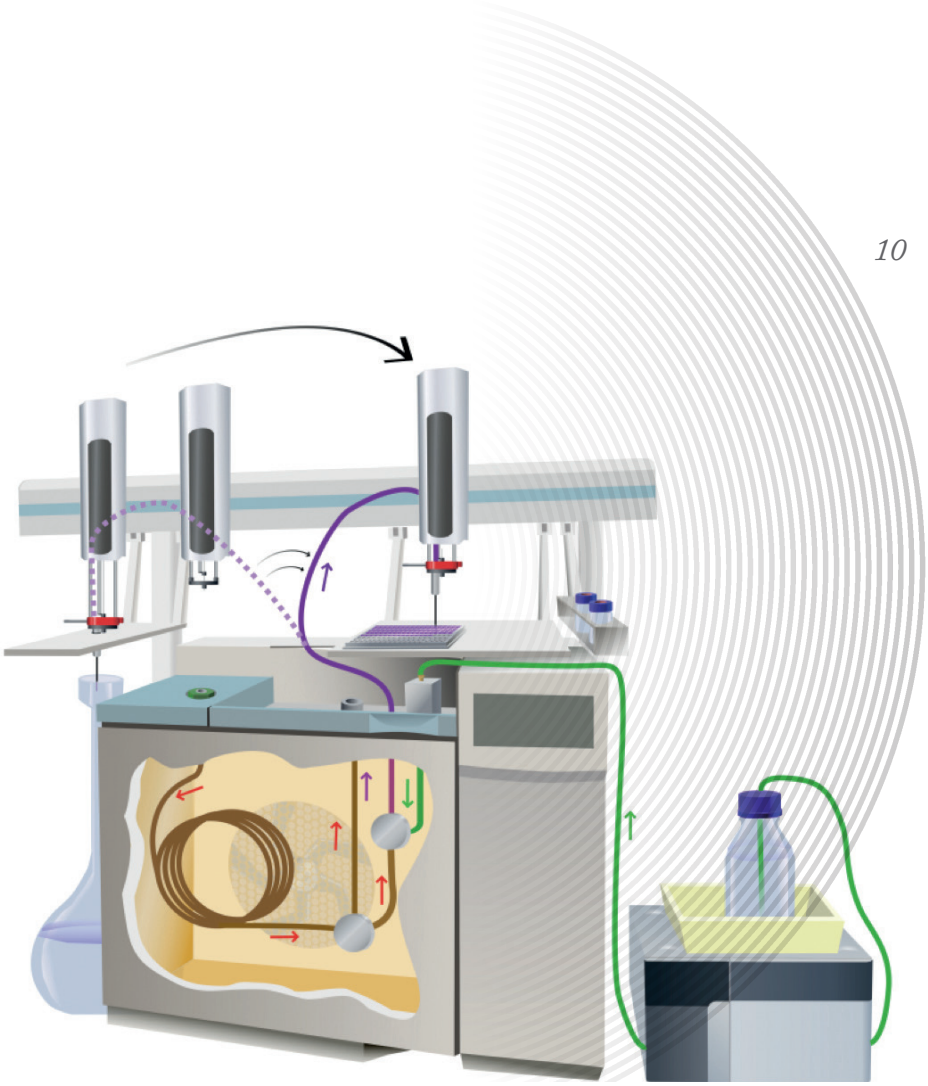

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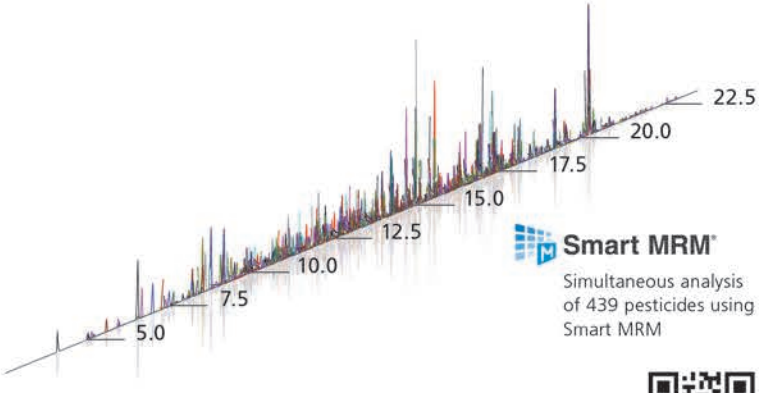

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Classic Chemistry

Combining art and science is becoming a bit of a theme for The Analytical Scientist. Lewis Carroll's reference to stereoisomers sent me down my own rabbit hole of discovery.

The cover of our first issue of 2015 features Lewis Carroll's Alice climbing through a mirror into another world. Inspired by early references to chirality noted by Christopher Welch in this month's feature on page 26, I went searching for additonal – more visual – representations and chanced upon Carroll's intriguing and thought-provoking nod to stereoisomers (1):

"How would you like to live in Looking-glass House, Kitty? I wonder if they'd give you milk in there? Perhaps Looking-glass milk isn't good to drink", says Alice to her black kitten in the first chapter of *Through the Looking-Glass*, before embarking on a new adventure.

Was Carroll (actually Charles Lutwidge Dodgson) accidentally referring to chirality or had he more deeply considered the fact that a stereoisomer of lactose – or any other nutritional molecule for that matter – could be indigestible, dangerous or simply tasteless? Perhaps we'll never know for sure, but given the timing of the novel (1871) and Pasteur's work on discriminating enantiomers in a mixture (1861), I think it's more judgment than luck. Dodgson's Oxford University background (in mathematics) probably saw him rubbing shoulders with the odd chemist or two...

Eager for more classic literature and chemistry, I came across another interesting use of chemistry in *Elective Affinities* where Goethe uses chemistry – specifically, the reaction between dilute sulfuric acid and limestone – as a simile for the unexpected but inevitable relationships that form when certain combinations of people come together:

"Suppose an A connected so closely with a B, that all sorts of means, even violence, have been made use of to separate them, without effect. Then suppose a C in exactly the same position with respect to D. Bring the two pairs into contact; A will fling himself on D, C on B, without its being possible to say which had first left its first connection, or made the first move towards the second." (*Elective Affinities,* Johann Wolfgang Goethe, 1809). Life and love, it seems, are a little more complicated than 'opposites attract'.

Finally, Charles Dickens really captures the spirit of analytical science with a wonderful simile: "Meanwhile the retainer goes round, like a gloomy Analytical Chemist: always seeming to say after 'Chablis, sir?' – 'You wouldn't if you knew what it's made of.' (*Our Mutual Friend*, Charles Dickens, 1864.) Thereafter, the servant is amusingly referred to as the Analytical Chemist.

Can you offer any classic references to your field?

Rich Whitworth
Editor

Reference

1. http://tas.txp.to/0115/alice

Upfront

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

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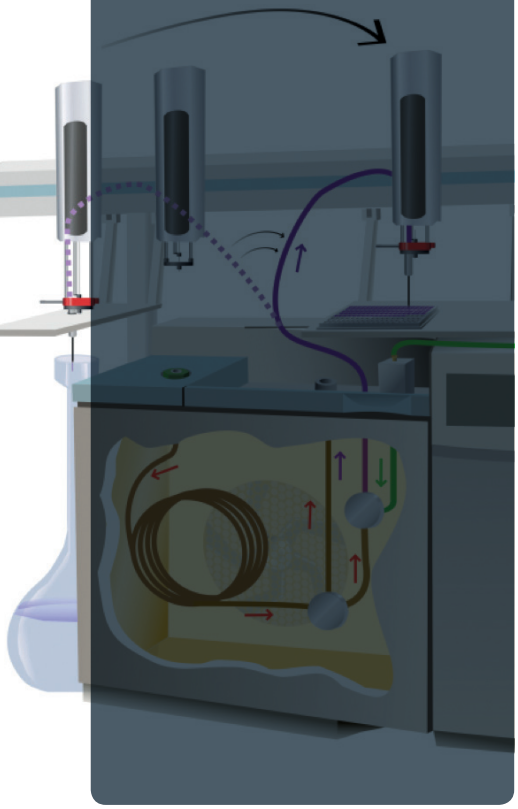

Redux: Preparative GC

A new approach to an old idea hyphenates FID detection with bioactivity assays

By Willem Jonker and Jeroen Kool

Gas chromatography's extremely high separation power makes it a popular analytical technique. And, in principal, GC could also be highly suited to compound purification and preparative purposes; for example, toxicity testing or flavor analysis. Unfortunately, going from an idea to a working platform is not always straightforward and fraction collection is complex – a gas is simply not easy to collect!

In the past, several GC fractionation systems have been developed, based on adsorbent traps or more popular rapid column eluate cooling, but complexity and limitations have slowed progress. For example, one of the issues with rapid cooling of the column eluate is potential precipitation of the analyte, meaning potential loss via aerosol formation. Another general problem is the limited number of fractions that can be collected.

We believe that the ability to collect each peak in the chromatogram would re-focus interest on this area. To that end, we developed a new approach that circumvents trapping by adsorption or cooling (see Figure 1). Furthermore, simultaneous flame ionization detection (FID) allows us to later correlate the content of a fraction to a peak in the chromatogram. The platform consists of a standard GC-FID with autosampler. First, the sample is injected onto the column. The end of the column is connected to a flow splitter that directs a small part towards the FID. The rest goes to a second split where "the magic" happens. At this split, a preheated volatile solvent (heated by a modified FID) is mixed with the column eluate and guided via a capillary outside the GC oven.

Once outside, the volatile solvent containing the separated analytes condenses and is collected in microtiter plates. The capillary is attached to a fraction collection tip that moves with short time intervals from one well to another, collecting the whole separation – up to 384 seven-second fractions. We are in the final stages of transforming our platform from a proof of principle setup into a robust system.

Currently, one of our applications is bioactivity screening of environmental matrices via a so called effect-directed analysis (EDA) approach. In EDA, bioassay testing is combined with chemical analysis for the detection of newly emerging toxicants. The flow scheme is illustrated in Figure 2. After fraction collection, the well plate is used for bioassay testing and a bioassay chromatogram is generated by connecting the response of each well to a retention time. The bioassay chromatogram can then be directly correlated to the FID chromatogram, enabling us to easily pinpoint bio-actives.

Thus far, we have successfully used the platform in combination with the acetylcholine esterase assay for the detection of carbaryl and aldicarb, and two functional gene reporter assays for the detection of dioxin-like and androgenic compounds. This direct comparison would not have been possible with previously developed setups because the number of fractions that can be collected are limited.

Besides EDA and toxicology studies, we also see potential for applications in other analytical research fields, for example, flavors and fragrances in the cosmetics and food industries. Another possibility is the purification of an organic synthesis product for

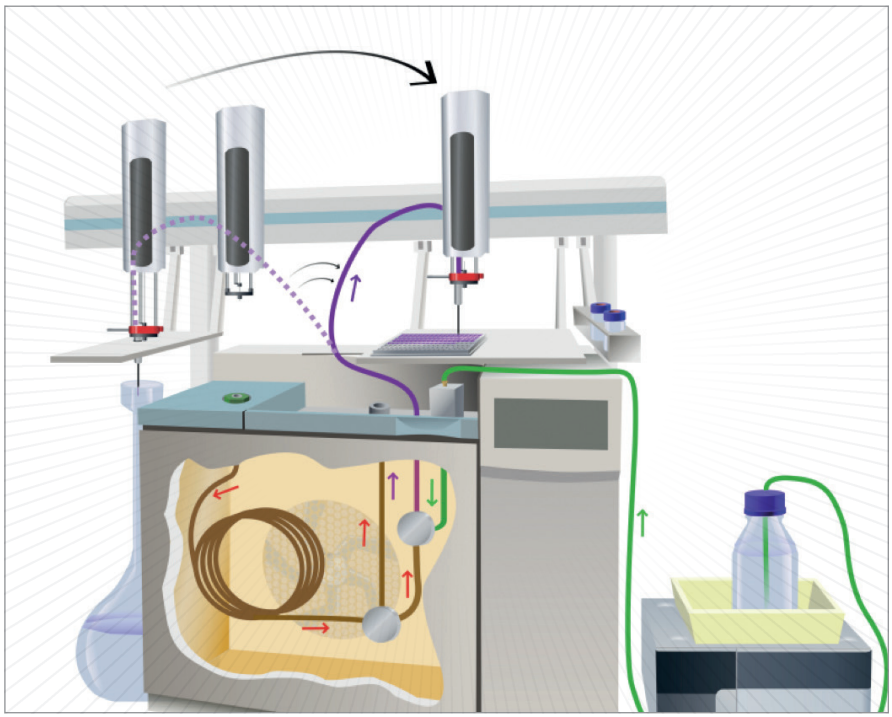

Figure 1. GC fractionation platform with parallel flame ionization detection.

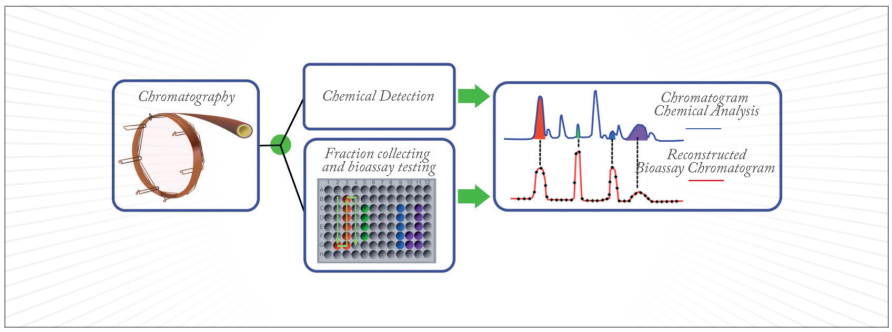

Figure 2. Hyphenating bioassay testing with chromatography and chemical detection. The collected fractions are biologically tested. The bioassay chromatogram can be directly correlated to the chemically-obtained chromatogram to rapidly pinpoint bio-actives.

NMR analysis; purification could be performed by repeated injections of the sample followed by fraction collection in the same well plate.

Furthermore, applications in drug discovery research can be envisioned in terms of bioactive compound screening. Using the platform we developed, sample complexity can be reduced and bioactive(s) of interest can be revealed.

Such use of gas chromatography opens up a whole new world of compounds for purification and bioassay testing. We hope that our platform may become a valuable tool for future research and that other analytical chemists share our interest.

Willem Jonker is a PhD student and Jeroen Kool is Assistant Professor at VU University Amsterdam, The Netherlands.

Swimming with Spectroscopy

Is it time to drop your NIR instrument in the pool?

Monitoring oxygen levels can provide feedback about whether muscles are receiving enough oxygen. near-infrared spectroscopy (NIRS) is already being used for this purpose in a variety of sports, but not in aquatic environments. Now, researchers at the University of Essex in the UK have developed a method of using NIRS underwater to measure muscle oxygenation in swimmers (1). But it's not just athletes looking to monitor their sports performance who could benefit; the technology could track and optimize water-based rehabilitation therapies for patients with muscle injuries.

One of the authors of the study, Benjamin Jones from the university's Centre for Sport and Exercise Sciences, says that the aim of the work was to really expand the application of spectroscopy in sport. "Advancements of wireless devices, device size and telemetric capabilities have all contributed to what are known now as 'portable' or 'wearable' NIRS devices that can be used on the sports field. Underwater NIRS is very limited," he says.

But waterproofing a suitable spectrometer is easier said than done, and proved to be a challenge for Jones and the team. First, the team experimented with a range of commercially available waterproof casings designed to protect cameras and phones, but a major issue was the effect the waterproof materials had upon the emitted light intensities. "Covering the NIRS light source caused a 'light piping effect', whereby the waterproof materials reflected a portion of the NIRS light along the material surface directly to the light receivers without entering the muscle tissue," explains Jones. "After attempting a variety of materials, we decided to manufacture an 'optical break'. We used a black polyvinyl chloride material acting as NIRS opaque layer, which seemed to sufficiently address the issue."

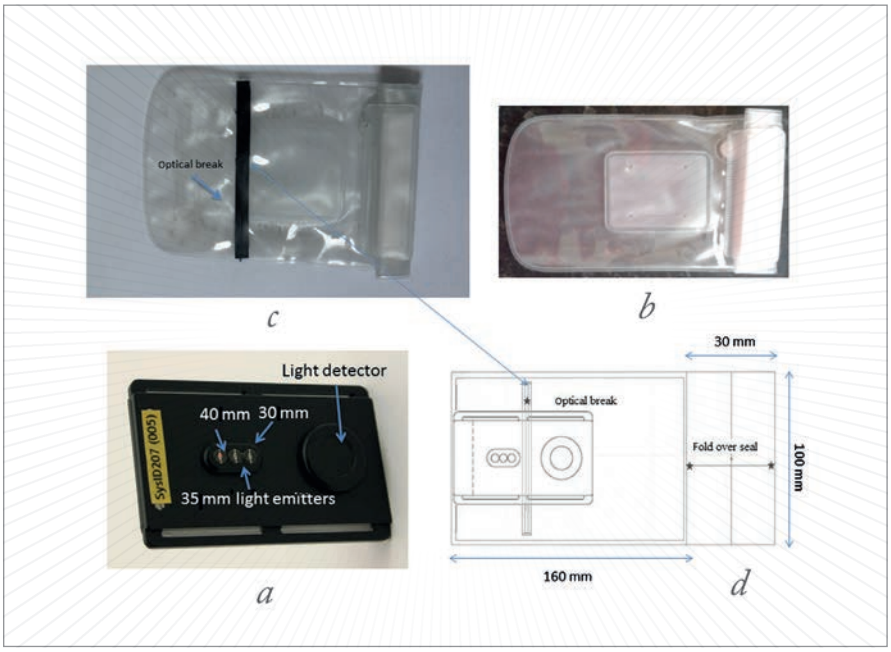

Figure 1. a) PortaMon-portable dual wavelength continuous wave near-infrared device; b) iSwim waterproof covering; c) iSwim with optical break medication; d) technical drawing of modification.

Another difficulty presented by the water was its effect on Bluetooth connectivity. Although the NIRS device had a telemetric capacity of 100 m on land, connectivity was lost after an immersion depth of just 15 cm, highlighting the difficulties of working in water.

Validation data from the study confirmed that the waterproof covering had no significant effect upon muscle oxygen measurements during exercise. The next step, according to Jones, will be to combine a peripheral measure of muscle oxygenation utilizing underwater NIRS with existing technologies that can provide a global measure of oxygen consumption. "This will allow us to observe a complete oxygen profile during swim exercise and the changes that occur in response to exercise," he says.

Reference

1. B. Jones, M. Dat, and C. E. Cooper, "Underwater Near-Infrared Spectroscopy Measurements of Muscle Oxygenation: Laboratory Validation and Preliminary Observations in Swimmers and Triathletes", J. Biomed. Opt. 19 (12), (2014). Doi: 10.1117/1.JBO.19.12.127002

Finding Phytase

Recombinant blood protein contamination is discovered by chance. NMR to the rescue?

When a research team accidentally discovered plant enzymes in a batch of supposedly pure, commercially available recombinant human blood protein, it kicked off a new project into using nuclear magnetic resonance (NMR) spectroscopy as a quality control test (1).

"We were conducting binding studies between the blood plasma protein, albumin, and adenosine 5'-triphosphate (ATP) and

other ligands," explains Robert Brinson, a chemist at the Institute for Bioscience and Biotechnology Research, which is part of the US National Institute of Standards and Technology (NIST). "We purchased recombinant human serum albumin (rHSA) without consideration of its source. Commercial biotechnology-grade rHSA is typically produced in yeast. However, the new rHSA product that we purchased was produced in rice. Albumin does not have the ability to degrade ATP, but when we performed binding experiments, we found that the ATP rapidly degraded."

The problem turned out to be contamination with phytase – a plant enzyme that hadn't been spotted by the manufacturer in its quality control tests. Probing the problem a little further, the group conducted a systematic ^{31}P NMR study of HSA and rHSA products, which included rice-derived products from various vendors and control products, human serum pooled HSA and yeast-derived rHSA. Varying levels of phytase contamination were found. Such residual host cell protein impurities can cause toxic or immunogenic responses in patients.

Could similar contamination occur in biotherapeutics? Brinson and the team didn't test any drug products, but if the purification process for clinical, rice-derived rHSA is similar to biotechnology grade products, then there is a risk. Brinson says, "It is not the mission of NIST to test specific clinical products for companies. Our goal in the field of biomanufacturing is to develop measurement methods to aid industry and regulators in the characterization of biotherapeutics." And he believes that ^{31}P NMR could step up to the plate. ^{31}P NMR only measures phosphorylated molecules, which means that measurements can be conducted on complex, buffered protein mixtures. In addition, ^{31}P NMR does not require knowledge of the phosphatase – the general name for any enzyme that cleaves a phosphate from a small organic molecule, such as ATP.

The two standard methods for contamination detection are ELISA and two-dimensional liquid chromatography coupled to mass spectrometry (2D-LC-MS). "The specifics of performing these assays are markedly different, but the purpose of both techniques is to determine host cell protein and other protein contaminants. And while these are indeed very sensitive techniques, the biggest drawback is the need to have an idea of potential protein contaminants. Otherwise, the mass level of an unknown impurity may fall below the threshold of detection," says Brinson.

In essence, a highly active enzyme that is only present in trace amounts could be missed by traditional techniques; however, the resulting cleavage reaction can be seen with ^{31}P NMR. Brinson notes, "We see our method as complementary to the established methods. The ^{31}P NMR method does not supplant them. NMR is not a high-throughput technique. While the ^{31}P NMR assay is only 20 minutes, the other methods are truly high throughput and can analyze products in parallel."

Reference

1. *R.G. Brinson et al, "Detection of contaminating enzymatic activity in plant-derived recombinant biotechnology products", Analytical Chemistry, 86 (23), 11508–11512 (2014).*

Jurassic Park Parchment

DNA from ancient archive gives researchers a window into animals of the past

OK – Jurassic was an exaggeration. Nevertheless, mass spectrometry (MS) has been used to analyze DNA extracted from ancient animal skin parchments obtained from an archive in York, UK; researchers are hoping to identify variations in animal livestock that have long since been lost through agricultural reform and breeding programs (1).

"I was invited to visit the new Borthwick Institute for Archives. Everyone was impressed with the high-tech nature of the facility – but all I could think about were the racks and racks of dead animal skins that I wanted to analyze," says Matthew Collins, a professor in the University of York's bioarchaeology department and one of the study authors. "I'd had a student working for three years to see if it was possible to look into a particular economy through the pattern of animal bones. But during that time, we only had around 20 or 25 animals to work with. Suddenly, there were thousands of animals before me – and most had a date written on them."

Collins and his colleagues used a combined proteomic/genomic approach to identify the source species of various parchments. And though it isn't the first time that researchers have sought to obtain DNA from parchment, previous attempts have not always been successful because of limitations in controlling and estimating contamination, according to Collins. Analysis can also be tricky if multiple skins have been washed, cured and depilated together.

Fortunately, technology has opened up new possibilities. "We used MALDI-TOF MS to fingerprint the proteins in a sample," says Collins. "A protein called collagen dominates skin and different species have different collagen sequences. MALDI-TOF can rapidly and cheaply discriminate these, and we used next generation sequencing to analyze millions of fragments of DNA from a sample in parallel," says Collins. "DNA exists in parchment as short fragments, which are ideally suited for this type of analyses. One of our next goals is to perform the analysis non-destructively. We're working on a new paper about that right now…"

Collins estimates that there could easily be over one million parchment documents in the UK alone, representing a vast, untapped resource into understanding the development of livestock across the centuries.

Reference

1. M.D. Teasdale et al, "Paging Through History: Parchment as a Reservoir of Ancient DNA for Next Generation Sequencing", Philosophical Transactions B, doi: 10.1098/rstb.2013.0379 (2014).

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Why?

Imaging of single DNA molecules is useful for various applications in physics, chemistry and biology, including the study of diseases associated with genomic alterations, such as cancer and Alzheimer's disease. Most current systems are expensive and bulky, unable to be used in point-of-care applications or in resource-limited institutions and many developing countries. Mobile phone based imaging is field-portable, cheaper and more accessible.

How?

The mobile attachment uses a laser-diode (450 nm, 75 mW) for oblique angle excitation, combined with thin-film interference filters to suppress background noise. A miniature dovetail stage is used for depth-of-focus adjustment and an external lens magnifies the image of the specimen onto the phone's image sensor chip. The set up creates a very high contrast dark-field.

Who?

The work was conducted by a team of researchers based in various departments and institutes at the University of California.

Where?

The research paper has been published as an open access article: Qingshan Wei et al., "Imaging and Sizing of Single DNA Molecules on a Mobile Phone", ACS Nano, 8 (12), 12725–12733 (2014), doi: 10.1021/nn505821y

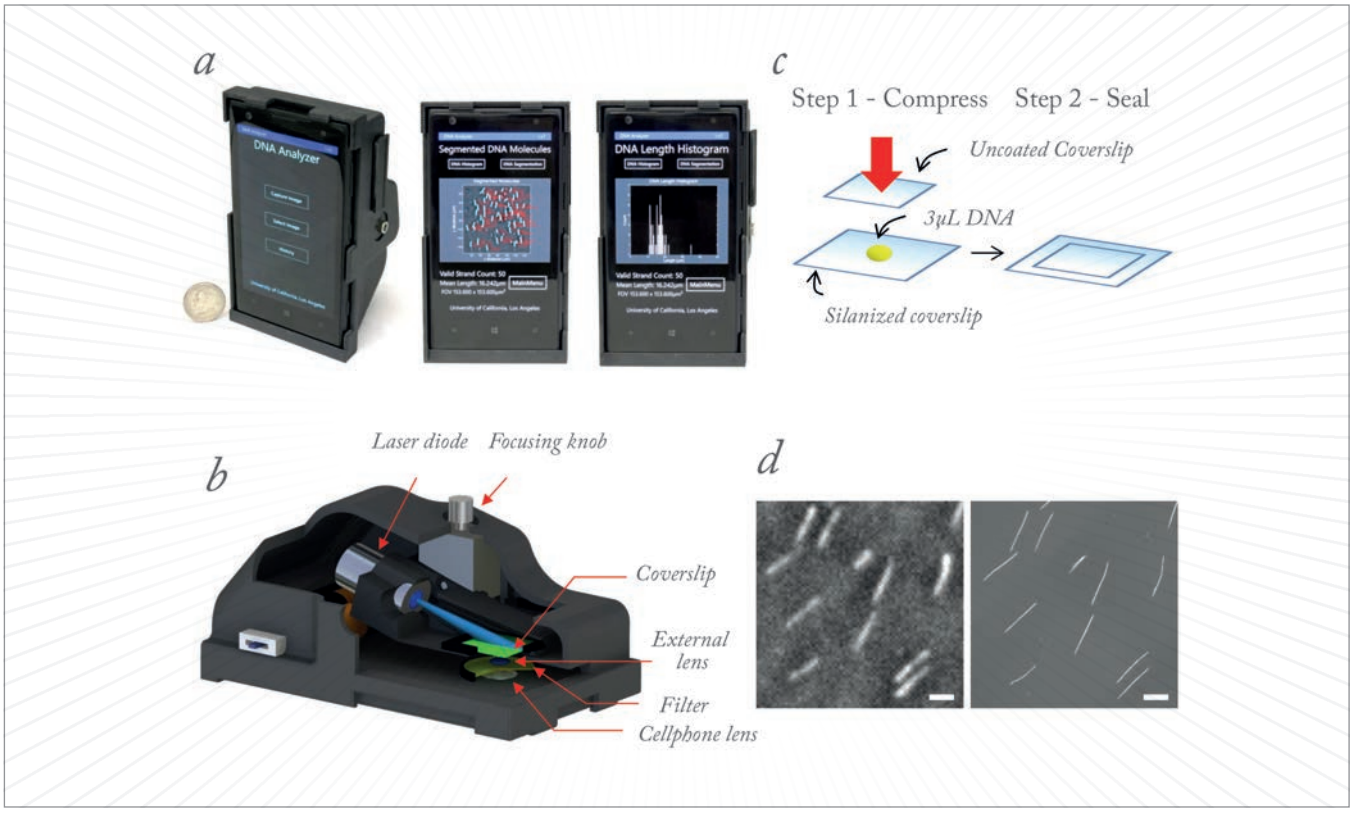

Figure 1. a) Smartphone in fluorescence microscope attachment. b) 3D illustration of opto-mechanical attachment. c) Illustration of simple DNA stretching method. d) Representative fluorescence microscope images (scale bar: 10 μm).

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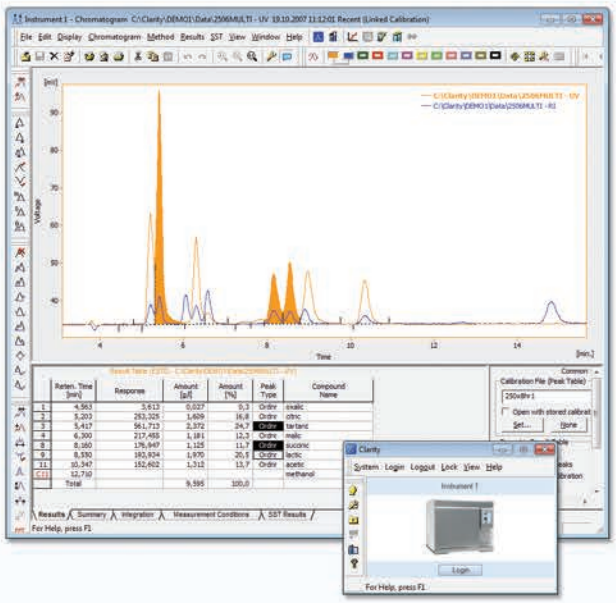

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In My View

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

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

Contact the editors at
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Fishing with Ion Chromatography Suppressors

Combining knowledge from multiple fields inspired a solution to a problem in ion chromatography. What a shame the manufacturer didn't take the bait...

By Purnendu (Sandy) Dasgupta, Jenkins Garrett Professor of Chemistry and Biochemistry, University of Texas at Arlington, USA.

I actually started using ion chromatography (IC) – of all things – to measure sulfate. Many people, including Hamish Small, say that IC became a commercial success primarily because it could measure sulfate. And that's true. Sulfate is a compound with no optical handle and before IC, people were measuring it with the barium chloranilate method, which took a lot of effort. And although ion chromatographs of that generation didn't come with autosamplers, the fact that you could measure sulfate in parts-per-million concentrations within 15–20 minutes was wonderful. Importantly, the whole concept and execution of IC depends on very understandable and exploitable chemistry.

One thing that really attracted my curiosity in IC was the suppressor. The suppressor was both the unique differentiator that made IC possible in that form, but also the weakest link in the chain. Indeed, suppressed IC suffered from i) the need for suppressor column regeneration, ii) variable retention of weak acids (as a function of suppressor column condition), and iii) suppressor column induced band broadening.

I was using Nafion tubes in air sampling work as dryers, and their properties piqued my interest. I knew that Nafion (a sulfonated fluoropolymer made by DuPont) was a cation exchange material and I wondered if, instead of using packed suppressor columns, I could use a Nafion fiber and pump chromatographic eluent through it. Bathing the fiber in acid would allow continuous exchange.

"One thing that really attracted my curiosity in IC was the suppressor. The suppressor was both the unique differentiator that made IC possible in that form, but also the weakest link in the chain."

"I wondered if, instead of using packed suppressor columns, I could use a Nafion fiber and pump chromatographic eluent through it."

I also knew the mass transfer equations applicable to denuders, which are used to capture gas molecules while transmitting the aerosol, from my work in atmospheric sampling. It's not intuitive, but the equation states that mass transfer to the wall is independent of the diameter of the tube given the same overall volume flow rate. That truth seemed to be of great significance to me. After all, band dispersion in chromatography depends acutely on the diameter of the tube. Therefore, the solution to improving suppression columns in this regard was simply to use tubes with a smaller diameter.

At the time, the tubes available were about 0.9mm in diameter. I had just moved to Texas Tech University, and I was talking to DuPont about the fibers. I asked if they could make 0.3 mm tubes. Yes, they could, but agreement would be needed from "upstairs," so I wrote a letter to Jack Kirkland. Six months later, I received a reply (I still have the letter) stating that DuPont was independently interested in the project. Jack suggested that I contact The Dow Chemical Company; Stevens and Small from Dow had just published a paper on using sulfonated polyethylene fibers as suppressors. Ironically, they were using exactly the same diameter that I wanted.

However, it turns out that sulfonated polyethylene isn't a great ion exchange membrane. I knew that Nafion would be far better – and yet DuPont would not make what I wanted. I don't know the full story, but in any case, like all good assistant professors, I was concerned about the safety of my idea. So I decided to go solo and attempt to make what I needed myself. I got up one morning and suddenly realized that I knew how to do it. I put fishing line inside a Nafion tube, coiled it and then boiled it in water, thermosetting the fishing line. The process produced beautiful coils and, more importantly, created suppressors with ten times less dispersion. My first two papers as an assistant professor featured the "annular helical suppressor" and were published simultaneously in Analytical Chemistry in 1984 (1, 2).

I take great pleasure in stating that, despite failing to pursue my idea with the manufacturer, I was able to take an alternative route by patenting the process of putting DuPont fishing line inside DuPont Nafion tubes. What's more amazing is that someone is still selling those suppressors – SeQuant in Sweden! Those two papers also kicked off an excellent relationship with Dow and then Dionex – but that's another story.

The moral: if you have an idea you believe in, don't be afraid to fly solo or go fishing.

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Making Room for Improvement

To make way for more flexible approved analytical methods, we need a better understanding of the underlying scientific principles.

By Dennis Åsberg, PhD student, Department of Engineering and Chemical Sciences, Karlstad University, Sweden.

Wolfgang Lindner's "Breaking out of the Black Box" article in the March issue of The Analytical Scientist highlights the problem with users not understanding the underlying science in analytical chemistry (1). The article caught my attention because black box thinking is one of the problems I've been working on for more than a year. I am looking for more flexibility in developing regulatory-approved analytical methods for the pharmaceutical industry. Approved methods are locked and don't require much scientific knowledge – the user must follow them rigidly and there is little opportunity for improvement.

"Our first and most important step was to investigate the differences between HPLC and UHPLC in depth."

The core of this project, therefore (which was part of a larger study on molecular interactions) was to shift the focus to a more science-based approach that requires an understanding of the analytical methods, thereby enabling continuous improvements.

Anders Karlsson (AstraZeneca R&D in Mölndal, Sweden) came up with the idea that launched the project. He wanted to continuously improve his quality control procedures after the original methods had been approved by regulatory agencies. Actually, this is already possible to a certain extent, but only if the analytical method is filed according to the European Medicines Agency's (EMA) Quality-by-Design guidelines. That is to say, the guidelines do allow post-approval changes if – and only if – the changes are inside the original design space, which can be limiting.

For example, a pharmaceutical company that had developed and filed a HPLC-based quality control method a few years before the commercialization of UHPLC would find it difficult, if not impossible, to upgrade its quality process simply because it would not have been able to include UHPLC conditions in the original design space. Done correctly, switching from HPLC to UHPLC is a minor modification because the essential difference lies in column dimensions and particle size – and yet it would offer significant improvements on analytical performance. However, according to the regulations, the changeover is not possible without resubmitting the method to the EMA.

Working with Karlsson and Mikael Nilsson, Cambrex Karlskoga, and my supervisors, Jörgen Samuelsson and Torgny Fornstedt, I launched a project with the goal of finding a way to develop analytical methods that allow minor post-approval changes – even if they are outside of the original design space.

"Approved methods are locked and don't require much scientific knowledge – the user must follow them rigidly and there is little opportunity for improvement."

We modified an original quality control method for esomeprazole magnesium (Nexium), by switching from HPLC to UHPLC. Our first and most important step was to investigate the differences between HPLC and UHPLC in depth, which allowed us to explain the differences scientifically, making method transfer easier. I believe that one of the reasons behind the success of the project was the diverse backgrounds, perspectives and skills of the people who became involved as it progressed.

To sum up, I clearly agree with Lindner that we should strive to understand the underlying principles behind analytical methods. Greater understanding is often the solution to creating smart and efficient analytical methods. I also think that it is important for the industry to encourage scientific understanding. On a personal note, the project has also taught me that you can't be an expert on everything and that cooperation is needed to produce good and robust analytical methods.

Reference

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Computer-aided Chromatography

We need to extend the limits of gradient condition optimization beyond reversed-phase separations.

By Eva Tyteca, predoctoral researcher at Vrije Universiteit Brussel, Belgium

The chromatographic simulation of reversed-phase separations has become widely used for the optimization of gradient conditions. Such simulations use the linear solvent strength (LSS) model developed by Lloyd Snyder and John Dolan. However, elution modes such as hydrophilic interaction chromatography (HILIC) and supercritical fluid chromatography (SFC) are becoming more popular for the analysis of ionizable and/or polar compounds, and for the analysis of a wide range of analytes. Here, the conventional LSS-model can no longer be used to predict retention under various gradient conditions. In these elution modes, retention is a result of a combination of several mechanisms, including partitioning and adsorption, which results in non-linear retention relationships. Clearly, more elaborate retention models are needed to describe the curvature.

"Clearly, more elaborate retention models are needed to describe the curvature."

I understand that this suggestion might seem frightening for the "hands-on" chromatographer, but I sincerely believe that existing modeling software, such as Drylab or Chromsword, dedicated to reversed-phase approach, could – and should – be extended to "new" and emerging elution modes, using non-linear retention models. I am aware that the parameter estimation using such non-linear models becomes more difficult, especially when performing a limited number of initial gradient scouting runs. And the parameter fitting becomes really problematic for the non-linear models for which no analytical expression for the retention factor in gradient elution exists. However, these non-linear models can provide a significant improvement in the accuracy of (gradient) retention time predictions, compared to the conventional LSS-model – and the extra effort would therefore pay off during the optimization of gradient conditions.

Several research groups have initiated isocratic modeling for these non-linear elution modes for a wide range of compounds; for example, the groups of Pavel Jandera, Thomas Letzel and Xinmiao Liang are working on HILIC, and the groups of Caroline West and Eric Lesellier on SFC. Recently, I started investigating the possibilities of gradient retention modeling in these two elution modes, together with Davy Guillarme, using a mixed model (combining both an adsorption and a partitioning term, proposed by Liang) and an empirical (reversed-phase) model, proposed by Uwe Neue. The use of these non-linear models for the separation optimization in HILIC and SFC should in the near future be validated with real-life mixtures, including compounds with a wide range of chemical properties

"Several research groups have initiated isocratic modeling for these non-linear elution modes for a wide range of compounds."

I am aware that, before computer-aided method development can be used routinely for the optimization of HILIC and SFC separations, an evaluation of the accuracy and the robustness is needed. However, it would be great to see the promising retention models proposed by several research groups being implemented by the vendors of the existing reversed-phase optimization software packages...

Clearly, we have to tackle the problems that might arise and answer the concerns of the chromatography community, but I believe there is a strong future for computer-aided method development that goes beyond traditional reversed-phase separations.

Furthermore, don't we all want a greener future? The solvents saved by reducing the number of required experiments during the chromatographic method development has got to be a good thing.

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Adding Science Behind the Canine

Leveraging technology solutions to support rather than replace dogs in arson investigations.

By David Matthew, Research Coordinator, Napa, California; John DeHaan, Fire-Ex Forensics, Vallejo, California; and Gareth Dobson, Smiths Detections, Danbury, Connecticut.

Back in 2013, an article entitled "Every Dog Has Its Day" in The Analytical Scientist suggested that the canine could be replaced by emerging technologies. "A new 'sniff' test for explosives uses direct, real-time vapor detection in a bid to put our canine colleagues out of work in the screening of people, baggage and cargo," it boldly stated (tas.txp.to/0115/dogdays). The article focused on using ionization chemistry at the front end of a mass spectrometer that could measure in parts per quadrillion. Our research project takes a rather different approach...

Instead of comparing and contrasting dogs with the latest technology, we found it beneficial to associate and align the capabilities of the canine with the field application of gas chromatography-mass spectrometry (GC-MS) teamed with solid phase microextraction (SPME). Why? We were aiming to increase scientific capability in a very special application area: arson investigations. At fire scenes, investigators look for traces of flammable liquids using electronic sniffers or specially trained canines. Debris is collected and must be examined by a properly certified forensic lab – a process that can take weeks.

Our Phase I testing involved bench-scale fires, which demonstrated the ability of GC-MS to identify 13 different ignitable liquids. A sensitive volatile organic vapor detector utilizing photo ionization technology (PID) was used to locate the highest concentration of vapors, which were then sampled using an exposed SPME fiber, followed immediately by hand-portable GC-MS. The initial testing provided significant improvements in sampling techniques including a method proposed by Dr. DeHaan that increased vapor concentrations while reducing cross contamination. Phase II testing – with full-scale room fires and extended post-flashover exposures – confirmed that the identification of ignitable liquid vapors from fire debris was possible, with reported vapor concentrations as low as 5000 parts per billion.

Phase III tests have been designed to evaluate our method in actual fire scenes in Texas and California, putting science behind the canine. And although the testing will not be concluded until 2015, the results have already demonstrated the system's capability to identify ignitable liquid vapors at the fire scene when located by trained canine teams.

And so back to the canine... "The sensitivity of canines is unknown... This work represents the first direct vapor detection of explosives so it should be comparable to the canine response," said Robert Ewing, a senior research scientist at the Pacific Northwest National Laboratory in the aforementioned article. And the canine olfactory system certainly sets a high standard – it is estimated to be in the order of a few ppb for many compounds.

Some of our significant research findings link GC-MS data to the work of fire/arson canines. During a canine certification test in Texas, 10 microliters of gasoline was placed onto carpet in an open-air room, about 10-meters square. Eleven of the twelve canines were able to pinpoint the source, ranging from 7 cm to within 0.7 cm of the target. Instrument readings revealed canine hits below the detection limits of the PID detector. By utilizing a 15-minute SPME sampling time with the DeHaan sampling methodology, GC-MS data identified the components of gasoline. While the sensitivity of the canine olfactory system is remarkable, the canine alert obviously cannot discriminate volatile fuels present; critical identification of possible accelerants from the residues of products used as solvents in adhesives, insecticides, fabric cleaners, or those that are inherent to the consumer product or its raw materials, can only be made by with GC-MS analysis.

What have we learned during our research project? Well, we've found that it is much more advantageous to collaborate with the canine rather than try to compete with it! By combining traditional and new approaches, we can provide the decision maker with additional timely information. Our airports are in desperate need of improved detection of explosives. The predominant technology being used today – ion mobilization spectroscopy (IMS) calibrated to look for nitrogen based explosives – is much maligned. The use of ionization with mass spectrometry is a significant improvement, but it may never be evaluated. Combining canines with advanced instrumentation may be a better idea than arguing for their replacement. Dogs are faster and more flexible than any instrument-based technology alone.

Reference

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In Lewis Carroll's 1871 novel, Through the Looking-Glass, Alice suggests to her black kitten, "Perhaps Looking-Glass milk isn't good to drink..." And though our four gurus don't attempt to predict the potability of milk from a mirror world, they keenly address the broader significance of chirality, the major milestones in chiral analysis, and where the field is heading.

How would you define the broad significance of chirality?

Christopher Welch: Molecular chirality is very important in biomedicine. As the cellular machinery of life is asymmetric, the interaction of the two mirror image forms of chiral molecules can show different activities. Also, almost all new chiral drugs are prepared as pure enantiomers.

Yoshio Okamoto: The homochirality of many functional proteins means that most biosystems (including human beings) are very sensitive to the chiral nature of compounds. Therefore, care must be taken when using biologically active chiral compounds. Chirality helps us to understand most stereochemical processes in biosystems. The distinct differences in the biological functions between the enantiomers of a chiral compound have long been understood, and the use of single enantiomers of chiral drugs was recommended about 30 years ago.

Wolfgang Lindner: I believe chirality and, therefore, the three-dimensional (3D) orientation of dissymmetrical small molecules in relation to large molecular objects – and them being surrounded by and in contact with chiral objects (molecules) – is intrinsically important. The diverse elements of molecular chirality describe a 3D information system on a macro molecular scale, which is essential for regulating stereochemically driven biological processes.

Bernard Testa: The vast domain comprising the chemistry of stereoisomers includes all molecules that exist in two enantiomeric, chiral forms. However, stereochemistry extends well beyond chirality as it also includes an additional relevant property: diastereomerism. Many molecules have one or more diastereomers without being chiral, and there exists an even larger number of other molecules exhibiting both enantiomerism and diastereomerism (1).

Stereoisomers are defined as molecules containing the same atoms (identical composition) and the same connectivity

(identical constitution), yet they differ in their 3D-geometry (configuration). This implies that stereoisomeric molecules have an addition level of structural complexity compared to their non-stereoisomeric analogs. Therefore, I think it is better to focus on stereoisomerism rather than on the narrower field of chirality.

Can you touch on the early milestones in our understanding chirality?

YO: Biot discovered optical activity in 1812 and Pasteur discovered the first resolution of tartaric acid in 1848, but the importance of chirality was better understood when in 1874 van't Hoff proposed the tetrahedral arrangement of valence bonds in carbon.

WL: Although Pasteur's brilliant discovery of molecular chirality was more than 150 years ago (1848), it remains a hot topic encompassing diverse fields of natural, life, and material sciences, astrophysics, and even philosophy.

CW: Pasteur's, van't Hoff's and Kelvin's contributions are well known, but it's interesting to note that back in the late 1700s, Kant (Prolegomena to All Future Metaphysics, 1783) gave a very explicit definition of chiral objects.

"What can more resemble my hand or my ear, and be in all points more like, than its image in the looking glass? And yet I cannot put such a hand as I see in the glass in the place of its original..."

But the history of chirality goes back even further. Aristotle, in De Incessu Animalium states, "All animals which are able of themselves to make a change of place, have a further distinction of left and right."

There are even earlier examples – although not so clearly stated.

BT: A detailed and well-documented response to this question can be found in the September 2013 issue of Helv Chim Acta (5), which discusses pioneering work by Pasteur and Piutti. The latter of the two discovered D-asparagine and found that it tasted intensely sweet, in contrast to the tasteless L-asparagine, which laid the foundation for stereoselectivity.

When and where did the interest in the analysis of chirality begin?

CW: Biot's discovery of optical activity and polarimetry was the starting point. For many years polarimetry remained the way to study and measure enantiopurity.

YO: After Pasteur established several resolution methods

The Gurus

Christopher J. Welch is Science Lead for Analytical Chemistry within the Process and Analytical Chemistry area at Merck Research Laboratories in Rahway, New Jersey. He co-chairs the organization that oversees identification, acquisition and evaluation of new enabling technologies of potential value to Merck Research Laboratories. Welch has authored more than 200 scientific publications and patents and is co-founder of the journal, Enantiomer, and a member of the editorial advisory board for the Chirality, among others. Welch is also active elsewhere, including chair of the ACS Division of Organic Chemistry and a member of the Executive Committee for the International Symposia on Chirality. Welch's father was an artist, grandfather a merchant and great-grandfather a preacher, and he feels that his career in the chemistry profession nicely combines these disciplines.

Yoshio Okamoto is a Professor Emeritus at Nagoya University, Japan. In 1979, Okamoto's group obtained the first helix-sense-selective polymerization using triphenylmethyl methacrylate as a monomer and (–)-sparteine-n-BuLi complex as an initiator. The one-handed helical polymer (PTrMA) exhibited unexpected high chiral recognition ability. In 1982, PTrMA-coated silica gel was commercialized as the first synthetic polymer-based chiral column. In 1984, his group discovered excellent chiral recognition abilities with cellulose triphenylcarbamate and, in 1986, its 3,5-dimethyl derivative, which has been commercialized as Chiralcel OD. Okamoto's group also reported amylose 3,5-dimethylphenylcarbamte (Chiralpak AD) in 1987. The latter two, OD and AD, are

that led to efficient separation of enantiomers, many groups developed an interest in analyzing chirality – but that was before chromatographic separation, the importance of which Wolfgang notes below.

WL: The principle of discriminating enantiomers in a mixture goes back to Pasteur in 1861. After that it became the focus of diverse research groups worldwide, although it turned out to be a difficult task.

Real breakthroughs, however, are associated with the development of separation sciences – in particular with capillary gas chromatography and liquid chromatography. These technological, scientific developments accompanied the steadily increasing knowledge of the principles of molecular recognition and intermolecular interactions for selectivity tuning. This new learning includes a better understanding of fundamental issues related to the inherent stereochemically controlled 3D structure of small molecules, and also of macromolecules and chemically modified surfaces.

For direct discrimination or resolution of enantiomers a chiral source (a chiral selector – SO) is required. The SO enables the enantiomers (analytes, selectands – SA) to have close contact with predominantly stereo-controlled multiple intermolecular interaction forces, which creates diastereomerically behaving SO-(R)-SA and SO-(S)-SA molecule associates that differ in their Gibbs binding energies.

With the dominant liquid chromatographic enantiomer separation concepts we use today, the chiral selector is bound to a surface of a chromatographic support material called a chiral stationary phase (CSP). The difference between the free binding energies of the intermediate diastereomeric complexes is directly proportional to the observed enantioselectivity value α.

BT: Interest in chirality grew with the availability of suitable analytical tools. These early chiral handles included chiral counterions, derivatizing agents, chromatography columns and chiral shift reagents.

Who were the pioneering scientists that developed chiral analysis and how influential are their discoveries on science and society today?

CW: The origins of chiral chromatography are interesting and have strong connections with Tsvet's development of chromatography in 1904. That year, Willstadter speculated about selective adsorption of chiral dye enantiomers by enantiopure silk or wool. It was a good idea, and attempts to actually do it were described in scientific literature for about 20 years, but there was no real progress with the technique.

In the 1930s, Science reported on the separation of

still some of the most popular chiral stationary phases used in HPLC and SFC.

Bernard Testa studied pharmacy and undertook a PhD thesis on the physicochemistry of drug-macromolecule interactions and later attended to Chelsea College, University of London, as a postdoctoral research under the supervision of the late Arnold (Joe) H. Beckett. Testa's fascination with chirality and stereochemistry began during his doctoral work and became a full-time pursuit under the supervision of Beckett, one of the very first scientists who grasped and explored the significance of stereoselectivity in drug and xenobiotic metabolism. Testa has held many prestigious academic roles, and (co) authored six books and over 500 research and review articles in the fields of drug design and drug metabolism. Testa's Emeritus status has freed him from administrative duties and gives him more time for writing, editing and collaborating in research projects. Testa's dedication to the field has continued ever since; "Organic Stereochemistry – Guiding Principles and Biomedicinal Relevance" (Wiley-VCH 2014) bears witness to his enthusiasm.

Wolfgang Linder is a Professor Emeritus at The Institute of Analytical Chemistry, University of Vienna, Austria. Wolfgang describes himself not just as an analytical chemist, but also a materials scientist, and he was trained as an organic chemist. Therefore, he attempts to bridge many different fields. Wolfgang is author of over 300 original publications, 11 book chapters, and the winner of numerous prestigious awards, including The Chirality Medal in 2008. In 2014, Wolfgang was co-chair of the International Symposium of Chromatography and he is active on several editorial advisory boards, including the Journal of Chromatography B and Chirality. Wolfgang co-invented chiral stationary phase technology that was licensed out to Chiral Technologies.

"It's very clear that the entire chirotechnology revolution in chemistry was enabled by the ability to keep score using chiral chromatography."

enantiomers of organometallic compounds using a column made from crushed single quartz crystals (quartz crystallizes in a chiral space group, and single quartz crystals are enantiopure). However, I think that when Prelog resolved the enantiomers of Troger's base on a lactose column in 1944, the world saw the true start of chiral analysis.

During the 1950s, Japanese researchers reported the separation of amino acid derivatives by paper chromatography, and in the 1960s, Gil-Av prepared several synthetic chiral stationary phases – that launched the modern era of the technique.

Baczuk et al. reported on the first chiral stationary phase designed for a particular molecule (DOPA) in 1968, and in the late 1970s, Cram described the development of a crown ether stationary phase with remarkably high enantioselectivity for amino acids.

Throughout the 1970s and 1980s, my mentor at the University of Illinois, William Pirkle, designed and tested several chiral phases, which laid the foundation for future development of the field. I was an undergraduate in the Pirkle labs in 1981 – just at the time that the first chiral stationary phases were being commercialized – and I have been involved with this intriguing area of science ever since. Also, in the early 1980s, Yoshio Okamoto developed the polysaccharide based stationary phases that remain the most widely used columns today. Other important contributions in chiral stationary phase design during the 1980s came from Daniel Armstrong and Wolfgang Lindner, among others.

YO: I think the first baseline separation of enantiomers using gas chromatography by Gil-Av in 1966 was an important breakthrough. Although it took many hours to complete the separation of enantiomers by capillary GC columns, it showed clearly that chiral analysis was possible by chromatography.

Another important milestone was the first baseline separation of amino acids by Davankov in 1971 using liquid chromatography. It indicated the future possibility of efficient enantiomer separation by HPLC.

WL: Yoshio and Christopher have touched on the main names – I'll provide a little more technical insight. In the 1960s, Davankov described the first fully designed and synthesized CSPs that enabled enantioselectivity for amino acids with intermediate diastereomeric metal mediated chelate complexes using proline derivatives as SO motifs. Then, in the early 1970s, Pirkle published an alternative concept of chiral discrimination based on intermolecular hydrogen bonding and π-π interactions between SO and SAs.

In the late 1970s and early 1980s, the use of specifically modified (for example, polyacrylamides of amino acids and polysaccharides) chiral polymers as SOs and CSPs were discovered by Blaschke and Okamoto. Such polysaccharide type CSPs dominate the market today, as noted by Christopher.

However, in the 1970s it was discovered that intrinsic stereochemically controlled 3D structures, such as proteins, could be considered as well as chiral polymers, and when bound to a support material they could be used successfully as CSPs.

These early developments – mainly driven by Hermansson and Allenmark – led to the commercialization of the first chiral columns offering a broad spectrum of enantioselective resolution for chiral drugs and intermediates. Those early pioneers enabled further SO types and principles to be described using various macrocycle type SOs introduced by Armstrong. In addition, the development of chiral ion exchangers is also significant to the success of CSPs and SOs.

I also agree that credit should be given to the development of enantioselective capillary GC, pioneered in the late 1960s and early 1970s by Gil-Av, which was developed further by Schurig and König.

The pioneering efforts made in enantioselective chromatography throughout the 1970s and 1980s and into the 21st century has enabled the technology to mature to a standard that allows us to resolve more than 90 percent of all chiral compounds (except carbohydrates) analytically – and also preparatively.

How important is chiral analysis?

YO: Chiral analysis is the basis for developments in chirality. Most research and developments dealing with chiral compounds and materials use this efficient method. Without analysis, advances in chirality would be significantly retarded. For example, many papers have been published on asymmetric synthesis, and most of them used chiral analysis methods developed in the past three decades.

CW: It's very clear that the entire chirotechnology revolution in chemistry was enabled by the ability to keep score using

chiral chromatography. This is now widely accepted, and one of the first masters of enantioselective synthesis, Meyers, was fond of making this point. I had the good fortune of being able to collaborate with him on some early alkaloid enantioseparations during my undergraduate days in the Pirkle lab.

WL: For all the diverse chiral technologies in use and being developed by industry, academia, and government laboratories, the availability of stereoselective and quantitative analysis methods and tools are essential. Breakthrough contributions into the field of chromatographic sciences have established the basis for the high technical standards we have today. There are so many contributors and we should be grateful to them all for getting us to the level we are at today.

BT: I'll conclude this point on a higher level: basic research depends on better and new technological tools as much as technology needs fundamental discoveries to progress. Applied and basic sciences therefore complement each other in a hopefully never-ending spiral of progress.

What are the most important current trends in chiral analysis

BT: One important trend that springs to mind is the constant improvement in computational resources for maximizing response-to-noise ratios.

YO: Development of new stationary phases is still continuing both on molecular-type and polymer-type stationary phases. The sensitivity of chiral detectors is still not sufficient; better detection of chirality is highly expected and miniaturization is a recent trend in separation science.

WL: Although we are achieving a high standard of chromatographic resolution of enantiomers, further advancements will continue. Such advancements are chemically driven in terms of the search for new chiral selectors (CSPs) but also technologically driven in terms of the implementation of materials with higher efficiency and higher loadability.

Success from Tragedy

By Wolfgang Lindner

The last 30 to 40 years of enantioselective chromatography is a true success story. Unfortunately, it was essentially driven by the pharmaceutical industry's Thalidomide (Contergan) tragedy in the late 1960s and early 1970s...

The commercial availability of these chiral columns stimulated research on the pharmacological difference of chiral drugs leading to modern guidelines that each stereoisomer of a drug substance with chiral centers needs to be produced as stereochemically integer drug substances for pharmacological testing. These stereoisomers also need analyzing stereoselectively in terms of enantiomeric purity.

The success of the pharmaceutical industry also inspired the food and agrochemical industries, together with those involved in pure and applied chemistry, to investigate the new analytical possibilities of chiral analysis.

As robust enantioselective separation methodologies improved rapidly, they became an indispensable method for the characterization and analysis of any small molecule type chiral compound that was newly synthesized or was becoming part of a multi-step synthesis protocol. This relates to the extremely dynamic field of asymmetric catalysis for organic, pharmaceutical, and agrochemical chemistry, and the further progress of reaction performance should be monitored and analysed.

Preparative chromatography technology for resolving enantiomers from a racemic mixture has also improved significantly. In this context, the establishment of techniques such as simulated moving bed (SMB) chromatography needs mentioning; this has become the method of choice for the chemical and pharmaceutical industries for isolating chiral compounds on a multiton scale for resolving enantiomeric species. We should also mention technological advancements of super critical fluid (or super-fluid) chromatography (SFC) for (semi)prep chiral resolution from the mg to the kg or even higher level. Economically, enantioselective SFC is the first choice for such resolutions.

CW: Speed is important. Many chiral chromatographic separations are possible in less than a minute, and we have recently shown that a number of separations are achievable in seconds. This is very important for high throughput analysis, where hundreds or thousands of samples must be analysed in a single day. In addition, there is continuous progress in increasingly efficient chiral columns – the sub 2-micron columns combined with the new Waters UPC2 instrument are pretty amazing. Finally, there is an ongoing quest for additional universal stationary phases – columns that will work well for many different analyte structures.

Where are the current hot beds of research?

CW: We have some excellent work progressing in our labs, and there are a lot of good things appearing from both academic and industrial labs worldwide. For example, we are seeing some important work on chiral sensors – either optical or MS-based. The key problem of interference with matrix components is beginning to be addressed, and these techniques show some pretty good promise. In particular, the work of Anslyn (University of Texas) and Wolf (Georgetown University) stands out.

YO: I think Armstrong (University of Texas) and Lindner (University of Vienna) are excellent examples. Also, companies such as Chiral Technologies and Phenomenex are helping advance research. Within China, this area is rapidly advancing too.

WL: I find it difficult to answer the question objectively. However, the most advanced chiral columns are provided by Chiral Technology Europe, Phenomenex, Macherey & Nagel, Sigma-Aldrich, Regis, AkzoNobel, and so on.

The Big Question

By Bernard Testa

The big question: is chirality (specifically) and stereochemistry (more generally) essential for the emergence and evolution of chemically based life? We still seek the answer, but do know that the additional level of complexity caused by stereoisomerism also implies additional/emergent properties (2).

My answer is: stereoisomers contain additional and highly valuable information compared with their non-stereoisomeric analogs. The supplementary information provides a broader range of responses (outcomes) in the interactions and reactions of stereoisomers, suggesting that stereoisomerism indeed contributes – perhaps critically – to the chemical complexity underlying life.

The operational concept here is stereoselectivity, namely the differential interactions of stereoisomers with enzymes, transporters, receptors, and other functional macromolecules. Such stereoisomers can be endogenous compounds (such as hormones and neurotransmitters) or indispensable exogenous compounds (for example, nutrients and vitamins), and these usually exist in a single stereoisomeric form progressively selected by evolution to support life from among a mixture of stereoisomers. Casual exogenous compounds are known as xenobiotics and include drugs, recreational compounds, natural toxins, and pollutants. When present as stereoisomeric mixtures, xenobiotics interacting with biosystems may elicit an overall response that results from the combination of two or more distinct outcomes.

The above scenarios outline the concept of substrate stereoselectivity. But there is more to the story, since the enzyme-catalyzed metabolism (or biotransformation) of endogenous and exogenous substrates may generate new stereogenic elements in a metabolite. For example, a center or an axis of chirality, or (E,Z)-diastereomerism. Such a reaction needs prostereoisomerism in the subtrate molecule, for example, a prochiral center.

In most cases, the stereoisomeric metabolites are not generated at identical levels, a phenomenon that gave rise to the concept of product stereoselectivity (3), which was developed further by Prelog (4). Since the chemistry of life is consistently more complex than we expect, product stereoselectivity may also differ between stereoisomeric substrates, a phenomenon known as substrate-product stereoselectivity.

Therefore, the additional structural information contained in stereoisomeric molecules may elicit an increased number of outcomes compared with non-stereoisomeric molecules. Stereoisomeric molecules allow more information to be gained from their interactions with biosystems, which themselves are endowed with chirality and stereoisomerism.

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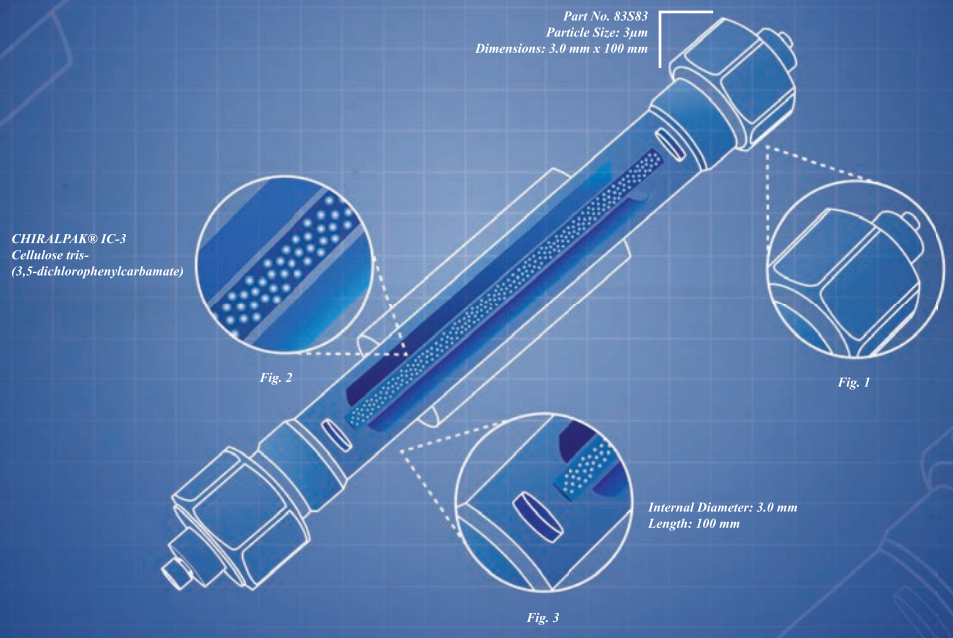

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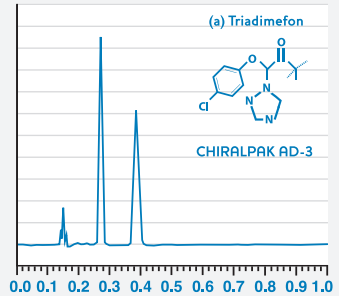

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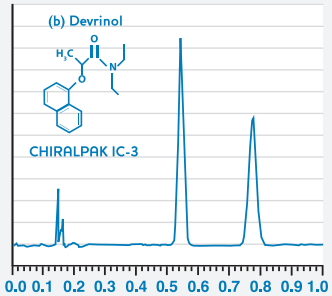

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Is the importance of chirality sufficiently understood by industry and government?

CW: I think the importance of chirality and chiral analysis is pretty well understood nowadays. I remember the strange looks I used to get in the early 1980s when I tried to share my research interests with others; now, high school students are learning about enantiomers, racemates and resolutions.

YO: The importance of chirality is understood sufficiently within the chemical and pharmaceutical industries. However, it is not fully appreciated by other industries and by governments. But I must say the situation has improved since the Nobel Prize award to Sharpless, Noyori and Knowles for asymmetric catalysis in 2001.

BT: Definitely not. There is a general ignorance in official and medical circles of what stereochemistry is, does, and contributes to scientific understanding.

We need to adopt a common language when communicating about chiral analysis and its benefits to others; such a common language already exists within chemistry, stereochemistry and chirality (6).

Some biomedical workers ignore or misuse stereochemical nomenclature in their publications. For example, by using ambiguous lower case d and l, either for optical rotation or for fake descriptors of absolute configuration. Some authors may be at a disadvantage due to a lack of instruction at pre- or postgraduate levels, plus there may be a lack of thorough in-house training within their place of research or work. And what about copy editors whose expertise or allocated time is wanting?

Also optical purity does not receive the attention it deserves, considering how much it may affect pharmacological outcomes (1).

Where will chiral analysis be in five to ten years?

CW: A bold prediction: how about handheld device offering same day method development for wide variety of compound structures, delivering a <15 s assay time for all but the most difficult separations? But maybe that will take 11 years...

YO: Success rates for chiral analysis (separation) are already high, and many chiral compounds can be analyzed by GC and HPLC using various CSPs. However, the chiral recognition mechanism of these phases, particularly polymer-based phases, is not obvious. This situation will be improved over the years; finding the solution may lead to the design and synthesis of new chiral phases. From an environmental improvement viewpoint, separation by supercritical fluid chromatography will become more important and popular.

"Progress in chiral analysis methodology will involve separation sciences, spectroscopy, physics, and also, more broadly, chemistry, material sciences and biochemistry."

WL: Progress in chiral analysis methodology will involve separation sciences, spectroscopy, physics, and also, more broadly, chemistry, material sciences and biochemistry. Chirality related research is, therefore, interdisciplinary, and analytical sciences provide essential tools to tackle the manifold challenges.

Although we have achieved high technological standards, the prediction of enantioselectivity and resolution values for certain chiral selectors and CSPs for resolving some chiral analytes remains largely trial and error. So, the full understanding of the underlying mechanisms and dynamics of molecular recognition and stereoselective discrimination processes remains challenging and is an extremely exciting field of ongoing and future research.

As a driving element that plays a central role in chemistry, biology, and material sciences, chirality requires continuous research to further advance its impact. The tools and methodologies required to analyze chirality, to discriminate between stereoisomers, and to resolve the enantiomers of a mixture, are indispensable in moving chirality-driven science forward.

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Chromatography: Winning Every Battle, Losing the War?

Other techniques are beating chromatography and mass spectrometry to the hero's reward all too often. Are they better? No. Are they faster to first result? Yes. Is it time to acknowledge that – on the frontlines – fit-for-purpose triumphs over best-in-class? You decide.

By Hans-Gerd Janssen

I recently gave a presentation to a group of separation scientists from the Royal Dutch Chemical Society about my chromatography concerns. Many people found the lecture amusing, which was my intention. After all, I used irony and humor to hammer home the message. Irony is useful in approaching our problems, failures and shortcomings, and original humor is about creativity. Creativity, of course, leads to new ideas and combinations. So, while the delivery was amusing, my real intention was to provoke and inspire people about a topic I am passionately serious about. Here, I share the same message with you.

Chromatography all around us

Chromatographers are everywhere. We are at the core of every scientific discipline; we work with people in the environmental field, in the food field, in the medical field… There is hardly any science that does not need us and that is our main strength. Science is about building hypotheses; people use analytical chemistry – and chromatography – to gather information for hypothesis generation. Later, they use analytical chemistry for hypothesis testing. As a consequence, we are continuously faced with great challenges that should allow us to grow.

But...

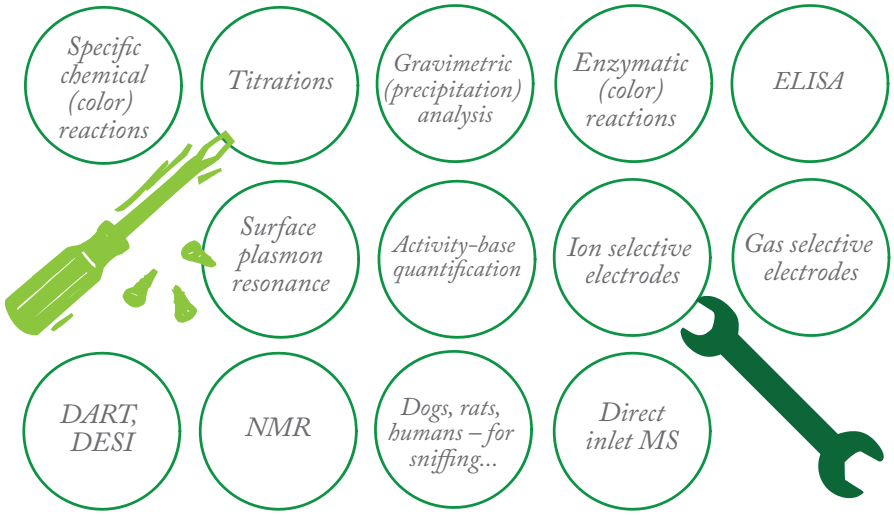
We don't really get any recognition even though we typically do a great job. If you consider chromatography and mass spectrometry (chrom-MS), we are always faster and always more sensitive than other techniques. And yet, even though we solve every problem on "the analytical battlefield", we rarely get any of the credit.

The danger? Well, we're on a sinking battleship – one that's being eaten by sharks. Certain elements of chromatography have been completely transferred to the users – who can do a lot of their chromatography themselves and don't need us anymore. In principle, we should have more free time to work on high-end techniques that will be essential in solving the problems of tomorrow. But because we typically don't get enough credit or attention, we don't get the funding we need either.

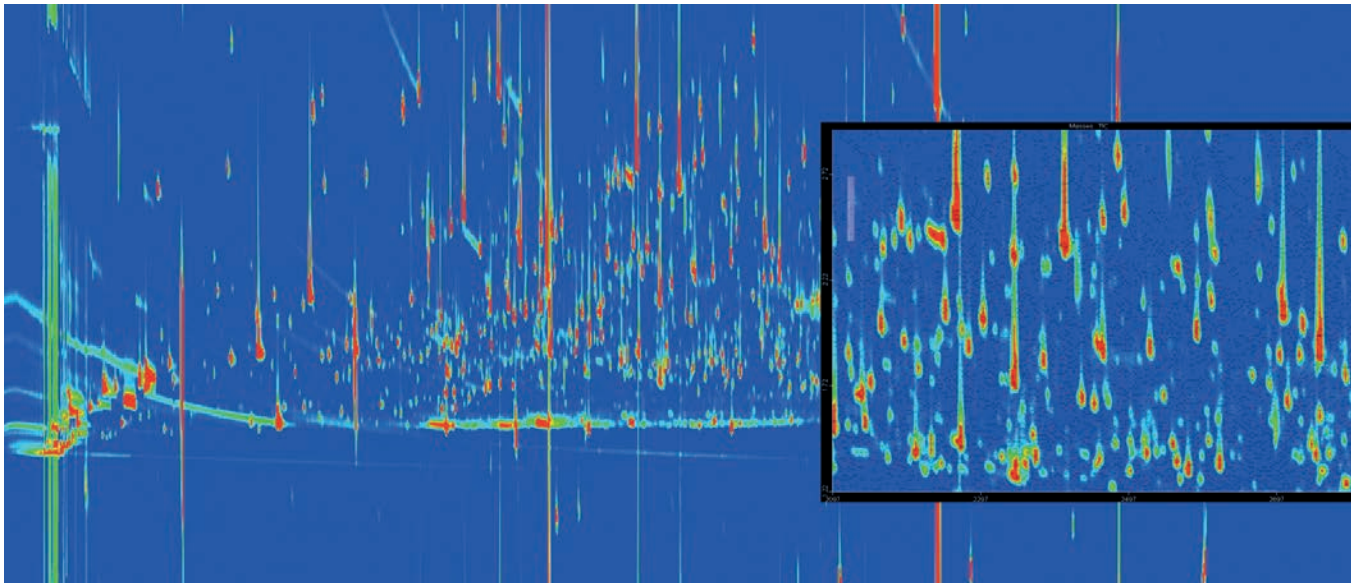
The sharks are very effective – in other words, we have become very efficient at transferring simpler methods and tools to users – and that's a good thing, isn't it? They are eager to use our techniques because they work and are very useful – it's chromatography's success story. I'm not here to challenge that. The problem is that we are not building the front end of the ship fast enough to stay afloat.

Let's not follow in a long line of disciplines that have sunk and disappeared: the typing department, the calculation department,

Considerations



Competitive Tools



Chromatography can be sexy too... (SPME–Comprehensive GC×GC of a green tea infusion).

the organic chemistry department... In all cases, standard work was passed to the requesters themselves, without any thought on developing new, high-end tasks. The problem is the same for all chemical analysis methods, but to a different extent (less so if your method is considered more difficult – spectroscopy, for example).

Competition in the toolbox

The toolbox is a problem for the industrial analytical scientist. A question comes in that needs to be answered. Most of us have a very heavy toolbox with (too) many techniques – chromatography is just one of them. A good analytical scientist needs to know what every single technique can do and needs to be able to select the best method. And we must be able to use the chosen technique. It's about knowing many methods and being able to get the maximum out of every single one of them. That is difficult.

I use the toolbox analogy because it also implies a potential hazard. If you only have a hammer, you cannot solve all problems. And, if you only have experience of working with hammers, you will approach every problem as if it were a nail.

We could consider developments in chromatography as increasingly big hammers. Think about GC where we can now inject 100 µl instead of 1 ml, or UPLC where we are now faster, or comprehensive chromatography that offers us multiple advantages. Handling a bigger or heavier hammer requires more from the analyst. And yet it remains a hammer, and it will never be suited for precision screw driving. Many analytical challenges exist, and some of them could absolutely benefit from state-of-the-art chromatography. Others will never benefit from chromatography – and a few might inspire new research in chromatography, if we are listening.

What is the chromatography hammer used for? It's largely used to find out what is present in a sample and how much there is. To a lesser extent, chromatography is also about finding composition – property relationships. There are numerous other techniques that can do the same, but chromatography is unsurpassed in terms of sensitivity, reliability, availability, cost and speed. So yes, chromatography is a very important technique in the toolbox, but it is not the only tool.

There are numerous other techniques available for quantitative analysis. Most of them overpromise and under deliver, or are of limited general relevance and applicability. Capillary electrophoresis is not reliable enough, surface plasmon resonance not selective enough. ELISA does not work for small molecules, near-infrared spectroscopy is too matrix dependent, should I go on?

Several other methods allow very rapid analyses of a reasonable quality, but require many extras and add-ons when more accuracy is demanded - much like buying a cheap computer and realizing that several upgrades are needed before it is up to the task. For me, NMR is an example.

Quantitative NMR is very fast in generating a reasonable number, offering very fast initial 'quick and dirty' answers. But getting the analysis to the same quality as chromatography requires at least the same time for method development and time per sample as chromatography, if not more – and with no guarantee you will ever get there.

Ten Solutions to Faster First Results

1. Universal extraction sample prep methods (QuECheRS…)
2. Universal derivatization methods (oximation-silylation).
3. Higher resolution mass spectrometry.
4. Universal gradients (GC: on-column injection, non-polar thin film, to very high temperature; LC: C18, Acetonitrile/water (acidified), wide range with DAD and ESI+).
5. Use Comprehensive LC×LC and GC×GC (or even LC×GC). Why? i) two phases are better than one, ii) gain peak capacity, and iii) able to see groups and trends.
6. Develop a flow chart (to avoid having to think hard every time a new sample arrives).
7. Improve library-searching skills.
8. Start a database of questions.
9. Create your own spectral databases.
10. Stop at 'fit-for-purpose', even if you can easily do much better...

Here's a sentence from an abstract from Yulia Monakhova's upcoming keynote lecture at 2015's PANIC (Practical Applications of NMR in Industry Conference):

"Nuclear magnetic resonance (NMR) spectroscopy is now of growing importance in the field of qualitative and quantitative food analysis. In this regard, NMR allows analysis in two ways within one experiment: namely, using targeted and non-targeted approaches. NMR provides qualitative and at least semi-quantitative information even without pure compound standards more rapidly than with any other currently available spectroscopic or chromatographic method."

Is NMR really that fast? Remember, the world record in GC is nine compounds in 0.6 seconds. Now that's fast. Calibration is certainly a very strong point for NMR – you do not need standards. And in chromatography, response factors between different compounds can differ up to, well, about infinity...

But what annoys me is that NMR is often presented as an alternative for chromatography in scientific literature. Apart from a few niche applications, it is not; the technique is complementary to chromatography. If the quick and dirty answer suffices then that's great. If not, chromatography can raise the level of analysis.

Staying relevant

I am absolutely aware that chromatography and mass spectrometry are enabling techniques. We need to work with those scientists who have the real challenges to develop our field, but in practice many people do not choose to work with chrom-MS initially because it takes us too long to get a first result. Instead, they choose other enabling techniques, like NMR, where the route to a first result is faster. Typically, those initial techniques are not good enough in the long term and chrom-MS is drafted in again (when the initial credit has already been taken).

Users want their ideas confirmed quickly. With chrom-MS, you first have to develop a method and then it takes weeks to get it running and validated before producing good (or the best) results. Because of our slow response, we are not part of the mission to explore new fields. For example, NMR has been widely used in initial metabolomics studies and is still presented as the key technique in this area. In reality, more or less all metabolomics platforms nowadays are based on LC-MS and GC-MS. The same happened in food safety and the detection of pesticides. There was a lot of work done on sensors to detect pesticides, PCBs and dioxins - certainly sensors work – but if you really want the best results, it's back to chrom-MS.

We need to be on the frontlines of the battlefield, not mopping up and minesweeping. In other words, we are not part of the discovery, but a service hired in afterwards. If we could have delivered results quicker we would have been part of the reconnaissance mission and included in the victory celebration.

Battling history

We have another problem: the hangover of previous experience. Every chemistry student – and some in other scientific disciplines – have used chromatography at some point in their education. They worked on a lousy instrument somewhere as a graduate student and did some experiments. They think: "I can do chromatography. Chromatography is simple. Chromatography is slow." I have a brush, so I can paint. But they are not aware of the vast improvements we have seen.

When people say the technique is fully developed, they often mean: "I cannot develop it any further." But an expert can! I've heard statements about our technique being mature for at least a decade or so, but look at what we've seen since then: large volume injection, MS connected to every LC or GC, comprehensive chromatography, UPLC – so 10 times faster, 100 times more sensitive and 1000 times more certain. Such growth rates are seen in infants, not mature adults. Catalysis is mature, engine development is mature, organic chemistry is mature. My car still uses 6.5 liters of gasoline per 100 km – like my previous car, and

the one before. Where is the progress?

I remember my first chromatography experiments over 30 years ago – yes, the instrument looked somewhat similar, but the accuracy, selectivity, sensitivity, and reliability today is far, far superior. Unfortunately, we fail to tell people that we have much more to offer. In fact, because we have to fight against a misunderstanding of what analytical chemistry – and in particular chromatography – can offer, telling is not enough. When someone comes to you with a sample and you tell them that you are 10 times more sensitive and 100 times faster than in the past, they may not care or even believe you.

We need to show what we can do. We must convince others of our ideas and abilities, and then give them some form of preliminary results very soon afterwards. Clearly, we are not adept at this last aspect. Preparing our first result takes us far too long. The analysis of the second sample hardly ever takes over an hour, but that first sample? It can take us weeks, if not months.

“We need to show what we can do. We must convince others of our ideas and abilities, and then give them some form of preliminary results very soon afterwards”.

Faster first results

I've done a lot of thinking about why chromatography is so slow. There are a number of reasons, but I think the biggest problem is that we simply have too few universal methods. Why? Because we have developed 'the best method' for specific applications. Unfortunately, such top-class methods don't help when proving our initial capability in a new project or application.

If we want chromatography to be taken seriously in the early stages, we don't need a perfectly validated method with the best in selectivity, sensitivity and so on. We need to show that we can do 'quick and dirty' too – and that we can do quick and dirty better than the rest. We desperately need more universal methods for sample preparation, more universal methods for chromatographic separation, and broader compound coverage.

In many ways, it's about accepting imperfect results, which is a psychological barrier for us. We chromatographers always strive to get the best accuracy, reliability and sensitivity achievable. But while we're busy working on that – the competition has swept across the battlefield and stolen victory. In the initial 'show and tell' phase we need to move towards a fit-for-purpose mentality rather than a world-record beating mentality.

A call to arms!

As chromatographers, we are too critical and too shy about our own work. We fight against each other instead of joining forces and comparing our technique with other methods. We have a lot to be proud of so we should raise the banner for our achievements. We have instruments in space, in submarines and everywhere in between. But our success in making our techniques available for anyone who needs them is now working against us. Chromatography is no longer 'sexy'. Sexiness is related to how many instruments are around (preferably as few as possible), how big they are, how expensive are they, how shiny, how difficult to operate...

In chromatography, the difficult step is wet chemistry, which is seen as low-level work. And yet, once you have a good chromatogram, getting information out of it (the high level desk work) is simple and short. In spectroscopy, it is the other way around. The lab work (the wet chemistry) is short, but getting any information out (the intelligent desk work) takes time.

In some ways it's also about PR and marketing. In society, there are numerous examples of failed sophisticated tools and techniques, but also examples of mediocre technologies that shot to fame. We cannot rely on fellow chemistry academics from other disciplines to give us a better name. We should work with experts from other fields, such as physics and big-data sciences, to deliver new concepts and insights.

Ten Targets for Change

1. Shorter time to first results
2. Localized compositional analysis
3. Molecules in context (neighbours and interactions)
4. Time-resolved analysis
5. Taking the instrument to the sample
6. Fewer methods and instruments
7. Easier calibration
8. More 'dirt-resistant' systems
9. Reduced environmental impact (chemicals and power)
10. Reliable black-box operation

That said, we must always keep benefit to society at the forefront. We must find answers to challenges, eliminate hurdles by generating solutions, cash and intellectual property. We must cooperate with those who have challenging problems and work with instrument manufactures to generate equipment to solve current problems for users. We must make the effort to join in on new discoveries.

Let me be perfectly clear: the experienced chromatographer is not under threat. If you have good chromatography and MS skills and high competency, you will be of great value to industry. Just don't forget that we are on a battleship that is being eaten by sharks – fortunately, we have plenty of room for improvement (see Ten Targets for Change).

As an industrial scientist, I am realistic; analytical chemistry is an enabling science and, for me, there is no discussion about that. But enabling has nothing to do with being irrelevant. We are not getting the credit for what we do. We are everywhere in science and society, but we lack recognition and, as a result of that, don't have sufficient funding support.

In summary, we need to provide first results quicker or we will always be last onto the battlefield. We need to improve our PR and marketing. And finally, perhaps we should not give credit away so easily when we had a crucial part to play in the victory.

It's time to turn the tide of war and get recognized.

Hans-Gerd Janssen is Science Leader Analytical Chemistry, Unilever Research Vlaardingen, and Professor of Biomacromolecular Separations, van 't Hoff Institute for Molecular Sciences, University of Amsterdam, The Netherlands.

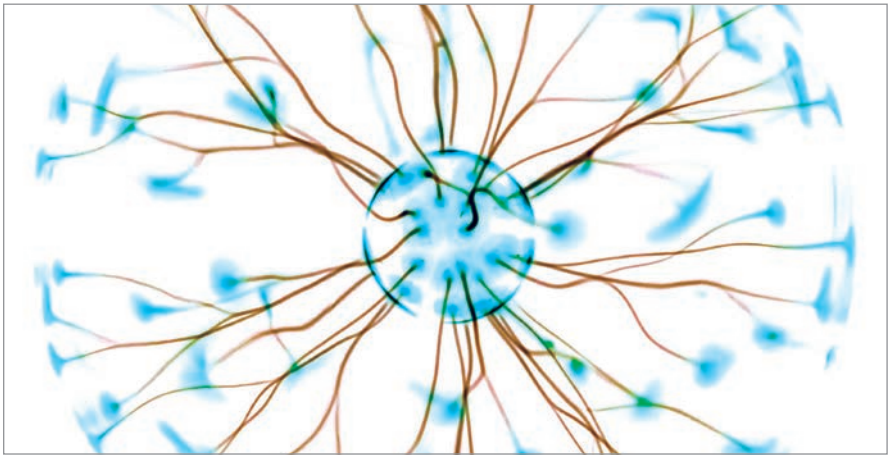
Solutions

Real analytical problems
Collaborative expertise
Novel applications

Electrifying Analysis in R&D

Electrochemical reaction cells are finding new applications in the pharma R&D lab that could offer big time and cost savings. Here, we share our experiences using electrochemistry with on-line mass spectrometry to study pharmaceutical stability and oxidation products – and explain why we're electrochemistry converts.

By Mark R. Taylor and Susana Da Silva Torres



Electrochemical reaction cells have been used in analytical chemistry for over 50 years (1). Exploiting nature's redox reactions of organic species, these systems have enabled us to achieve selective and sensitive detection in chromatography (2) as well as benchmarking stability of products susceptible to oxidative degradation (3). However, until recently, the use of electrochemistry as a method of producing redox products for further study has not been routinely applied in the majority of pharmaceutical research and development laboratories.

The pioneering work of Professor Uwe Karst and his co-workers at the University of Münster has shown that commercial lab-scale electrochemical reaction cells (in our case a Roxy Potentiostat system from Antec, the Netherlands) can be routinely applied to generate pharmaceutical metabolite profiles very similar to those observed in vivo and in vitro using enzymatic digestion (4). There are obvious advantages to being able to produce metabolites of a drug substance quickly and cleanly in real-time using electrochemistry with on-line mass spectrometry (EC-MS) rather than traditional in vitro methods using expensive xenobiotic metabolizing enzymes or cell digestions, where lengthy sample preparation for MS is often required due to potential interference from the sample matrix. Use of a reaction cell with on-line MS or LC-MS system allows for much more rapid and convenient study of redox metabolites and the EC-MS technique is starting to be applied more widely as a result, giving faster access to key data on metabolite profiles and structure. The oxidized species produced in these reactions are often unstable; by studying them in real-time, we can avoid bias from product decomposition during sample preparation and storage.

Stability testing

As well as in vivo metabolism, oxidation plays a major role in pharmaceutical stability and, along with hydrolysis, is one of the most common mechanisms of drug degradation. Pharmaceutical companies go to great lengths to understand and control these potential degradation mechanisms in their products. Gaining an understanding of the theoretical and real oxidation product profile of each new pharmaceutical product is a regulatory expectation and of fundamental importance to protecting patient safety and ensuring robust and relevant stability indicating methods, to provide a basis for

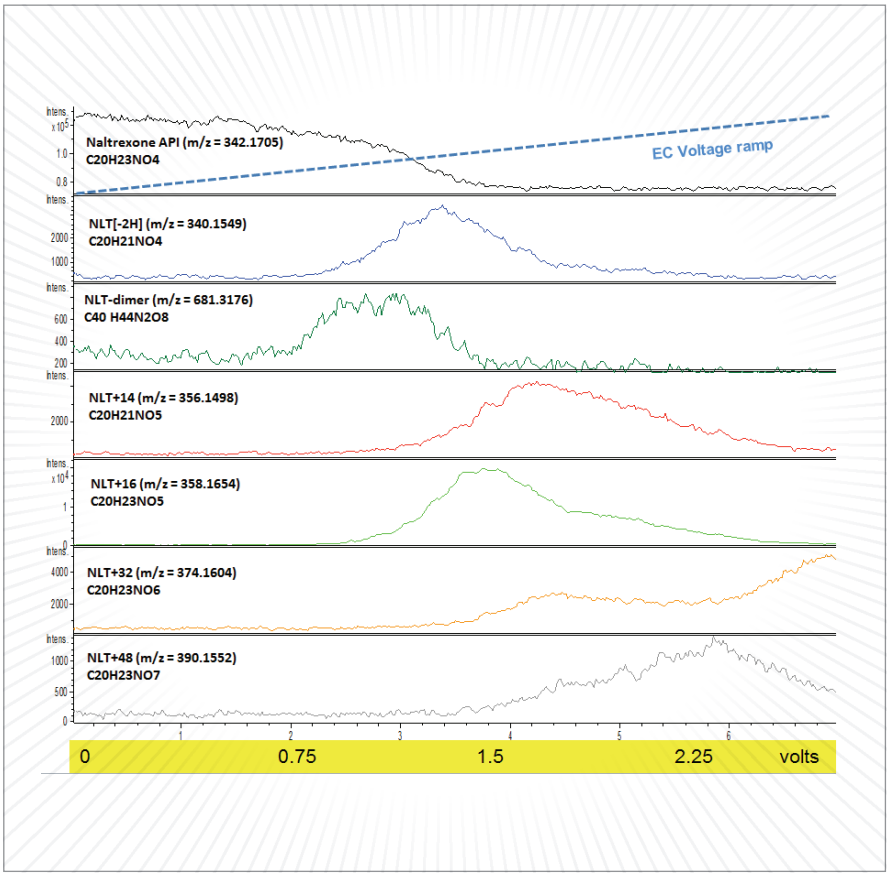


Figure 1. EC-MS ion intensity plots of infused naltrexone and electrochemical cell oxidation products using a magic diamond electrode and applied voltage ramp (0-3V in 7 minutes).

stability studies. Extensive stress testing through forced degradation is routinely applied using a range of in silico and in vitro methods designed to cover all possible routes of potential degradation including thermal, humidity, photo and chemical (hydrolytic and oxidative) stress tests (5).

Just like the metabolic studies we mentioned earlier, electrochemical reaction cells coupled to MS and LC-MS give us a new and convenient way of studying the redox stability of pharmaceutical products. We can now study reactions on-line and in real-time using high-resolution accurate mass MS, which is able to churn out proposed chemical formulae of products in a matter of seconds (6). The use of electrochemical cells obviates the need for lengthy and often hard-to-replicate chemical treatments using caustic reagents, such as hydrogen peroxide. By fine-tuning the applied cell potential we can optimize the process to achieve the maximum yield of specific target reaction products prior to on-line or off-line analysis by complementary spectroscopic techniques, such as nuclear magnetic resonance (NMR) or bioassay (7).

An addictive example

We used EC-MS in direct infusion mode to study naltrexone, a potent narcotic antagonist used in maintenance treatment for opiate and alcohol dependence, which is known to degrade by oxidation (9). Figure 1 shows an overlaid ion intensity

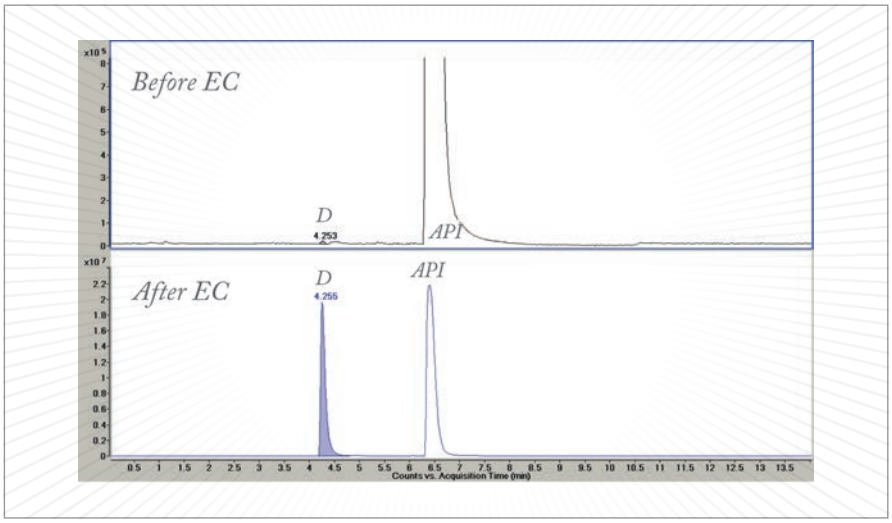


Figure 2. Overlaid LC-MS chromatograms of a pharmaceutical API sample showing enrichment of a trace degradant (D) following electrochemical oxidation (EC).

plot of a naltrexone standard solution, prepared in a simple ammonium acetate electrolyte buffer, as it is syringe-pumped through an electrochemical reaction cell into a high-resolution mass spectrometer. As the applied cell potential was linearly increased, the intensity of the naltrexone substrate mass ion (m/z = 342.1705) decreased and ion intensities from naltrexone oxidation products increased and decayed as new oxidation products are formed. Mass ions consistent with formation of a dimer (2-2'-bis-naltrexone), dehydrogenated naltrexone and associated dimer (M-2H) and from the addition of one, two and three oxygen atoms (M+16, M+32, M+48) to naltrexone were observed as the oxidation potential was increased. It was interesting to observe the relationship of the different products to applied voltage – you can see the formation and decay of reactive intermediates and products in real-time as voltage was increased. Analysis of the redox cell effluent by LC-MS suggests that isomers of the oxygenated reaction products are formed from different sites of hydroxylation. The results were consistent with what we already know about the drug – species formed at lower potentials are observed in laboratory stability studies and special stabilization agents have been proposed to mitigate against their formation in pharmaceutical products (8). The ability to gather data by EC-MS without the need for specialist reagents and reaction time-course sampling is hugely attractive and gives us a head start to stability-relevant data.

Amplifying oxidation products

Once pharmaceutical oxidation products cross a particular concentration threshold relative to the active pharmaceutical ingredient (API), regulators expect their structures to be identified. Markers of the oxidation products are often needed to validate stability indicating methods and to ascertain their relative response factors in the selected quantitative analytical methods. It can be quite a challenge to identify trace (≤ 0.1 percent) levels of oxidation products, as they can easily be drowned out by the relatively large quantities of main-band API. In our laboratories, we have used EC-MS and, more recently, preparative synthetic electrochemistry to accelerate identification of the oxidation products. By electrochemically depleting the amount of API relative to that of the oxidation product it is quite possible (by selecting the optimum cell potential) to increase the concentration of an observed trace oxidation product to 50 percent or more of the total chromatogram peak area in just a few minutes (Figure 2). This provides enough material for more sophisticated LC-MS-MS experiments to be performed and for structure confirmation and concentration measurement using NMR, without having to resort to complex sample preparation techniques.

Making oxidation markers

Synthesizing API oxidation product markers for method development and validation can be technically challenging using traditional wet chemistry approaches, often requiring weeks of chemist time, plus sourcing and evaluation of starting materials and reagents. We wanted to see if we could use an electrochemical synthesis cell to produce oxidation product markers rapidly and directly from solutions of the API dissolved in electrolyte (9). Using fesoterodine as a model compound, we were able to produce the oxidation products directly from the API without the need to source special reagents (Figure 3). Better yet, we observed that the reaction was much more rapid and selective than in-house attempts to produce these oxidation products using traditional approaches. Attempts to produce larger amounts of material (starting with 80 mg substrate) were successful too, with the trade-off that the total conversion rate in two hours was reduced to approximately 75 percent. A mixture of products was produced so individual pure products were recovered using preparative HPLC and centrifugal evaporation.

As early adopters, we approached the use of EC-MS to support pharmaceutical stability and structure elucidation studies with trepidation. We were concerned that the technology would fail to deliver in a high-pressured

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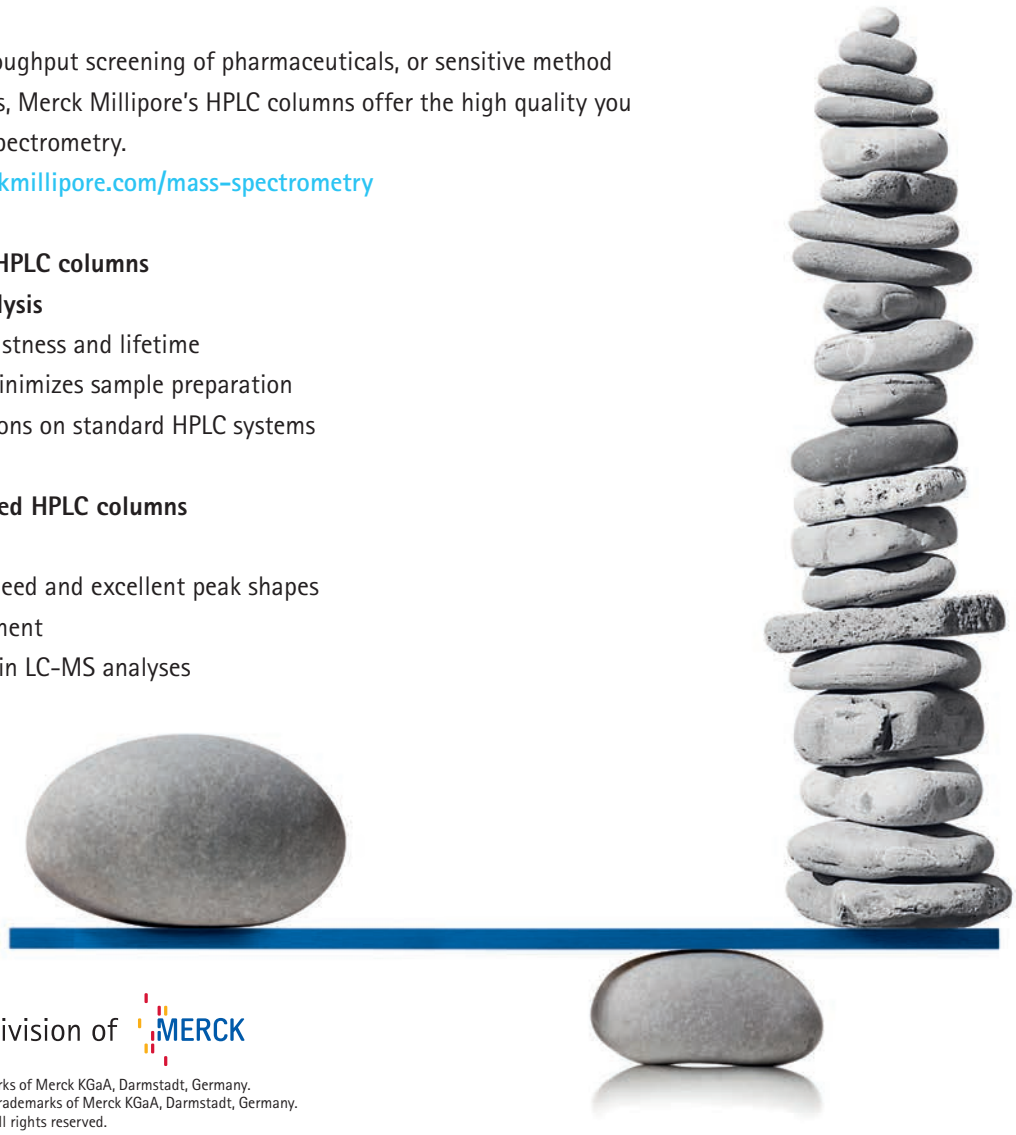

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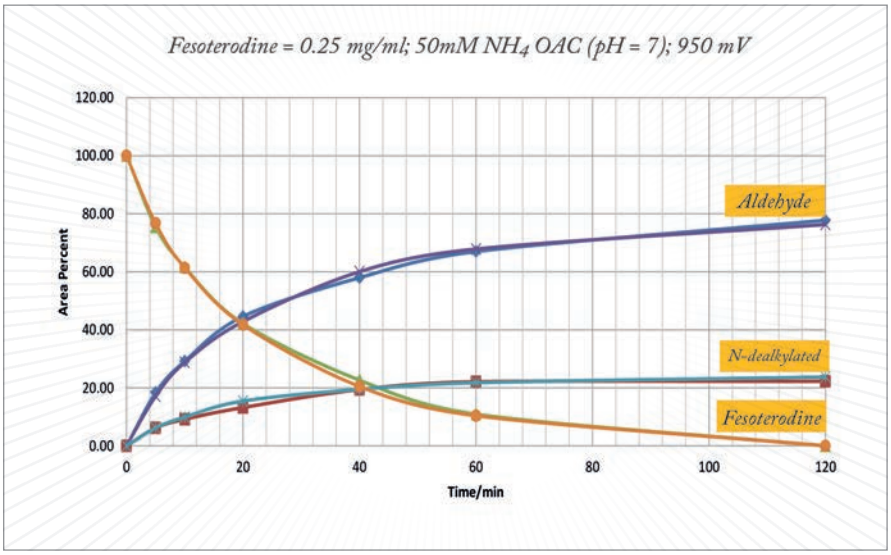

Figure 3. Overlaid curves showing the rate of formation of two target oxidation products produced by electrolysis of 20 mg fesoterodine fumarate (9).

and busy laboratory. However, in every case, electrochemistry has provided a time and labor cost saving that has easily repaid the capital investment. We are now using the technique routinely to facilitate understanding of pharmaceutical oxidative stability, enable structure elucidation and simplify synthesis of oxidation product markers, and we expect to see more and more laboratories joining us in the years ahead.

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Ultrasensitive Detection of Mercury: Beyond FRET

Rapid screening for total mercury content of water, soils and fish takes to the field

Cicely Rathmell, MSc

Are You Sure You Should Eat That?

Contamination of groundwater, soil and fish with mercury has been increasing steadily for a decade, even in the most remote lakes and wetlands. Excess mercury is a particularly pervasive environmental problem due to its tendency to accumulate in tissue, leading 33 states in the U.S. to issue fish consumption advisories. In fact, 140 countries worldwide have regulations regarding mercury content in food. Ingested mercury acts as a neurotoxin, and can affect growth, development and reproduction. Rapid, accurate and portable measurement of mercury at the ppb level is needed to validate the safety of drinking water and fish destined for human consumption. Most current analytical techniques for quantification, however, are time-consuming and are limited to a lab setting.

No Need to FRET

Förster Resonance Energy Transfer (FRET) occurs when two chromophores get close enough to transfer light nonradiatively. FRET can be used to create sensitive fluorescence assays, but typically offers limited signal and requires an interaction length (chromophore proximity) of <100 Å. Researchers at Jackson State University (Jackson, MS) have found a way to overcome these limitations and achieve a thousand-fold enhancement in fluorescence signal, allowing mercury (Hg) to be measured with a portable system.

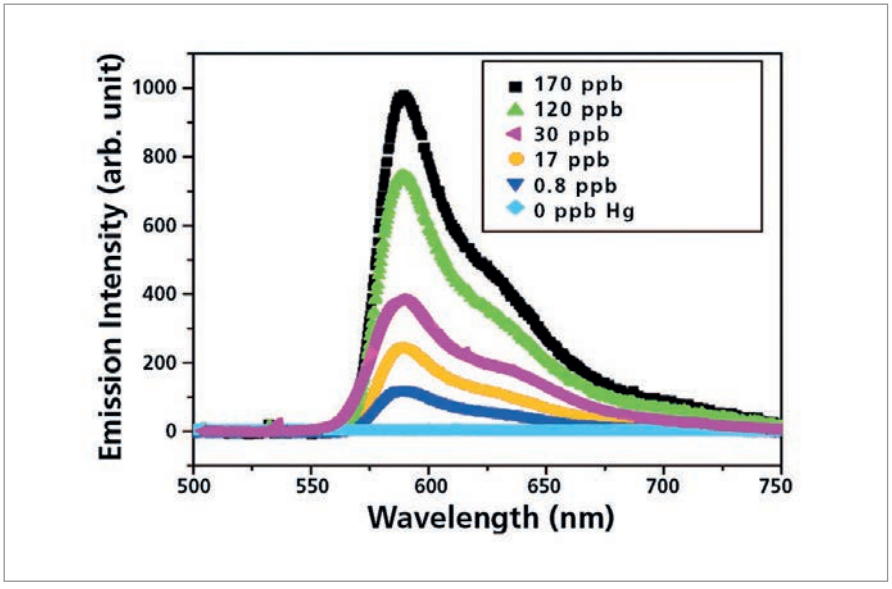

Figure 1: Fluorescence response of RhB adsorbed onto gold nanoparticles increases linearly with concentration of mercury, and is zero in its absence.

The researchers created a chromophore pair by adsorbing rhodamine B (RhB) onto a gold nanoparticle surface. While adsorbed, the colloidal gold quenches the RhB fluorescence, a phenomenon they've dubbed Nanomaterial Surface Energy Transfer (NSET). In the presence of Hg(II) ions, RhB molecules are released from the gold nanoparticle surface and quickly begin to fluoresce as they bind to the mercury, yielding a signal that correlates directly to the concentration of Hg(II) ions. The sensitivity and range demonstrated are ideal for testing against the U.S. EPA limit of 2 ppb of mercury in drinking water.

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With a 532 nm laser pointer as the excitation source, a 4-way cuvette for the sample holder, and our QE-series spectrometer for detection, the entire system fits into a small case and can be run using battery power and a laptop computer - extremely useful for on-site measurements. The system achieved accuracies of 85-95% for samples of water, soil and fish collected from the Mississippi River, demonstrating its ability to also distinguish between safe and toxic seafood according to the U.S. EPA's 0.55 ppm standard.

If You Teach a Man to Fish …

Just as this technique enables innovative environmental monitoring, it is equally applicable to field detection of other toxins. The enhanced efficiency of NSET over FRET can be applied to detection of biothreat DNA agents such as anthrax, and foodborne pathogens like E. coli, offering tremendous potential for the development of a multitude of biosensors and bioassays to make our world a safer and healthier place.

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Free Spirit

Sitting Down With Purnendu (Sandy) Dasgupta,
Jenkins Garrett Professor of Chemistry and Biochemistry,
University of Texas at Arlington, USA.

We noticed you had a busy end to 2014.
Yes – it was rather momentous for me because I had three papers published in the same issue of Analytical Chemistry about the theory, workings and exploitation of a $100 detector for open tubular ion chromatography. I've had "twins" before, but that was my first "Trifecta" as my friend Dan Armstrong would say. I also got elected a Fellow of the IEEE, much to my surprise.

Such success must reaffirm your life choices...
Yes, I was very pleased. I recently visited China, Korea and Japan where I had the chance to see some of my overseas students – that's something else that tells me I made the right choice by staying in academia. Many of us who work on the more practical side of science have a great deal of opportunity to work in industry, and I've had my fair share of temptations. But I can play in as many sandboxes as I want where I am in academia, and I know when I travel to many parts of the world there will be someone at the airport who is truly delighted to see me. That's hard to beat.

Could you walk us through your early years?
I went to Presidency College in Calcutta at 15, which was very different to my experience as a relatively younger student at an all-boys high school. I discovered women for one thing! On a much more serious note, I also got very deeply involved in (militant) politics and it became unsafe for me to stay there ("The Lowland" by Jhumpa Lahiri offers a glimpse). Those times consumed a great number of young people and I feel fortunate that I am alive and well. In essence, we wanted to reform India. I laid low for a while and then went to a small town where I majored in chemistry and got my masters in organic chemistry.

So, you stayed in chemistry after that?
Actually, I wanted to be a poet (I've since published a book of poetry – and a novel), but I realized that I couldn't make a living from it, and ended up as a photographer... My magazine sent me to take shots of Professor Santiranjan Palit, who delved into my past and told me I was wasting my life. Three days later I'd quit my job and joined his lab.

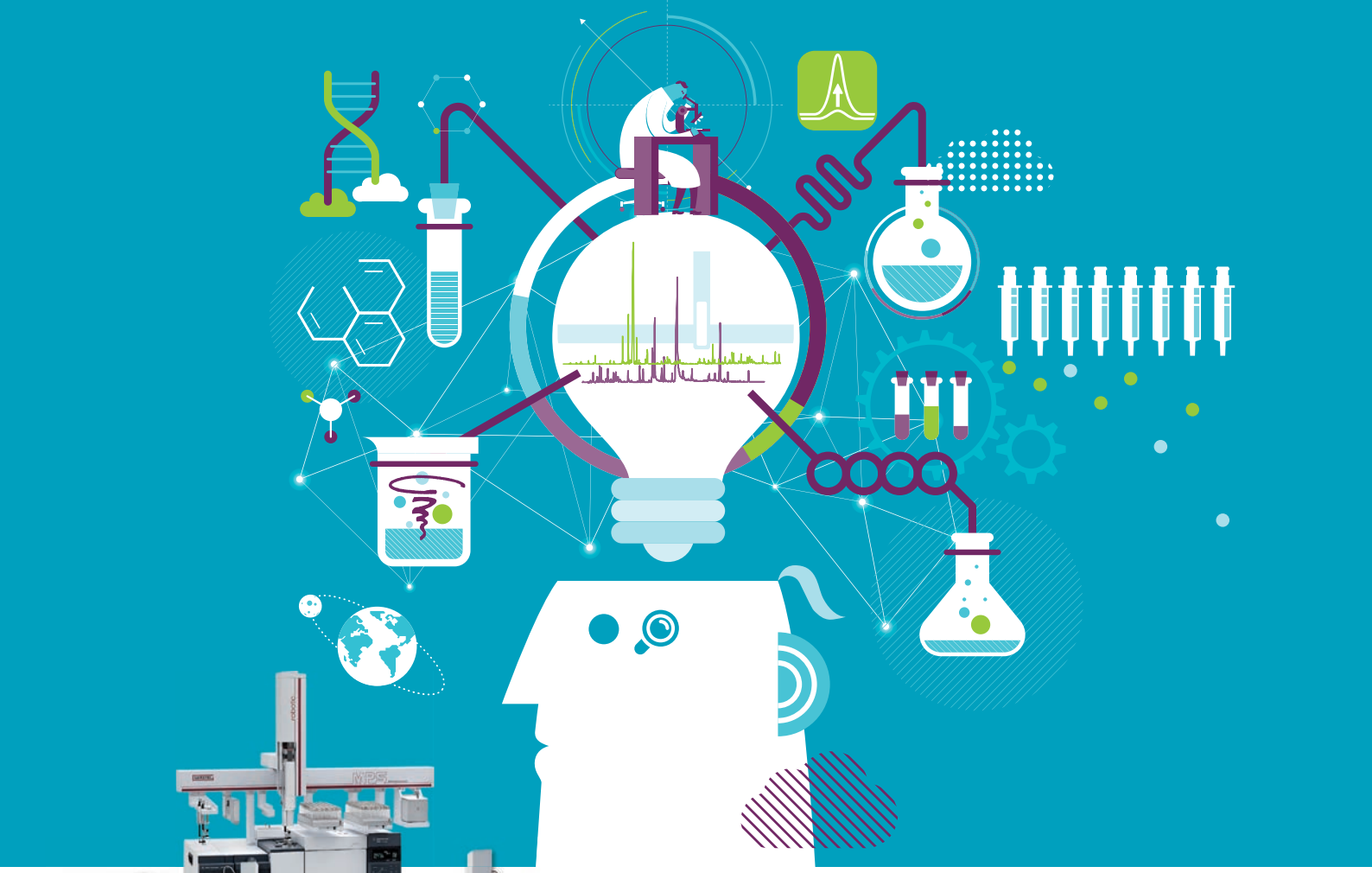
What did you do there?
I was investigating the limiting nature of Faraday's laws of electrolysis – in particular, the fact that they are only accurate when the solutions are relatively concentrated. I wrote up my hypothesis of why this was happening– and it came back with a sea of red ink. Palit would often tell me, "You're such a bright young man – why are you so stupid?" I didn't understand what he meant back then – I certainly do now. Anyway, he encouraged me to find a good electrochemist to work with abroad and I moved to the US – Louisiana State University – in 1973. I only stayed two years with my first mentor there – a bitter-sweet early experience with electrochemistry. Nevertheless, it made an impact – we're still doing electrochemistry of a different sort.

And then?
I was lucky enough to be welcomed into the group of Philip West. After my PhD, I returned to India to find a job, but got a call from Phil about a teaching position. One thing led to another and I never ended up returning to India. I moved from LSU to work as an "Aerosol Research Chemist" at the California Primate Research Center – a very clinical setting – at UC Davis. It was a wonderful experience and ignited my significant interest in ion chemistry (see page 18). However, I was also teaching, which showed me my true calling, and in 1981 I joined the Chemistry Department at Texas Tech University where I stayed for 25 years before coming to UT Arlington.

"Those times consumed a great number of young people and I feel fortunate that I am alive and well."

You come from an academic family...
I've been in academia my whole life – and the same can be said for my father (a medical doctor whose PhD happened to use a great deal of analytical chemistry). And my grandfather was in academia most of his life too – a historian and by far the most famous of my family (try Googling Surendra Nath Sen or his book "Eighteen Fifty-seven"). Therefore, I am a third-generation university professor.

How did your family's academic past influence your choices?
Well, my father always encouraged my curiosity; I don't know many people anywhere in the world who would have had access to a full-fledged lab in their family home in those days or even now. That lab was purpose built because of my interest in chemistry. And I made good use of it; at the age of 14–15, I was doing experiments worthy of publication today. For example, I developed a scheme of analysis that replaced hydrogen sulfide in metal precipitation – and it was solely on the basis of that work that I was given a national science scholarship, which set me on my winding scientific path. I've picked up something useful at every twist and turn; no experience should be thrown away in this life.



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