NOVEMBER 2015 # 34

the Analytical Scientist

Upfront
Are all your GC-MS data really wrong?
10 – 11

In My View
Investigating the six dimensions of GC
19 – 20

Feature
Five gurus debate the true potential of CE-MS
34 – 41

Sitting Down With
Detlef Günther, VP of Swiss Precision Research
50 – 51

Landmark Literature: 2015

Six experts reveal the half dozen research papers that stood out from the crowd this year.

26 – 33

www.theanalyticalscientist.com

built

Biotherapeutic protein characterization demands reproducible, robust and reliable instruments. Introducing **Thermo Scientific™ Vanquish™ Flex UHPLC System**, designed purposely for biopharmaceutical workflows; charge-variant profiling, aggregation analysis, peptide mapping and glycan characterization. Built with engineering excellence, we deliver all the pieces to meet your requirements.

for biopharma

• Enjoy chromatography • thermoscientific.com/BuiltForBiopharma

QVQLKQSGPG
PGKGLEWLGV
KMNSLQSNDT
SIMPLICITY
NSGALTSGVH
TKVDKRVEPK
KPKDTLMISR
YNSTYRVVSV
ISKAKGQPRE
DIAVEWESNG
GLYCANS
LSVSPGERVS
ASESISGIPS
GTKLELKRTV
PREAKVQWKV
VYACEVTHQG

CONFIDENCE
IWSGGNTDYN
AIYYCARALT
APSSKSTSGG
TFPAVLQSSG
SPKSCDKTHT
TPEVTCVVVD
AGGREGATES
PQVYTLPPSR
QPENNYKTTP
SVMHEALHNH
FSCRASQSIG
RFSGSGSGTD
AAPSVFIFPP
DNALQSGNSQ
LSSPVTKSFN

LVQPSQSLSI
TPFTSRLSIN
YYDYEFAYWG
TAALGCLVKD
LYSLSSVVTV
VERSATILITY
VSHEDPEVKF
LTVLHQDWLN
DELTKNQVSL
PVLDSDGSFF
YTQKSLSLSP
TNIHWYQQRT
FTLSINSVES
PEPTIDE MAP
ESVTEQDSKD
RGA

TCTVSGFSLT
INTACT
QGTLVTVSAA
YFPEPVTVSW
PSSSLGTQTY
CPPCPAPELL
NWYVDGVEVH
GKEYKCKVSN
WORKFLOWS
LYSKLTVDKS
GK
NGSPRLLIKY
EDIADYYCQQ
SDEQLKSGTA
STYSLSSTLT
VANQUISH

NYGVHWVRQS
KDNSKSQVFF
STKGPSVFPL
PERFORMANCE
ICNVNHKPSN
GGPSVFLFPP
NAKTKPREEQ
KALPAPIEKT
TCLVKGFYPS
RWQQGNVFSC
DILLTQSPVI
VARIANTS
NNNWPTTFGA
SVVCLLNNFY
LSKADYEKHK
FLEX UHPLC

© 2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries

Online this Month

Constructive or Crass?

(tas.txp.to/1115/peerreview)

The principles of reviewing (I believe) are clear, but humans behave badly. Some will submit the same paper to two journals simultaneously. I know because I've been asked to review both of them. Some authors completely ignore reviewer comments and try the next journal on their list. I've also gotten these submissions to review 2 months after the first try, with no improvements in the manuscript in response to the first reviews. There are too many journals of low quality, too many authors wanting to publish pedestrian uninteresting science, and too much pressure to just publish "something" for a variety of incentives that have nothing to do with science, but more to do with a perception of personal success.

All of this floods the system. Thus there is too little time for very thorough reviews beyond the top few journals...

– *Pete, United States.*

Sign up online for free to have your say: theanalyticalscientist.com/subscribe

Deadline for Humanity

The closing date for Humanity in Science Award entries is November 27, 2015.

Analytical science has the power to change human lives for the better The Humanity in Science Award was launched by The Analytical Scientist and Phenomenex to recognize a recent breakthrough in analytical science that can have a true impact on mankind's health and wellbeing.

The next Humanity in Science Award will be presented on May 10 at Analytica 2016, with the winner receiving a grand prize of $25,000. Could it be you or someone you know?

We look forward to receiving your nominations at: www.humanityinscience.com

Tea With Rich

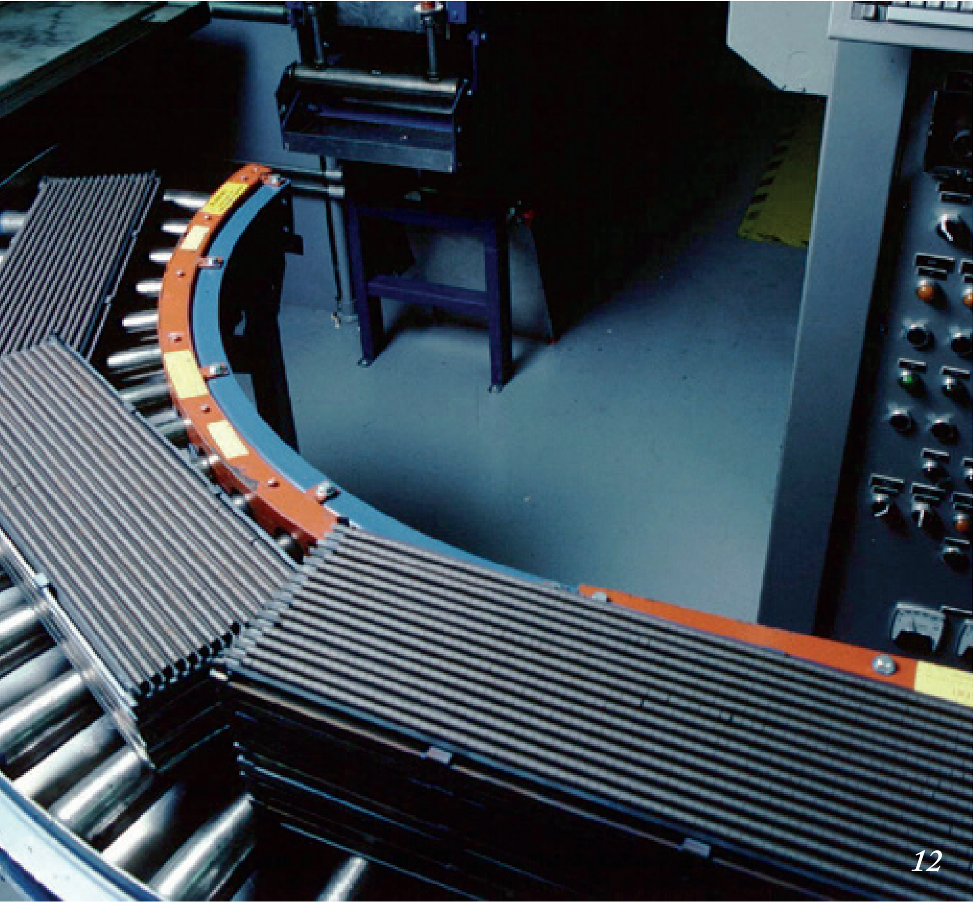
"Tea With Rich" – an ongoing series of informal interviews with key analytical scientists in glorious settings around the globe – makes a comeback with six scientists that feature on the 2015 Power List.

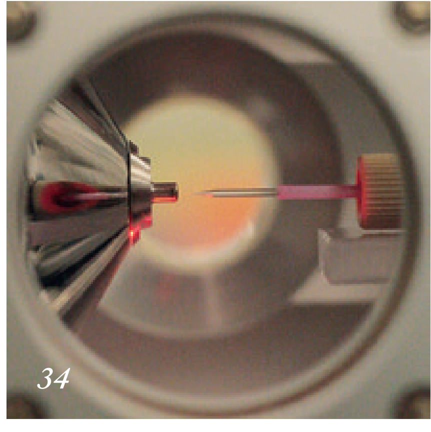
First up is Gérard Hopfgartner, who reflects on the responsibilities of chairing the ever-successful HPLC conference. Gérard then discusses his current research: multidimensional chromatography and its utility in finding answers to global and complex problems, before considering HPLC 2025.

Coming soon: Gert Desmet, Barry Karger, Mary Wirth and more...

Watch Tea With Gérard now: tas.txp.to/1015/teawithgerard.

Contents





03 **Online This Month**

07 **Editorial**
The Power of Passionate Debate, by Rich Whitworth

08 **Contributors**

Upfront

10 LC-MS Versus GC-MS

12 TXRF Goes Nuclear

13 Hot (Dog) or Hype?

14 Gold Rush

16 Name Your Poison

In My View

18 **Rob Haselberg** asks if CE-MS is in your toolbox – and if not, why not?

19 Are you still constrained by packed column thinking? asks **Nicholas Snow**

20 **Ryan Sasaki** wants his data alive – not dead – and even offers a handsome reward.

22 It's time to tackle the drug concentration conundrum, says **Florin Marcel Musteata**

On The Cover

Landmarks from author's cities and towns (Chicago, Milford, Bonn, Brussels, and Leiden) are decorated in abstract text – quite literally.

26

Features

26 **Landmark Literature: 2015**
Six experts select the best papers of the year – and tell us the reasoning behind their choices.

34 **Gurus of CE-MS**
Norman (Norm) Dovichi, Michael Knierman, Rob Haselberg, Tomoyoshi Soga, and Christian Wenz consider the future potential of capillary electrophoresis –mass spectrometry.

Reports

24 **The Analytical Scientist × Merck Millipore**
The Simple Beauty of TLC-MALDI-MS, by Jürgen Schiller

42 **The Analytical Scientist × Thermo Fisher Scientific**
The Never-Ending Challenge of Pesticide Analysis, by Kate Mastovska

Departments

46 **Solution: Finding a Piece of the Protein Characterization Puzzle**, by Tiemin Huang

49 Application Notes

Sitting Down With

50 **Detlef Günther,** VP Research and Corporate Relations, and Professor for Trace Element and Micro Analysis, ETH Zürich, Switzerland.

the Analytical Scientist

ISSUE 34 - NOVEMBER 2015

Editor - Rich Whitworth
rich.whitworth@texerepublishing.com
Commissioning Editor - Iestyn Armstrong-Smith
iestyn.armstrong@texerepublishing.com
Deputy Editor - Stephanie Sutton
stephanie.sutton@texerepublishing.com
Associate Editor - Roisin McGuigan
roisin.mcguigan@texerepublishing.com
Associate Editor - Michael Schubert
michael.schubert@texerepublishing.com
Scientific Director - Frank van Geel
frank.vangeel@texerepublishing.com
Senior Designer - Marc Bird
marc.bird@texerepublishing.com
Junior Designer - Emily Strefford-Johnson
emily.johnson@texerepublishing.com
Chief Executive Officer - Andy Davies
andy.davies@texerepublishing.com
Chief Operating Officer - Tracey Peers
tracey.peers@texerepublishing.com
Publishing Director - Lee Noyes
lee.noyes@texerepublishing.com
Sales Manager - Chris Joinson
chris.joinson@texerepublishing.com
Audience Insight Manager - Tracey Nicholls
tracey.nicholls@texerepublishing.com
Traffic and Audience Associate - Lindsey Vickers
lindsey.vickers@texerepublishing.com
Digital Content Manager - David Roberts
david.roberts@texerepublishing.com
Traffic and Audience Associate - Jody Fryett
jody.fryett@texerepublishing.com
Mac Operator Web/Print - Peter Bartley
peter.bartley@texerepublishing.com
Tablet Producer - Abygail Bradley
abygail.bradley@texerepublishing.com
Apprentice, Social Media / Analytics - Ben Holah ben.holah@texerepublishing.com

Editorial Advisory Board
Monika Dittmann, *Agilent Technologies, Germany*
Norman Dovichi, *University of Notre Dame, USA*
Gary Hieftje, *Indiana University, USA*
Emily Hilder, *University of Tasmania, Australia*
Ron Heeren, *FOM-AMOLF, The Netherlands*
Tuulia Hyötyläinen, *VTT Technical Research Centre of Finland*
Hans-Gerd Janssen, *Unilever Research and Development, The Netherlands*
Robert Kennedy, *University of Michigan, USA*
Samuel Kounaves, *Tufts University, USA*
Marcus Macht, *SCIEX, Germany*
Luigi Mondello, *University of Messina, Italy*
Peter Schoenmakers, *University of Amsterdam, The Netherlands*
Robert Shellie, *University of Tasmania, Australia*
Ben Smith, *University of Florida, USA*
Frantisec Svec, *University of California at Berkeley, USA*
Ian Wilson, *Imperial College London, UK*

Change of address:
tracey.nicholls@texerepublishing.com
Tracey Nicholls, The Analytical Scientist, Texere Publishing, Haig House, Haig Road, Knutsford, Cheshire, WA16 8DX, UK

General enquiries:
www.texerepublishing.com
info@texerepublishing.com
+44 (0) 1565 752883
sales@texerepublishing.com

Distribution:
The Analytical Scientist (ISSN 2051-4077), is published monthly, by Texere Publishing Ltd and is distributed in the USA by UKP Worldwide, 1637 Stelton Road B2, Piscataway, NJ 08854. Periodicals Postage Paid at Piscataway, NJ and additional mailing offices
POSTMASTER: Send US address changes to The Analytical Scientist, Texere Publishing Ltd, C/o 1637 Stelton Road B2, Piscataway NJ 08854
Single copy sales £15 (plus postage, cost available on request tracey.nicholls@texerepublishing.com)

Where Innovation Goes to Play.

PITTCON CONFERENCE & EXPO

Measure. Interpret. Apply.

March 6-10, 2016

Atlanta, GA
Georgia World Congress Center

www.pittcon.org

Follow us for special announcements

Pittcon is the leading conference and exposition for the latest advances in Laboratory Science. Attending Pittcon gives you a unique opportunity to get a hands-on look at cutting-edge product innovations from leading companies. Participate in any of the more than 2,000 technical presentations to learn about recent discoveries from world-renowned members of the scientific community. Improve or develop your skills by taking a short course taught by industry experts.

The Power of Passionate Debate

Peer reviews, Power Lists and the effects of heat (in GC and discussions).

Ever since I can remember, I've enjoyed a good discussion – or even a heated debate. Indeed, on countless occasions I've played 'devil's advocate,' hoping to elicit a more passionate response from my sparring partner. Some may argue that passion has no place in science – especially in cases where a totally objective viewpoint is needed. (Although whether anyone can claim total objectivity is a matter of philosophical debate in and of itself.)

For those who missed last month's "Critical, Constructive or Crass?", Victoria Samanidou used the In My View section entirely as we intended, writing in a focused and passionate way on the subject of peer review (tas.txp.to/1115/peerreview). I'm sure you can guess from the title the general gist of the article. Magically, her passion for the topic ignited a short discussion online (and hopefully a few more words over coffee or in corridors):

'Pete' states, "The principles of reviewing (I believe) are clear, but humans behave badly. Some will submit the same paper to two journals simultaneously [...] Some authors completely ignore reviewer comments and try the next journal on their list."

But 'Bob' fights Samanidou's corner: "[Reviewer] comments such as 'this is garbage', are not only rude and unhelpful, they also demonstrate (to the Editor) that the reviewer is either lazy or incapable of clearly listing and explaining the paper's limitations." It appears reviewing and total objectivity do not always go hand in hand.

The Power List reignited our 'women in science' debate on Twitter. @ClaireEEyers tweeted: "Really guys – only 15?" Meanwhile, @J_DoubleS really hammered home a couple of points: "Shame that the top 20 of the @tAnaSci #powerlist are all white men." And @HilderEmily wryly noted, "Now with almost 100% more women." But as I noted last month – it's your list. And in fact, the percentage of women on the final roster quite accurately reflected the percentage nominated...

Finally, on page 10, we interview Gary Siuzdak who has received both praise and criticism for a recent paper on thermal degradation in gas chromatography (1). An example of the latter is an online comment on C&EN (2): "This seems another unfortunate example of a peer-review failure." And thus, we have come full circle.

Debate is healthy. Discussions spark new ideas. Don't be shy.

Rich Whitworth
Editor

References

1. *M Fang et al., "Thermal degradation of small molecules: a global metabolomic investigation", Anal Chem, 87(21),10935–41 (2015). PMID: 26434689*
2. *http://cen.acs.org/articles/93/i42/Heated-Dispute-Over-Analytical-Method.html*

Contributors:

Peter Griffiths

Peter Griffiths is emeritus professor of chemistry at the University of Idaho. His research interests involved many applications of vibrational spectroscopy in analytical chemistry. His group pioneered the use of diffuse reflection spectroscopy in the mid-infrared, the coupling of various types of chromatograph (GC, HPLC, SFC, TLC) to FT-IR spectrometers, surface-enhanced infrared absorption and Raman spectroscopy, and open-path atmospheric monitoring by FT-IR spectrometry. Peter was formerly editor-in-chief and is now second editor of Applied Spectroscopy. Peter describes his hyperspectral Landmark Literature selection on page 30.

Nicholas (Nick) Snow

Nick is professor of Chemistry and Biochemistry and director of the Center for Academic Industry Partnership at Seton Hall University, South Orange, New Jersey, USA. He teaches advanced undergraduate and graduate courses in analytical chemistry and separation science. He has been recognized twice by the Seton Hall University Board of Regents for outstanding teaching and service to students. He maintains an active research group with projects involving rapid separations of complex mixtures, multidimensional separations, sampling techniques for chromatography, gas chromatography, and gas chromatography/mass spectrometry. He is especially interested in working with industrial and private partners in solving difficult analytical problems. Nick advises us not to be constrained by "packed column thinking" in GC on page 19.

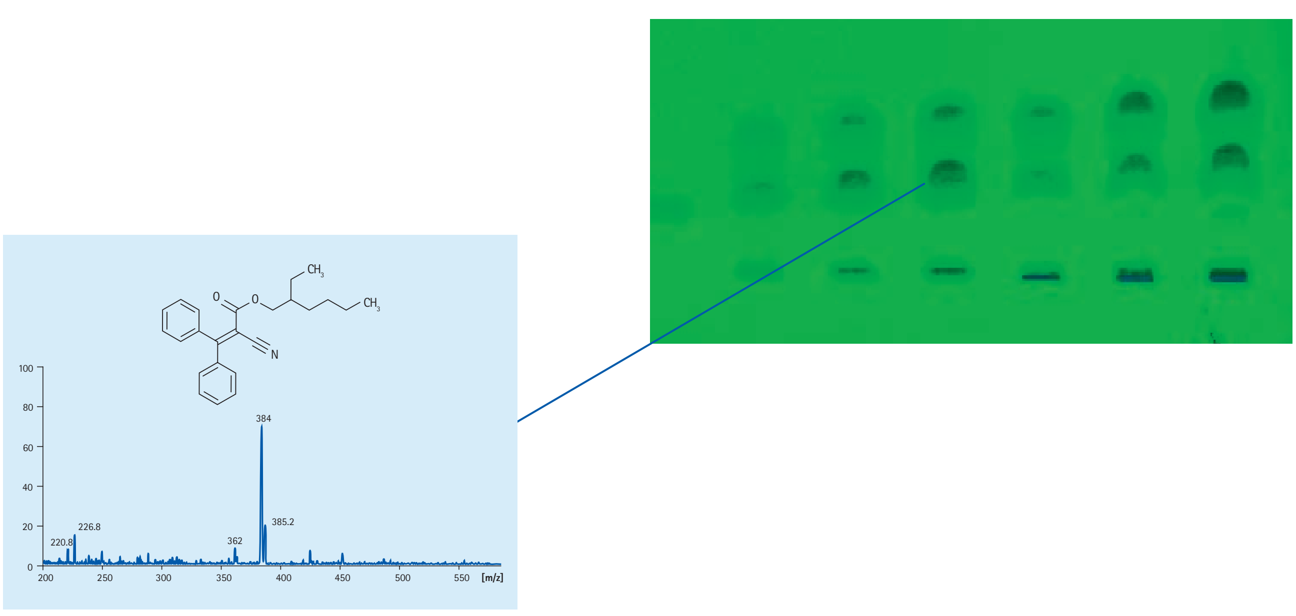
Hans-Gerd Janssen

Hans-Gerd is the science leader for compositional analysis at Unilever Research and Development in Vlaardingen, The Netherlands. "A chemical engineer is what I wanted to be," says Hans-Gerd, and so, decided not to go to a "regular, dull " university but to a University of Technology. "I then got annoyed by the approximate nature of chemical engineering." Of special interest to Hans Gerd now are food samples. "I want to understand why certain foods are safe and of high quality whereas others are poor. On page 28, Hans-Gerd puts 30 years of experience to use by selecting his Landmark Literature paper.

Florin Marcel Musteata

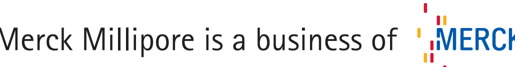
Marcel thought his career path was set after receiving his PharmD degree and beginning a pharmacy residency program in a major hospital. Little did he know that he would become intensely preoccupied by therapeutic drug monitoring. Collecting large vials of blood from fragile patients for single drug concentration measurements seemed particularly inefficient. Consequently, he decided to study microsampling and microextraction and got a PhD in analytical chemistry. Most notably, he has developed nanoporous microextraction coatings for in-vivo sampling and a mathematical model for individualizing drug therapy based on body composition. Florin attempts to address the drug concentration conundrum on page 22.

The perfect couple
Silica gel plates optimized for coupling TLC with MS



TLC-MS is a fascinating new field that combines the speed, simplicity and economy of thin layer chromatography with the powerful detection of mass spectrometry. To maximize the benefits of this innovative technique, Merck Millipore has developed TLC and HPTLC plates that are optimized for coupling with MS. They are exceptionally clean, allowing trace analysis in the nanogram range. Whether for pharmaceuticals, cosmetics, food or beverages, our plates are proven to offer enhanced sensitivity and extremely low background signals – for perfect results.

Start coupling TLC with MS on: www.merckmillipore.com/tlc-ms

Merck Millipore is a business of 

Merck Millipore and the M logo are trademarks of Merck KGaA, Darmstadt, Germany.
©2015 Merck KGaA, Darmstadt, Germany. All rights reserved.

Upfront

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

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

LC-MS Versus GC-MS

Is the heat applied in gas chromatography-mass spectrometry really responsible for false results? And, if so, how often?

What happens to small molecules during GC-MS analysis? Gary Siuzdak, professor and senior director of TSRI's Scripps Center for Metabolomics, Mingliang Fang, a postdoc and GC-MS specialist at Scripps, and other members of the lab's team have interrogated the data being generated by GC-MS by mimicking its conditions and performing LC-MS analysis to investigate the fate of molecules in the sample. The results were analyzed on the XCMS data analysis platform developed in house. They reported some cause for concern – the temperature involved in GC-MS can result in half the metabolites being degraded or transformed. We caught up with Siuzdak to find out why his study is generating quite a lot of its own heat.

What inspired you to take a closer look at the effect of heating in GC-MS?

Numerous previous studies have examined the effect of heat on specific types of molecules, so we simply applied a global LC-MS based approach to the issue to examine the extent of the effect. Interestingly, thousands of standards have been analyzed by GC-MS, and the observation that a signal was obtained was used as proof of the technology's robustness. The problem with this is that GC-MS is being used to critically examine GC-MS. Instead, we used LC-MS to investigate GC-MS.

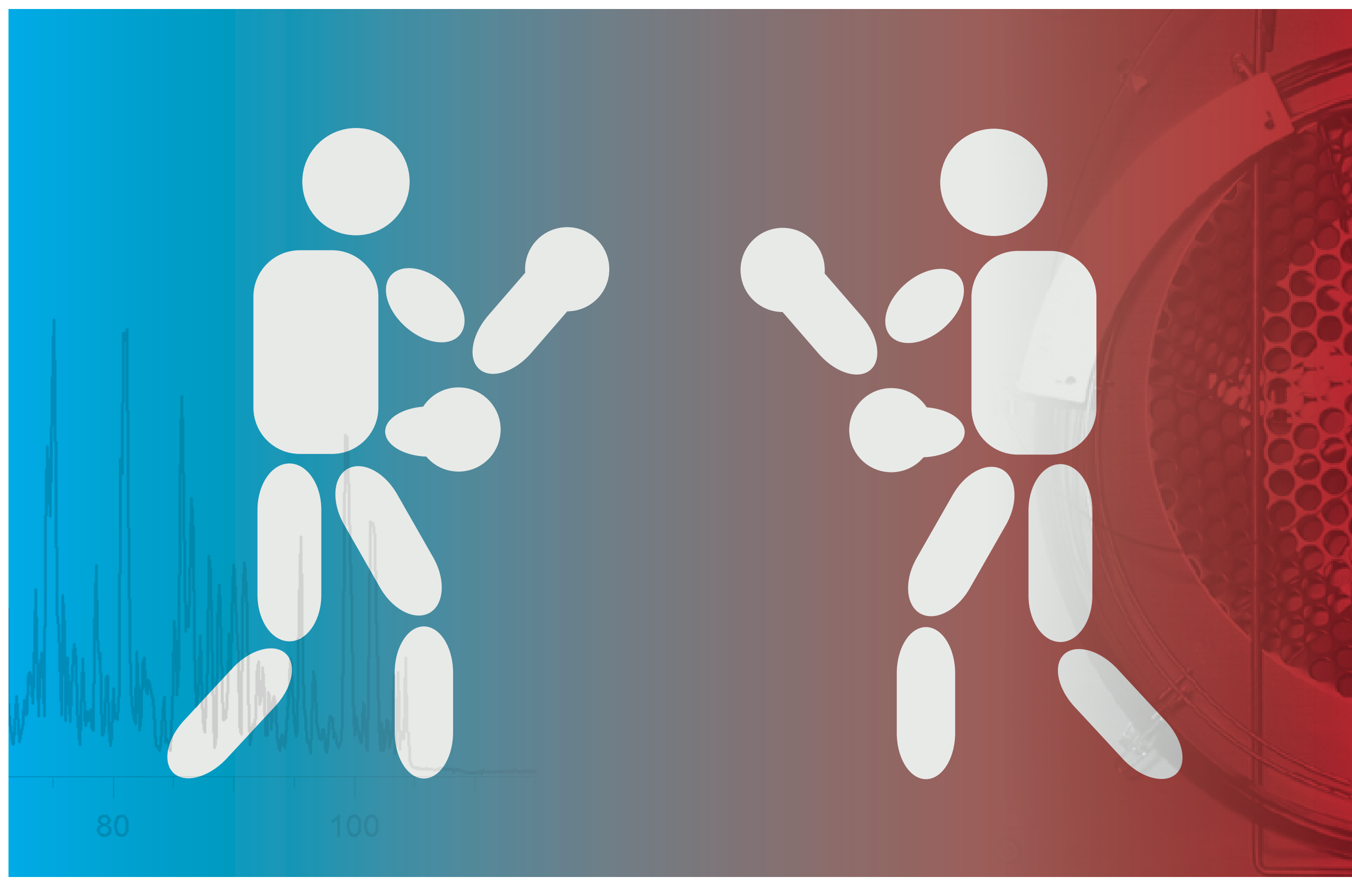
Could you give a brief overview of the study and the results you found?

The samples, both derivatized and underivatized, were heated at three different temperatures (60, 100, and 250°C) at different exposure times (30, 60, and 300 s). It is worth noting that we were careful to design the experiments to minimize effects that would not occur in GC-MS. Samples were prepared using a standard protocol, dried, heated under nitrogen, stored at -20°C, placed in solution at neutral pH to minimize hydrolysis, and analyzed by LC-MS at ambient temperatures using soft ESI-MS. We then exploited new informatic capabilities within our data analysis platform (XCMS/METLIN) to allow for global examination of these data sets.

XCMS (XCMSOnline.scripps.edu) is a free cloud-based data analysis program that we used alongside another free resource – the METLIN database (METLIN.scripps.edu). Both were developed in house to fill an unmet need for comparative pairwise and multigroup data analysis. METLIN is currently the largest tandem MS database in the world and XCMS currently has over 8500 registered users. Together, XCMS and METLIN allowed us to statistically examine the data output and identify the transformed molecules.

The results showed that heating had an appreciable effect on both the underivatized and derivatized molecules, with as much as 50 percent of the metabolites being degraded or transformed.

A challenge that we weren't quite prepared for was how derivatization efficiency dramatically varies from molecule to molecule – anywhere from 10–90 percent. That was documented in the paper but has also been well documented in other studies (referenced in our paper). Ultimately, when you do GC-MS experiments you are performing them on a complex mixture of underivatized, partially-derivatized,



and fully-derivatized molecules. These effects can add to the already increasing variance of the system.

Can you share specific examples?

Beyond the extent of degradation, one surprising result was data that was sent to me from a colleague. In developing an amino acid assay with GC-MS they observed an apparent transformation product. The derivatized form of arginine was injected into the GC-MS and the identified molecule was ornithine; we did some follow up work and there were already examples of this transformation in the literature.

How has the community reacted?

I wasn't sure what to expect but generally the response has been overwhelmingly positive. Most people were not surprised by the results but appreciated the examination of the thermal degradation process using an orthogonal technology (LC-MS). I also received multiple reminders that, beyond the papers we cited in our Analytical Chemistry paper, other more directed research has been published on thermal degradation, especially in the environmental field. It is now clear from these correspondences that even more research has been ongoing in other labs on this topic but have gone largely unpublished. One person told me that they were forbidden from doing the LC-MS experiments we performed as the group leader "was afraid of what they would find." Some individuals however have not been so open to the thermal degradation results, believing that the elevated GC-MS temperatures are not that detrimental to molecular stability.

I hope this study at least encourages students to think more critically about their results and to use standards for validation whenever possible. Ultimately, I think from a global perspective the point has been made, as it is hard to argue with the reality that some small molecules thermally degrade at high temperatures.

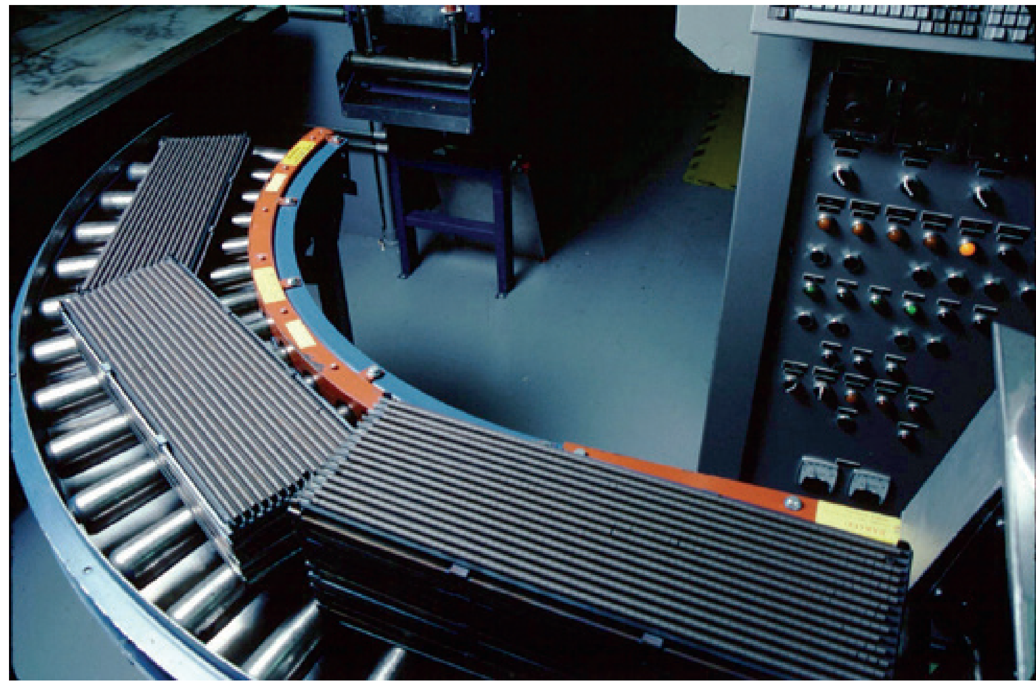
What are your thoughts? You can comment online: tas.txp.to/1115/LCversusGC

Reference

1. *M Fang et al., "Thermal degradation of small molecules: a global metabolomic investigation", Anal Chem, 87(21),10935–41 (2015). PMID: 26434689*

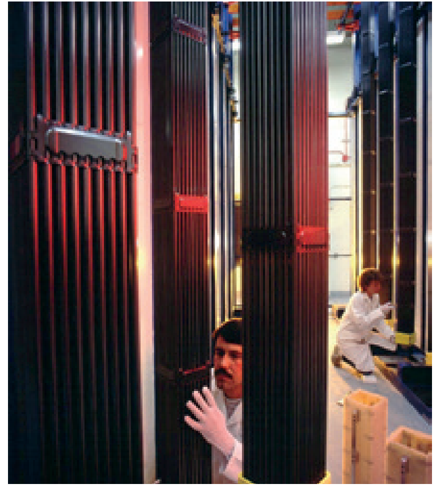
TXRF Goes Nuclear

Using total reflection X-ray fluorescence for the non-destructive analysis of nuclear fuel oxides



India's three-stage nuclear power program was devised in the 1954 to deliver energy independence to India, capitalizing on India's vast thorium reserves (25 percent of the global reserve) and recognizing its limited uranium stocks by using mixed oxides and advanced reactors. During the preparation of these fuels, physical and chemical quality control is critical to produce high-quality and high-performance fuels. Nand Lal Misra and co-workers at the Bhabha Atomic Research Centre (BARC), Mumbai, India, believe they have developed the world's first method for the direct compositional characterization of $(U,Th)O_2$ solid samples without sample dissolution using total reflection X-ray fluorescence (TXRF) analysis (1).

"Compositional characterization of these fuels by dissolution using conventional techniques is a very cumbersome process involving corrosive chemicals like hydrofluoric acid. With TXRF, the samples can be analyzed almost in a non-destructive manner. It only requires a few nanograms of sample," explains Misra, who is Head of the X-ray Spectroscopy Section, Fuel Chemistry Division. Indeed, sufficient amounts can be transferred by gently rubbing the $(U,Th)O_2$ samples on TXRF supports or by drying uniform slurry to make a thin film. "Though we assumed that some amount of $(U,Th)O_2$ will be transferred by gently rubbing the samples on supports, we were doubtful about such material transfer in the case of sintered pellets. Surprisingly, we observed a good TXRF spectrum of the specimen prepared from this composition, with quite strong U and Th Lα peaks, " adds Misra. In fact, the team found that all compositions of $(U,Th)O_2$ produced accurate TXRF spectra; RSD values obtained on replicate measurements were 2.6 percent and the average deviation of TXRF-determined values from the expected values was 5 percent.



Misra believes that the method could be used for characterization of other fuels (carbides, nitrides, and oxides) in different forms (pellets, powders, and microspheres) and notes that it could be equally useful for precious metals or even in forensic applications. However, given the radioactive nature of samples in the nuclear energy industry, the advantages the method brings – low sample amounts, negligible sample preparation, and reduced analytical waste generation – are particularly clear.

Reference

1. *S Dhara, P Prabhat, and NL Misra, "Direct compositional characterization of $(U,Th)O_2$ powders, microspheres, and pellets using TXRF", Anal Chem 87 (20), 10262-10267 (2015). DOI: 10.1021/acs.analchem.5b01824*

Hot (Dog) or Hype?

Kickstarter technology is nothing new – but Clear Labs is making in-roads into something less common: kickstarter analytical science

Clear Labs recently published a pretty damning report on US hot dogs through its consumer initiative Clear Food – the first of more to come, they say. Here, we introduce the company and present some of its findings – and ask what you think.

Who? Clear Labs, according to its own glossy website, are "The pioneers in food analytics" and it "analyzes food at the molecular level to help the world's best brands differentiate on quality and stand behind their value."

What? Clear Food is a consumer initiative that plans to publish "monthly reports that translate quantifiable molecular tests into actionable information consumers can use to find safe and healthy foods, at the best value." The first report? Hotdogs of course. A North American icon. Anyone who remembers the 1988 movie "The Great Outdoors" may remember Roman's (Dan Aykroyd) classic reference that suggested the main constituents were "lips and [...]" an unmentionable hindquarter part. The question, of course is, from what animal?

Why? The company uses an objective rating system called the "Clear Score" (trademarked of course) "to give consumers the information they need to make informed purchasing decisions". And it provides the reports for free – so fair play on that front. But if you want to make a suggestion for the next report? You guessed it: you've got to go through its Kickstarter campaign.

Behind every great (U)HPLC system…

is a great column

We pride ourselves in:

Scalability

• UHPLC ↔ HPLC ↔ PREP • easy method transfer

Robustness
• pH
• temperature
• 100% aqueous eluents

Reproducibility
• day to day
• column to column
• lab to lab

Selectivity
• silica, polymer, hybrid, core shell technology

www.ymc.de | Phone: +49 2064 4270 | info@ymc.de

How? Emphasis is placed on next-generation DNA sequencing at Clear Labs (the company purchased "every single food item" in the US, and ran them through an Illumina MiSeq) – especially for the Clear Food reports. But Clear Labs also uses non-specified "modern non-DNA tests" (presumably, referring to any other analytical technique). Apparently, the combined platform allows them to cover an impressive list: authenticity, GMO, contamination, gluten, heavy metals, allergens, antibiotics, hormones, nutrition, and pesticides. For the hot dog report, the company analyzed 345 samples (75 brands and 10 retailers) – now that's commitment.

Clear results?

- 14.4 percent of hot dogs and sausages had issues
- 2 percent of samples contained human DNA (the majority in vegetarian products)
- 10 percent of vegetarian products contained meat.

The report also offers a "Clear List" which only includes items with a Clear Score of 95 or more. Clear?

What do you think of Clear Labs and its initiative Clear Foods – hot or hype? Let us know online: tas.txp.to/1115/hotorhype

Gold Rush

Nanoporous gold helps 'sieve out' biological debris for fuss-free nucleic acid detection

Detection of nucleic acids in biological fluids is typically hindered by lengthy preparative procedures and the need for complex instrumentation. However, a new nucleic acid-detecting sensor developed by a team led by Erkin Şeker could potentially filter and detect in one go. The 'gold sponge' sensor can sieve out debris in biological samples allowing passage only to target DNA fragments, which may obviate the need for a separate purification step. The team believes that after some optimization of the initial prototype, the device could allow the sensitive, point-of-care molecular detection of DNA in complex biological samples, such as serum from whole blood (1). We caught up with Şeker, assistant professor of electrical and computer engineering at UC Davis, to find out more.

Why are complex biological fluids typically so problematic for sensors?

Biological fluids contain large proteins that would normally block-up other sensors (a problem known as biofouling). These large biomolecules adsorb non-specifically on the electrodes surface and prevent the transport of smaller target molecules. Normal electrode-based sensors for the detection of DNA often require external anti-biofouling coatings, but our gold sensor is composed of a network of nano-sized pores, so biological debris, such as proteins, don't get through, but the nucleic acids can enter easily.

Why use nanoporous gold (np-Au)?

Nanoporous gold has a multitude of desirable features, such as high surface area to volume ratio, ease of thiol functionalization, compatibility with conventional microfabrication techniques, and tunable morphology. From a scientific research point of view, it is a great material to study structure-property relationships in a wide range of fields. Technologically, these features make nanoporous gold an excellent choice for DNA sensing applications and enable facile integration and scalability for point-of-care molecular detection systems. However, I must admit that I was not even thinking about bioanalytical aspects of this material when I first put together the research plan of my faculty application package. My initial (and still on-going) research focus has been on multifunctional biomedical device coating. The entire project on bioanalytical sensors started as a result of a university-wide initiative called UC Davis Research Investments in the Sciences and Engineering (RISE) program.

How is the sensor made?

We use a self-assembly process to fabricate the np-Au sensor's gold surface. We then immobilize DNA 'capture' probes into the nano-surface. The np-Au electrodes enable detection of target DNA molecules (10–200 nM) in complex biological media, whereas the sensor with planar gold electrodes performed poorly under the same conditions.

Nanoporous gold is microfabrication-compatible and electrically-conductive, it is a versatile material to seamlessly integrate with sensing electronics – a problem for some nanomaterials as they cannot be easily integrated into devices by conventional microfabrication techniques. We hope our work on np-Au DNA sensors can serve as a guide for developing nucleic acid sensors using various other novel nanostructured materials.

What was the most challenging aspect?

Well, first I needed to learn some electrochemistry! And the main challenge was developing an electrochemical protocol that maintains a delicate balance between reaction- and mass transport-limitations, which directly dictate the sensor performance. Most redox markers mainly reacted with the top portion of nanoporous gold films even before diffusing into the pores to make use of that entire surface area underneath the first layer of pores. We (especially the key researchers, Pallavi Daggumati and Zimple Matharu) developed techniques to address these issues – and we were surprised by how well the variations in nanomorphology allowed for the control of the dynamic range of the sensor via influencing the molecular transport and target capture kinetics.

What's next?

We are about to start evaluating the performance of a np-Au-based sensor in detecting microbes in blood (in collaboration with research from UC Davis School of Medicine and School of Veterinary Medicine). We also have some spin-off ideas that should further assist analytical sample preparation – hopefully we'll share these results with the biosensor community in the near future.

There is a race to develop biosensors that are more sensitive, cheaper, and faster. Though these efforts are commendable, I think the fundamental science behind how the nanostructure dictates sensor performance is very important. We are grateful that the recent National Science Foundation (NSF) award will fund us in continuing to pursue this fundamental question.

Reference

1. *P Daggumati et al., "Biofouling-resilient nanoporous gold electrodes for DNA sensing", Anal Chem 87 (17), 8618–8622 (2015).*

Processing,
Not Programming

You shouldn't need a Degree in Computer Science to program your instruments. That's why we have created the Extrahera™ as an easy-to-use, time efficient SPE & SLE sample preparation processor for your laboratory.
www.biotage.com

Name Your Poison

A new DNA-based sensor shows promise for indirect detection of thallium

Thallium sulfate has no taste, smell or color but is highly toxic – and that's probably why it goes by the name of The Poisoner's Poison in certain circles. "I remember about twenty years ago there was a well-known murder case in China in which thallium was used as a poison – the mystery remains unsolved even today," says Juewen Liu, assistant professor at the University of Waterloo, Canada. Liu wondered about the potential of a thallium biosensor, but could find very little in the literature. "It seems strange that its two neighbors in the periodic table, mercury and lead, have been so extensively studied, but not thallium," says Liu, who has focused on developing DNA-based ligands for metal detection for the past 15 years. To redress the imbalance, Liu and colleagues decided to develop a method that can indirectly detect Tl^{3+} using a catalytically-active DNA molecule, known as a DNAzyme.

DNA is a highly stable, programmable, and versatile component in the design of sensors. And compared to current instrumental analysis methods, DNA-based biosensors are more cost-effective and portable. The big problem is that Tl^{3+} has very little interaction with DNA, which likely explains the lack of research, says Liu, who found a workaround.

The new sensor uses a DNAzyme called Tm7 isolated in recent work (2). Tm7 is an Er^{3+}-dependent RNA-cleaving DNAzyme with an unusual property. "We discovered that if we introduce a phosphorothioate (PS) modification into the RNA substrate, Tm7 becomes completely inactive – it cleaves only the normal phosphate (PO) RNA substrate," explains Liu. "This is the first DNAzyme ever reported with this property. For most other DNAzymes, partial activity is retained even with the PS-modified substrate."

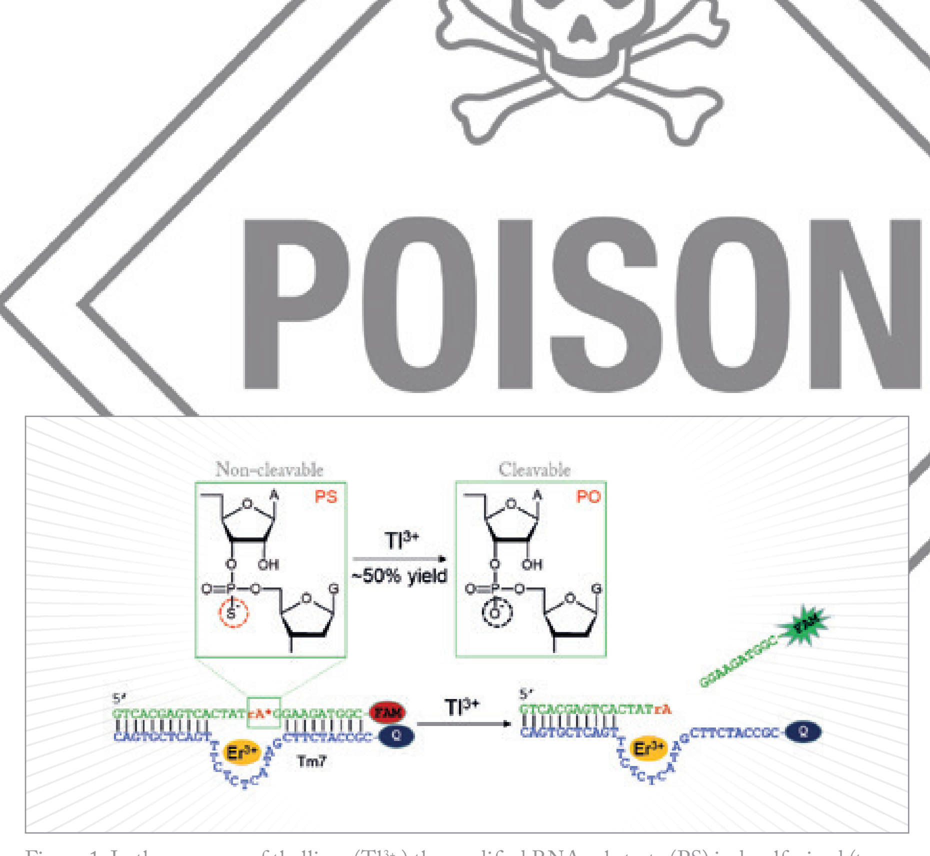


Figure 1. In the presence of thallium (Tl^{3+}) the modified RNA substrate (PS) is desulfurized (to PO), which allows the DNAzyme to cleave the RNA linkage, releasing the FAM fluorescent tag from its quencher (Q).

What does this have to do with thallium? In the presence of Tl^{3+}, which is strongly thiophilic, the RNA linkage is desulfurized, meaning that it can subsequently be cleaved by the DNAzyme (see Figure 1). The RNA substrate is labeled with a fluorophore that is quenched when bound to the DNAzyme. The cleavage reaction releases the fluorescent fragment and therefore results in Tl^{3+} concentration-dependent fluorescence enhancement. Liu notes that other approaches, such as colorimetric or electrochemical detection, may also be possible.

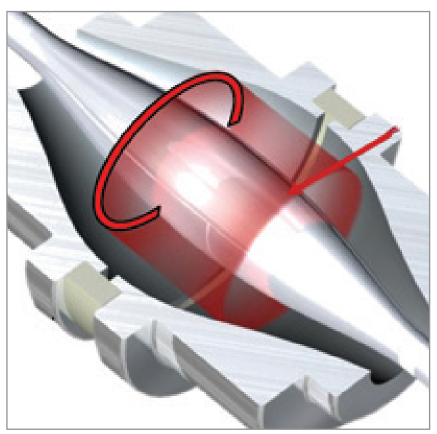
Liu adds that though Hg^{2+} and other thiophilic metals are able to desulfurize the RNA substrate, they also inhibit Tm7 – Tl^{3+} does not inhibit the DNAzyme, which offers some specificity. And according to Liu, sensitivity goes down to 1.5 nM – below the maximal contamination limit defined by the US Environmental Protection Agency (10 nM).

One current limitation is that the sensor can only detect Tl^{3+} and not Tl^{+}. "We are now trying to develop a Tl^{+} sensor using DNA and hope to use both sensors to study metal speciation. We are also interested in finding the difference between Tl^{3+} and Hg^{2+} to allow better separation of these two metals," adds Liu.

References

1. *PJJ Huang et al., Desulfurization activated phosphorothioate DNAzyme for the detection of thallium", Anal Chem, 87, 10443-9, (2015). DOI: 10.1021/acs.analchem.5b02568.*
2. *PJJ Huang et al., "A new heavy lanthanide-dependent DNAzyme displaying strong metal cooperativity and unrescuable phosphorothioate effect", Nucleic Acids Res, 43, 461-469 (2015). DOI: 10.1093/nar/gku1296.*

Using GC & LC Orbitrap mass spectrometry to confidently identify leached packaging and process impurities



Learning objectives:

1. Get insight into latest instrument technology for unknown impurity identification
2. Follow a leachable study for both GC and LC amenable organic impurities
3. Learn about the latest enabling software and libraries for unknown impurity identification

Wednesday 2nd December 2015, 15:30 UK Time
Register Free at: http://tas.txp.to/1115/EandL

Maintaining Chain-of-Custody Throughout the Analytical Workflow

The ability to successfully defend a laboratory's data audits or litigation is paramount to the overall mission of any environmental lab. Central to that, is maintaining chain-of-custody throughout the analytical workflow, not just during sample collection. One of the last frontiers to maintaining sample custody exists within the analytical workflow, specifically the labeling of analytical samples. For a large percentage of analyses, the samples are extracted for use in chromatographic instruments, and the sample is stored in a glass, 2 mL vial. Technology now exists to eliminate the manual nature of vial labeling by printing directly onto a vial with information taken directly out of a Laboratory Information Management System (LIMS).

Monday 7th December 2015, 10:00 am EDT
Register Free at: http://tas.txp.to/1115/Chain

Making Best Use of Your Ultra-high Pressure Instrument

Maybe you have invested in UHPLC instrumentation but are not making best use of it. Five simple rules will be given to maximize the throughput of your HPLC lab by optimally selecting column length and particle size as a function of the sample requirements or the goal of the method development process or analysis. The rules will be illustrated with a wide selection of contemporary examples. As some of these rules may go against intuition or general "wisdom", their physical background will be explained as well.

Thursday 10th December 2015, 15:00 GMT
Register Free at: ttp://tas.txp.to/1115/UHPLC

In My View

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

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

Contact the editors at
edit@texerepublishing.com

Is CE-MS in Your Toolbox?

Online hyphenation of capillary electrophoresis and mass spectrometry is nearly 30 years old, but has been ignored in certain camps. It's time to give CE-MS a chance.

By Rob Haselberg, senior post-doctoral researcher, AIMMS Research Group BioMolecular Analysis, VU University, Amsterdam, The Netherlands.

In March 2015, I attended the inaugural Analytical Technologies Europe conference in Berlin. Wassim Nasahbeh presented the opening lecture in which he explained that it can easily take up to 30 years for technologies to make it from initial academic publication until they become an accepted standard in industry. This made me think that it is close to 30 years since on-line hyphenation of capillary electrophoresis (CE) and mass spectrometry (MS) was first published. So, where does CE-MS stand today?

In academic research, CE-MS is definitely an established technique, with many research groups around the world using it in very diverse application areas. In today's industrial laboratories, however, CE-MS is applied only occasionally. But why?

Analytical chemists who are not familiar with CE often claim that it is not robust. Surprisingly, most of these people are unaware of the fact that CE is at the heart of the DNA sequencers that elucidated the human genome with unsurpassed accuracy and speed. Currently, CE is also well established and widely used in biopharma. In the last decade, CE has become a gold standard in monoclonal antibody analysis and is applied routinely from product development to release testing.

Published wide-scale interlaboratory studies have demonstrated robustness, efficacy, specificity and reliability of protein CE methods, which convinces the drug regulation authorities that it is up to the mark. Today, there are ready-to-use CE methods and kits on the market to evaluate protein charge heterogeneity, size distribution, and glycan profiles. Moreover, similar CE methods for the analysis of inorganic ions, low-molecular weight drugs, chiral compounds, and DNA show the enormous versatility of the technique outside of the protein field. Today, we can safely say that we have busted the lack of robustness myth!

So, what is holding back wider application of CE-MS? In my view, there are three important reasons for its narrow take up. First, CE is a younger sibling in the separation methodologies family. Consequently, like in every family, it had to first prove itself extensively and find the areas in which it excelled over its older brothers and sisters before it was considered a suitable alternative. This eventually happened in the DNA and biopharma fields, but it took a number of years. Simultaneously and quickly, its older brother liquid chromatography (LC) also developed in the protein field, because it provides a reasonable alternative for companies as they already have the equipment and knowledge.

Another factor is the compatibility of CE methods with MS detection. When the current gold standard CE methods were first developed, MS compatibility was not a priority. This means it can be difficult to make slight modifications to enable MS coupling. Significant redevelopment and validation often is required, which industrial laboratories are reluctant to do.

In contrast, LC and GC methods are often easily transferable to MS detection with only minor adaptation of the conditions.

Finally, CE-MS interfacing is technologically more challenging than LC- or GC-MS interfacing, with only two commercial solutions available today. Unfortunately, of the two, the one with the longest and best track record (20 years) is not a universal solution and can only be used in combination with two mass spectrometer brands. The second interface solution offers broader compatibility; however, it only became available last year and must enter the proving grounds.

So what about the future of CE-MS? Currently, due to the success of standalone CE, CE-MS methods are in high demand. Consequently, vendors, industry, and academics have put great effort in creating the required methods and showing their robustness. The first interlaboratory study on CE-MS is complete and the results are very encouraging. There has also been a renewed interest in technological developments over the last few years, which will definitely help to push CE-MS forward even more. In my view, with the current momentum and investments made, CE-MS has a very healthy future indeed.

On page 34, four gurus and I discuss the past and future of CE-MS in more detail.

The Six Dimensions of GC

The juxtaposition of simplicity through new technology with complexity in the chemistry keeps gas chromatographic method development exciting – despite GC being described by many as a "mature" technique. But are we still using packed-column thinking?

By Nicholas Snow, professor and director, Department of Chemistry and Biochemistry, Center for Academic Industry Partnership, Seton Hall University, South Orange, New Jersey, USA

Can we argue that today's analytical methods are both simpler and more complex than a generation ago – for example, when I did my first injection into a gas chromatograph? In 1985, many typical gas chromatographic methods employed a packed column with direct injection and detection by flame ionization or thermal conductivity detection. Although capillary columns had been in use for about 30 years, easy-to-use fused silica capillary columns had only been known for about five. Back then, most instruments for capillary GC were modified versions of their packed column cousins. The useful separation space on a typical packed column accommodated about 10 peaks, so chemistry optimization focused almost entirely on the column. There were hundreds of stationary phases available for packed columns, each with its own selectivity. Sample preparation chemistry was critical because interferences that would generate overlapping peaks or damage the column needed eliminating – therefore, packed column methods usually considered these two of the possible separation dimensions carefully.

GC's roots in packed columns still influences us today; I call this "packed-column thinking".

The practical consequences of the fundamental equilibrium theory driving separations has shifted. Due to the high mass of stationary phase present in packed columns, separations are performed at temperatures generally well above the normal boiling points of the analytes (thick stationary phases make analytes "stick" more strongly in the column). While in capillary columns, separations are usually performed at temperatures well below the normal boiling point (thin films favor evaporation into the mobile phase). This, together with engineering advances that easily allow hundreds of thousands of theoretical plates in capillary columns, has caused the range of possible analytes for GC to rise considerably.

Inertness was often a problem in packed columns. Indeed, the interior of a modern capillary GC column can be one of the most inert places in the entire field of chemistry. The column is manufactured from deactivated fused silica that is chemically bonded to a stationary phase usually made from a very high purity polydimethylsiloxane (silicone)-based polymer. And, the carrier gas is usually high purity helium. Simply put, if the inlet is kept clean, there is little available for labile compounds to react with, as long as air and water are kept out.

Today's methods employing comprehensive two-dimensional GC (GC×GC) have up to six (yes, six – can you name them?) possible dimensions in which separation can happen, or in which separation can be compromised. Most articles and reports still focus primarily on optimizing one or two dimensions – rarely all six, just like the packed column literature of yesteryear. Papers and methods that focus on the columns rarely focus on the injection (in fact, relatively few papers seriously discuss optimization of the injection), sample

preparation or detection. Papers that focus on sensitive detection, such as with mass spectrometry (MS) often treat the chromatography and sample preparation as merely an inlet. We are all guilty of this – a full optimization and discussion of all steps in the process could turn the average paper into a dissertation.

Here are some of the common statements about gas chromatography that, given the separation power available today in GC, should be challenged and definitely provide excitement in continuing the study of "basic gas chromatography" (1).

- Samples must be volatile. This is packed column thinking. Think about vapor pressure and equilibrium theory; only a few mmHg of vapor pressure is necessary to drive an analyte through a capillary column and the columns can be heated to more than 300 °C.
- For non-volatile and polar samples, derivatization is required. This is packed column thinking. With inert columns and inert inlets, the active sites that would adsorb polar analytes are much less common. Many analytes that once required derivatization no longer do.
- Injecting water kills columns. This is packed column thinking. Inert, chemically bonded stationary phases are much more resistant to polar solvents such as water. The more important challenge is solvent effects related to the inlet.
- LC-MS is more sensitive. This is packed column thinking. With careful consideration of sample preparation and injection chemistry, GC-MS based techniques can generate and surpass LC-MS sensitivity. And, we get classical electron ionization mass spectra.
- Use a polar column to separate polar analytes. This is packed column thinking. Highly efficient capillary columns and selective detectors, such as mass spectrometry, place much less pressure on tuning the column chemistry (selectivity) to the analytes. In my lab, we almost exclusively use 5 percent phenyl PDMS capillary columns, which greatly simplifies column management.
- Inject 1 µl of a liquid sample. This is packed column thinking. We have all been doing this since our first injection. There are myriad online sampling and injection techniques available. Split and splitless injection can severely limit the overall capability of capillary GC for trace analysis. Programmed temperature vaporization (PTV) injection has not yet become very popular although if sensitivity is the goal, it is clearly the method of choice.

In your own research and method development, I encourage you to challenge these and other common comments you may have heard about GC. The six dimensions of chemistry: sampling, sample preparation, injection, first dimension column, second dimension column (if GC×GC) and detection, that we can bring to bear on any problem will lead you to a pleasant surprise.

Reference

1. *HM McNair, Basic Gas Chromatography, Palo Alto: Varian, 1965 (several editions); HM McNair, JM Miller, Basic Gas Chromatography, 2nd Edition, New York: John Wiley and Sons, 2009.*

Data: Wanted ~~Dead or~~ Alive

Can R&D organizations really afford to kill off analytical data that could be used for future decision-making? Perhaps it's time to breathe life back into analytical data environments.

By Ryan Sasaki, former Director of Global Strategy, ACD/Labs, Toronto, Canada.

Looking back to my time in the lab as a process chemist for a pharmaceutical company, I remember my daily laboratory routine like it was yesterday. Outside of meetings and discussions on our target API, it was very much a design-synthesize-analyze-report routine. We didn't have an electronic lab notebook (ELN) at the time, so everything was on paper. Even back then, when I stocked away yet another lab notebook on a shelf with hundreds of others, I couldn't help but wonder, "Does anyone ever look back at these notebooks? And if they do, how can they possibly ever find what they are looking for?"

Several years later, the old routine involving the paper lab notebook has disappeared, especially in large pharmaceutical and biotech laboratory environments with the emergence of the ELN. The movement had huge intellectual property reasoning behind it, but there was also the expectation that it would help shed light on the dark data and experiments that were buried away in old paper lab notebooks. But among all the scientific data being

"Dead data is difficult to search, and impossible to re-process"

generated in the industry to support chemical and biological workflows, a significant amount still ends up in a place where it can never be re-accessed or re-used. Indeed, a joint Scientific Computing and IDBS survey from 2011 claims that, despite the emergence of modern laboratory informatics systems, 88 percent of R&D organizations lack adequate systems to automatically collect data for reporting, analysis, re-use, and future decision making.

In the old days, our data transactions were very much of the "One-and-Done" variety; we'd acquire data, print it out, review it and then either throw it away – or if it was really important, glue it into our lab notebooks. Unfortunately, the workflow hasn't changed much, even though we have much better technology and much fancier ways to process and visualize the data. For example, chemists will run a reaction and acquire some analytical data (for example, NMR and/or LC-MS) to confirm they have made the right stuff. After that, they will generally convert their information-rich data into a PDF for proof of the transaction and attach it to their ELN. The analytical data is being treated almost exactly the same as 20 years ago; the processed and interpreted data is buried, never to be interrogated again.

What value does this legacy transactional data really have? Imagine you are in the lab doing a separation or purification on your compound of interest. What happens when you discover that dreaded new impurity peak in your chromatogram? Why have you never seen it before? Has someone else seen it before? In reality, data is often acquired and interpreted from scratch, without any knowledge of previous investigations. Perhaps, someone else has actually fully characterized and studied the very impurity you are concerned about. This unproductive environment is the result of the one-and-done data lifecycle. Given the technology we have at our disposal today, this really should not be the case.

Managing in-house data is one thing, but what if 70 percent of your data is being generated by contract research organizations in various locations? In our increasingly outsourced world, data is dispersed all over the globe and, therefore, is commonly shared and distributed via PDF files, text documents, and spreadsheets. Certainly, the file sizes are kept pretty small and the applications for viewing the data are universal, but when I was at ACD/Labs we called it 'dead' data. Why? Because all of the rich information within the file has been completely stripped away, reducing it to a series of text strings, tables, or images. Dead data is difficult to search, and impossible to re-process, re-analyze, or compare with newly acquired 'live' data sets.

Crucially, such dead-data workflows are preventing scientists from re-using, re-purposing, and re-leveraging legacy and existing data sets. In the past, if we had questions, we could turn to our colleague across the hall, or take a short walk to another department to speak to a long-term specialist. But the world has changed. We are being asked to collaborate with colleagues across the globe with whom we've never met; sometimes, we may not share a common language. I strongly believe that the future of medicine, science, and technology demands that we evaluate our changing landscape and tackle emerging issues head on with the myriad of technologies already available. So, is your data dead or alive?

Ion chromatography? Metrohm is the better deal!

- Because we make sure your applications run on your Metrohm IC – **guaranteed!**
- Because when you have a question, our specialists are just a phone call or visit away – **guaranteed!**
- Because we train you to become an IC expert yourself – **guaranteed!**

www.metrohm.com

The Drug Concentration Conundrum

When it comes to measuring drugs in biological samples, what is needed, what is quantifiable – and what role should analytical scientists really be playing?

By Florin Marcel Musteata, associate professor, Department of Pharmaceutical Sciences, Albany College of Pharmacy and Health Sciences, Albany, New York, USA.

Making progress in treating diseases with pharmacotherapeutic agents demands that we study the interaction between drugs and living organisms at the molecular level. Measuring drug concentrations in biological samples to understand the relationships between drug dose, concentration, and effect is an important part of such efforts. To find meaningful relationships that are applicable for all individuals, we really need to know the drug concentration at the site of action; unfortunately, measuring this concentration can be exceedingly complicated due to the high compartmentalization of living organisms. Furthermore, drug concentrations are usually very low and can change rapidly over time.

For decades, the method of choice has been to collect a small part of the investigated organism followed by sample analysis in the laboratory. And the biological sample of choice is blood plasma because it is in close contact with all tissues. Once the sample gets to the laboratory, analytical scientists – for whom the overall purpose of the study is generally unknown – treat the sample from a purely analytical point of view, finding the concentration of the target analyte in the sample. The sample is effectively separated into fractions and the total drug concentration is measured in the portion that exhaustively contains the analyte.

While the established approach is very reproducible and has become the gold standard in most pharmacokinetic-pharmacodynamic studies, it does not contribute much to the overall purpose of the study: finding a relationship between dose, concentration, and effect. This is because the drug in plasma is several biological membranes away from the site of action – the surroundings of the biological receptor. Plasma composition varies between individuals and contains numerous components – especially proteins – that interact with most drug molecules, affecting the freely diffusible drug concentration and making it very difficult to predict drug effects in specific individuals based on total concentrations alone.

In stark contrast, methods developed for minimally invasive in-vivo sampling and analysis – such as microdialysis, microextraction, ultrafiltration, and biosensors – directly measure the freely diffusible drug concentration (1). These methods are based on partial extraction of the target analyte, usually through a semipermeable membrane. Although these methods can also be used to analyze sample aliquots, they are rarely applied in this way as they are sensitive to changes in temperature, pH, and even dissolved gas content. Moreover, these partial extraction methods can be time consuming and difficult to calibrate, and they tend to have lower sensitivity, accuracy, and precision than methods based on measuring total concentrations in sample aliquots. Accordingly, they have not gained widespread acceptance in clinical practice and are used mostly for research purposes.

One of the main impediments to finding good correlations between drug concentration and effect at population levels is the high inter-individual variability in drug distribution between body components and target receptors. The total concentration does not compensate for this variability, while the free concentrations does. This has been demonstrated for antibiotics, antiepileptics, immunosuppressants, and even endogenous hormones, such as vitamin D metabolites and testosterone. On one hand, we have the highly accurate and reproducible methods based on measuring total concentrations in sample aliquots that are poorly correlated with therapeutic effects; and, on the other hand, we have the less accurate but better correlated methods for measuring free concentrations. The result? Progress in finding good population-level correlations between drug concentrations and effects has been slow.

One solution to avoid concentration conundrum is to determine an effect-normalized concentration based on free or total concentration and the composition of the investigated organism (2). The normalized concentration would be the total drug concentration that produces the same therapeutic effect in an organism with average chemical composition.

As the sensitivity of analytical methods continues to improve and sampling is performed with minimal interference close to the site of drug action, analytical scientists will be able to provide highly accurate data that is much more closely related to therapeutic effects. However, for this to happen, the specialists in measuring drug concentrations – analytical scientists – have to be supported in their fundamental research efforts and asked for input in interdisciplinary drug development teams.

References

1. FM Musteata, "Recent progress in in-vivo sampling and analysis", TrAC, 45,154–68 (2013).
2. FM Musteata, "Calculation of normalized drug concentrations in the presence of altered plasma protein binding", Clin Pharmacokinet, 51:55–68 (2012).

Why to buy polymer-based columns?

- The great chemical stability leads to an extended pH range (2 to 13).
- The low bleeding allows the use of sensitive detection.
- The large variety of material properties creates a higher resolution.
- They are available for almost all separation techniques.
- The price per injection is cheaper than in silica-based columns due to their extended lifetime (2 to 3 times longer than silica-based).

Why to choose Shodex as supplier?

- We offer a comprehensive and high standard technical support (pre- and after-sales).
- We provide 50 years of experience.
- Our experience leads to a great knowledge and a huge application database.
- Full production control: own production of polymer gels & own column packing.
- Strong partnership with customers and distributors.
- We offer you demo columns for free during 30 days.

Register on our new website and get lots more of information:

www.shodex.de

The Simple Beauty of TLC-MALDI-MS

Thin-layer chromatography's ability to simply and inexpensively provide high-quality separations in parallel makes it the perfect partner for MALDI mass spectrometry – especially when it comes to the analysis of complex (and sometimes "suspicious") biological samples.

Yulia Popkova (left) and Katharina Lemmnitzer (right) prepare for TLC-MALDI-MS analysis at the Institute of Medical Physics and Biophysics at Leipzig University.

By Jürgen Schiller, Principal Investigator, Institute of Medical Physics and Biophysics, University of Leipzig, Germany.

I've always found chemistry a fascinating field, and I recognized early on that it's a prerequisite for many other studies, for example, in biology and medicine; after all, biological molecules stem from chemistry. In my opinion, without a detailed knowledge of chemical aspects, it's very difficult to understand physiological features. Nevertheless, today I find myself conducting research in a medical physics/biophysics department, which in some ways emphasizes the need for multidisciplinary approaches to solve complex problems.

In my early days, I had no knowledge of mass spectrometry. I was more fascinated by nuclear magnetic resonance (NMR) spectroscopy (which we still rely heavily upon today, as you will see). In fact, my diploma thesis focused on the application of NMR spectroscopy in inorganic chemistry. And my PhD continued my work with NMR but familiarized me with its clinical application (my thesis was: "NMR investigations of synovial fluids and contributions to the modelling of cartilage destruction during rheumatoid arthritis").

My introduction to mass spectrometry was somewhat accidental. During my postdoctoral research, I was involved in a much bigger project that aimed to use both NMR and MALDI MS. At first, we attempted to analyze proteins released from cells and then moved onto carbohydrates – both efforts were unsuccessful, to be honest. But when we looked at lipids (specifically, phospholipids), we found great success. And so, the application of MALDI-time-of-flight (TOF)-MS to the study of lipids and phospholipids has been my major research focus for around 15 years.

We are particularly interested in modifying lipids using oxidation reactions to understand what is possible from a chemical point of view; even with a very simple lipid, the introduction of reactive oxygen species (ROS) can result in many different species. Our ultimate goal is to understand what that means in the context of an organism, among other things.

TLC: tried and tested – and simple

I first used thin-layer chromatography (TLC) many years ago in organic chemistry to monitor the number of products after our numerous chemical reactions. Even nowadays, I believe students of chemistry use TLC in this way. And I suspect many people may consider TLC just as a simple separation technique for chemistry students.

In fact, it is TLC's simplicity – and its low barriers to entry – that make it so attractive in combination with mass spectrometry. In mass spectrometry, you are typically faced with the problem of ion suppression when it comes to complex mixtures – and that means you don't detect all analytes with the same sensitivity. Therefore, some form of separation is desirable if not essential. In lipid analysis, sensitivity in mass spectrometry is determined by the lipid headgroup (that is to say, its charge) and we quickly ran into the effects of ion suppression in complex lipid mixtures. We were not specialists in chromatography, so you might say we chose the simplest option, but in fact, TLC is actually very well established in lipid separations and seemed like the obvious choice.

For us, the main advantage of TLC is the ability to gain high quality separations with unsophisticated equipment (and with little expertise). In fact, all you need is a TLC plate, a sample syringe, and a developing chamber. And yet, despite its simplicity, it allows many samples to be analyzed in parallel, increasing throughput. Suspicious samples are also no problem – I am sure you would not want to inject some of our

samples into your HPLC system... Indeed, the ability to load large sample volumes or highly contaminated samples can be very advantageous in our work.

Ten years ago, we used to separate our phospholipid classes using TLC, and then scratch the corresponding spots off the plate for re-elution ahead of MS analysis. It actually worked well, but you can probably imagine that with large numbers of samples, the process was somewhat tedious. At the same time, researchers were using MALDI-MS to analyze biological tissues, and the parallels between TLC plates and slices of tissue became apparent. Could we not detect our samples directly on the TLC plate? Yes, we could – and we are still doing that today, though there have been improvements along the way.

TLC-MALDI-MS 2.0

Advances in commercial instrumentation are certainly making the combination of TLC-MALDI-MS even more attractive (for example, Bruker Daltronics has produced a TLC-plate adapter than can be inserted directly into the system and software that facilitates analysis). But quieter innovations have been occurring in the actual TLC plates themselves.

Dedicated MS-grade TLC plates that have been optimized for use in MALDI-MS are now commercially available. Such TLC plates use a thinner layer of extremely pure silica (standard plates typically use 200 μm layers, but the MALDI-grade plates use 100 μm silica layers), and we have shown that stationary phase thickness determines the quality of MALDI-MS spectra for lipids (1), which is illustrated in Figure 1. The matrix background is significantly reduced with thinner layers, and though we do not fully understand the reason, I believe the advantage is conferred by an analyte concentration effect and the fact that the UV laser of MALDI-MS does not penetrate deeply into the surface. In any case, if we look at a diluted extract from cells (as we so often do), we will likely see very poor spectral quality with standard plates, but remarkably good spectra with optimized plates.

TLC-MALDI-MS in action

In terms of our own research, we are applying TLC-MALDI-MS in a number of different research areas. For example, we are currently looking at the effect of lipid composition on spermatozoa samples with a view to discovering lipid biomarkers of fertility. Of course, this could have a clinical impact, but it's also of interest in the world of animal breeding, where artificial insemination could benefit from a prediction of sperm quality.

Another big focus area for us is in understanding the role of lipids in the mechanisms of obesity. In particular, we are interested in learning how different compositions of fat in the diet correspond to uptake of fatty acids in fat tissues or other organs. The third field, which I mentioned earlier, is the area of lipid oxidation.

Oxidative stress is well-known terminology in biology, but it is difficult to assess the contributing factors to lipid oxidation in physiology. We began by trying to understand which oxidizing species lead to which products in an isolated system, but we have also started researching the physiological aspects. There are many different enzymes (lipases and phospholipases) and one of our questions is: are oxidized lipids/phospholipids metabolized to the same extent as their native equivalents. Here, we are applying a combination of different mass spectrometric methods, as well as NMR.

In all of these cases, we might begin with a mixture of lipids from a cellular extract (for example, neutrophils or spermatozoa) and use NMR (in particular, phosphorous-31 NMR) to gain quantitative information about the lipid classes. Why NMR and not mass spectrometry? Well, if you are typically running the same samples (for example, blood samples in clinical chemistry), you may already have an idea about the concentrations of expected phospholipids and can therefore use dedicated deuterated standards to obtain quantitative information. However, we deal with many different kinds of samples and cannot easily make such predictions, which makes NMR a very useful analytical technique as it avoids additional time-consuming experimentation but gives reliable quantitative information. However, NMR does not provide detailed information about the fatty acid composition, which is where the use of TLC-MALDI-MS comes to the fore. We can run the same samples and investigate the MS spectra to complete our investigations.

Conversely, with TLC-MALDI-MS it is difficult to gain quantitative information about the compounds separated on the plate. Why? Because the distribution of analytes is not also homogenous across a spot, so the mass spectra produced are dependent on the position of the laser irradiation zone (which is significantly smaller than the TLC spot to offer increased resolution in MS imaging). One possible solution may be the option to adjust the laser spot size depending on the application – but that is a question for the instrument vendors...

What I think our projects emphasize is the fact that to answer complex questions, the use of complementary techniques can be hugely beneficial – and the simplicity and utility of TLC-MALDI-MS fits perfectly in our research. Finally, a book entitled "Planar Chromatography - Mass Spectrometry" (2) will be at available at the end of this year - and really emphasizes the relevance of this scientific field.

References

1. *H Griesinger et al., "Stationary phase thickness determines the quality of thin-layer chromatography/matrix-assisted laser desorption and ionization mass spectra of lipids." Anal Biochem, 451, 45–47 (2014). PMID: 24530848*
2. *T Kowalska, M Sajewicz, J Sherma, "Planar Chromatography – Mass Spectrometry", CRC Press, 2015, ISBN: 9781498705882.*

Landmark Literature: 2015

We ask an impossible question as 2015 comes to a close: which piece of literature stood out from the crowd and showed the greatest potential for pushing the field of analytical science forward?
Here, six experts boldly share their answers.

SFC Star

By Davy Guillarme, Senior Lecturer, School of Pharmaceutical Sciences, University of Geneva/University of Lausanne, Geneva, Switzerland

"Some of the findings in this study were particularly relevant and unexpected."

Let me begin by setting the scene for the paper I've chosen. In the last few years, people are regaining interest in supercritical fluid chromatography (SFC) at the analytical scale. This is largely due to the commercialization of new generation SFC systems. These systems offer enhanced sensitivity, robustness, and quantitative performance. In addition, they present a better compatibility with modern stationary phases, such as those packed with sub-2 μm fully porous particles or sub-3 μm superficially porous particles.

In our laboratory, we have evaluated ultra-high performance SFC systems (UHPSFC) and have found that both reliability and performance has significantly improved. However, the extra-column band broadening observed on such instruments is non-negligible because of the long, wide connection tubes employed and the UV cell, which represents a volume of up to 8 μL. Therefore, it would be very interesting to use short 2.1 mm ID columns – today's standard in ultra-high performance liquid chromatography (UHPLC). Not only would such columns reduce band broadening, but solvent and sample consumption would be reduced, making UHPSFC a much greener strategy than UHPLC. In addition, the mobile phase moves at relatively high linear velocity under UHPSFC conditions, so narrow bore columns would be appropriate to stay within the flow rate range of current instrumentation (upper flow rate limit of 4 to 5 mL/min on UHPSFC systems). Unfortunately, short 2.1 mm ID columns are not well suited to UHPSFC because of the significant loss of efficiency.

Now to my landmark paper for 2015: "Understanding and diminishing the extra-column band broadening effects in supercritical fluid chromatography" – by Ruben De Pauw, Konstantin Shoykhet, Gert Desmet and Ken Broeckhoven. The authors identified and quantified the different contributions to extra-column band broadening, including the influence of sample solvent, injection volume, detector cell volume, and tubing volume. Rather than calculating the extra-column variances for different system geometries, which is not an easy task when using a compressible fluid, the authors decided to evaluate the change in performance by tracking the plate count for a few model compounds with different retention factors. After complete optimization of the UHPSFC instrumentation, the plate count was drastically increased and the optimized system was found to be fully compatible with narrow-bore 2.1 mm ID columns.

Some of the findings in this study were particularly relevant and unexpected. First, the authors have shown that, when using a commercial modern SFC system, minimizing the tubing ID from 250 μm to 170 μm and then to 65 μm was not a good strategy for reducing extra-column variance, since the achieved plate count was not modified on a standard system. These findings contradict what is generally observed in LC, where this parameter is one of the most important for improving peak shapes. The different behavior can be attributed to the higher molecular diffusion coefficients in SFC, leading to a lower contribution of tubing to extra-column band broadening, thus allowing the use of larger ID tubes compared with LC. Secondly, the UV cell volume was reported as an important parameter for minimizing peak broadening, and so using UHPSFC systems with a UV cell volume of <1 μL is highly relevant. However, you need to take care when reducing the UV cell, since turbulent flow conditions are much more common in UHPSFC than UHPLC and can cause an unexpected increase in pressure in the narrow flow path of the low dispersion flow cell. Finally – yet importantly – the authors prove that the most important parameter to achieve suitable peak shapes on narrow bore UHPSFC columns was the nature of the injection solvent and its volume. The sample diluent should indeed match the mobile phase in terms of viscosity, elution strength, and polarity, while the injection volume should be minimized.

In conclusion, this study provides some useful tips to chromatographic instrumentation vendors that should help with improving their UHPSFC systems. I expect the next generation of commercial UHPSFC systems to work optimally with narrow bore 2.1 mm ID columns. For this to happen, UV cell of less 1 μL in volume that withstand pressure up to 400 bar need developing, and we need autosamplers that offer precise injection at very low quantities (< 1 μl). However, reducing tubing ID below 120 μm doesn't appear to be a useful strategy, since it increases the system pressure, with limited benefits in terms of achievable plate count.

Davy's Landmark Paper

R De Pauw et al., "Understanding and diminishing the extra-column band broadening effects in supercritical fluid chromatography", J Chromatogr A, 1403, 132–137 (2015). PMID: 26054561.

The Birth of Data Fusion

By Hans-Gerd Janssen, Science Leader, Unilever Research and Development, The Netherlands

Science is about generating new knowledge and understanding. To do so, we develop hypotheses and then perform experiments to either accept or reject them. The process of hypothesis testing centers around data; indeed, the data are collected with a specific purpose in mind: to test the hypothesis. However, data collected for one purpose can be valuable for numerous other situations, especially given the dramatically improved data-density of our current analytical measurements. And with the improved systems we have for storing and transferring large data sets, we are now ready for recycling, re-using, and reinterpreting data. To allow this, the data must be aligned, which is defined as "made laboratory independent". My choice of landmark analytical paper describes a method to do so (1). More importantly, it expresses and emphasizes this idea of not just using (selected) data for a single purpose, but to store and combine them, and use previously measured data to speed up hypothesis testing, possibly with very different purposes in mind.

> *"The authors describe the use of re-measuring a subset of samples from different studies to obtain transfer models."*

The paper was written by a team of analytical experts from the Netherlands Metabolomics Centre (Leiden) led by Thomas Hankemeier alongside co-workers from several Dutch academic hospitals wrote the paper. And although the work focuses on metabolomics, the recommendations made in the article reach far beyond into other fields. It is a plea for making data amenable to data fusion – merging of data to a seamless data set – and describes the precautions that need to be taken to allow doing so.

Fusing datasets is easy when the data are absolute or quantitative. The analysis of a given sample should give the same absolute concentrations irrespective of where it is measured. But modern instruments deliver much more data than just those for a few target compounds that are normally quantified. The paper examines those additional untargeted data, the much high number of compounds for which only relative, semi-quantitative information is available.

The authors describe the use of re-measuring a subset of samples from different studies to obtain "transfer models". Application of a transfer model then enables matching and integration of semi-quantitative profiling data between different studies and sample sets measured at different times and/or in different laboratories. Alternatives for a possible lack of transfer samples (for instance, due to limited sample volume) are given. Reference samples such as those available from the US National Institute of Standards and Technology (Gaithersburg, Maryland, USA) are a first option, but other solutions are also included.

Ideally, after completing each set of measurements a set of transfer samples is analyzed. This set provides the bridge to a new study, and enables integration of all the studies performed. Reanalysis of approximately 6–7 percent of the total number of samples was found to be optimal for establishing the transfer model, but it is possible to do less.

There is still a long way to go before we can simply look on the Internet for the data we need to test our hypotheses. I sincerely believe, however, that in the future we will not only Google for information, but also for data. Data transfer strategies such as those developed in the paper will help that happen – just like good laboratory practice in putting data on the Internet.

Hans-Gerd's Landmark Paper

AD Dane et al., "Integrating metabolomics profiling measurements across multiple biobanks", Anal Chem, 86, 4110–4114 (2014). PMID: 24650176.

Dimension X

By Martin Gilar, Principal Investigator, Waters Corporation, Milford, Massachusetts, USA

The potential of multidimensional separations has always interested me. Just imagine how many analytical problems would be solved if we could increase the resolution of liquid chromatography (LC) from hundreds to thousands or perhaps a hundred thousand compounds? Two-dimensional (2D)-LC has that potential, which is why groups led by Peter Carr, Dwight Stoll, Peter Schoenmakers, Pavel Jandera, Paola Dugo, and Mark Schure, among others investigated it. Whether the goal was practical or academic, the drive towards multidimensional separations is noticeable. Instrument manufacturers picked up the trend and they now offer systems suitable for targeted or comprehensive 2D-LC.

The paper that caught my eye features several "big hitters" in separation science – Gert Desmet, Sebastiaan Eeltink, and Peter Schoenmakers, with their talented students Bert Wouters, Herman Terryn, and Jelle De Vos – who investigated the concept of spatial 2D-LC as a first step towards the ultimate goal of 3D-LC (1). (Schoenmakers has presented his 3D-LC separation vision at several HPLC conferences.) It isn't a simple concept. For example, if the first dimension separation is performed within an hour, the second dimension separation must be done within tens of seconds, and the third should not exceed seconds. Schoenmakers has proposed a concept of spatial chromatography that overcomes this problem. Similar to thin layer chromatography (TLC), he proposes that the sample components are separated spatially in two dimensions and then resolved in the third dimension using a "separation cube".

The concept of spatial separation evaluated in the paper is a stepping-stone towards a 3D-LC solution. The concept is equally as intriguing as challenging to execute. However, collectively, the authors have the necessary expertise to do it. They first describe a design and construction of a planar chip for the 2D separations. They borrow the expertise for chip building from Desmet's earlier microfluidic work and add to it. Formulating separation media in the chip channels was a problem the authors dealt with by filling the channels with a monomeric mixture to create the monolith stationary phase in-situ. They also had to work out additional technical details such as connection to pumps, isolation of the flow in the first dimension, and the transfer of the analytes to second dimension. They stop at this point without showing any actual 2D separations, but they promise more results in subsequent papers. It remains for us to see how well the 2D-LC chip works with test mixtures and real life samples!

Several problems with the 2D-LC chip need solving. In the current version, both the first and second separation dimension use the same monolithic stationary phase. This means that orthogonal separation selectivity needs inducing by the mobile phase, for example by altering the pH, ion-pairing, or solvent type in both separation dimensions. Also,

"The concept of spatial separation evaluated in the paper is a stepping-stone towards a 3D-LC solution."

the spatial separation concept works well for small molecules in the isocratic separation mode, but may fail for proteins and other (bio)polymer applications. The solution to the problem – gradient elution – does not translate well from time domain to spatial chromatography. In other words, weakly retained peaks will inevitably leave the chip boundary, while the more strongly retained compounds will remain clustered at the 2D-LC chip inlet. Executing a gradient within a single column volume could evenly spread the peaks along the separation plane, but such separations are a tricky proposition.

The authors aim to apply the 2D-LC chip to protein separation (similar to 2D gel electrophoresis). If the 2D-LC chip offers a straightforward interface to mass spectrometry, this is a worthy proposition.

Despite the technical difficulties, this paper describes exciting research. The potential of multi dimensional separations always has interested me and I hope the prospect of a hundred thousand-peak capacity will be possible in the not too distant future.

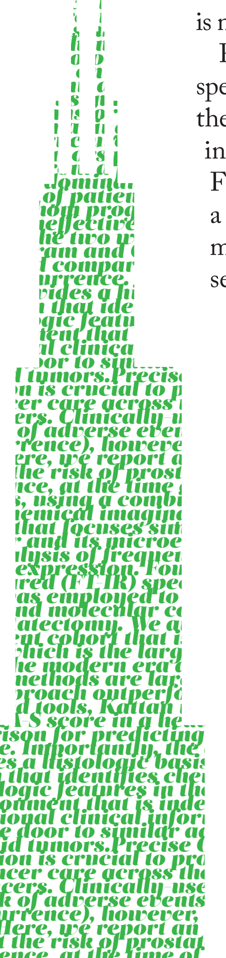
Martin's Landmark Paper

B Wouters et al., "Design of a microfluidic device of comprehensive spatial two-dimensional liquid chromatography", J Sep Sci, 38, 1123–1129 (2015). PMID: 25598051.

Hyperspectral Disease Diagnosis

By Peter Griffiths, Professor of Chemistry Emeritus, University of Idaho, Owner, Griffiths Consulting LLC, Moscow, Idaho, USA.

The diagnosis of cancers at an early stage is critical for the long-term survival of patients. For solid cancers, such as lung, breast and prostate cancer, this is currently accomplished by staining tissue samples with hematoxylin and eosin (H&E) dyes followed by histopathological examination; time to results is typically days rather than hours. Furthermore, diagnoses performed in this way are quite subjective. Indeed, if four histopathologists examine a stained tissue sample, there could be four different diagnoses! Clearly, a technique that is faster, more accurate and less subjective than H&E staining is needed.



For at least two decades, vibrational spectroscopists have attempted to demonstrate the feasibility of using infrared spectroscopy in medical diagnosis. In the early studies, a FT-IR microspectrometer equipped with a single-element detector was used in the mapping mode, where spectra were measured sequentially, with the sample being moved in steps of a few micrometers. Although the results showed promise, the time required to acquire enough spectra to allow tissue samples to be fully classified was too long. Furthermore, an insufficient number of samples were usually tested, so that any results were rarely statistically significant. As a result, optimism for such measurements was not justified.

Hyperspectral imaging achieved by the interface of mercury cadmium telluride array detectors to a standard continuously-scanning FT-IR spectrometer allows thousands of spectra of tissue samples to be measured in a couple of minutes with a spatial resolution of between 1 and 10 µm. The spectrum measured at each pixel can be classified by several different chemometric algorithms (sometimes known as chemical imaging). Several research groups have demonstrated the applicability of such methods in predicting different types of cancer. For example, groups led by Rohit Bhargava at the University of Illinois (USA), Max Diem at Northeastern University (USA) and Nick Stone at Exeter University (UK) have all made remarkable progress in area.

My paper of choice details the results of a very careful collaborative study of the prediction of prostate cancer recurrence by scientists from the US Center for Interventional Oncology at the National Institutes of Health, the Department of Pathology at the University of Illinois at Chicago, and the Computer Science, Bioengineering, and Electrical and Computer Engineering, departments and Cancer Center of the University of Illinois, Urbana-Champaign (1). Their results significantly outperformed those found using the most commonly applied approaches – H&E staining with classification using the Kattan nomogram or the Cancer of the Prostate Risk Assessment (CAPRA-S) score.

> *"My paper of choice details a very careful collaborative study of the prediction of prostate cancer recurrence."*

The paper stands out because of the combination of very high-quality spectroscopy and data processing and the collaboration of scientists from different disciplines. The paper described an approach that, in the words of its abstract "provides a histologic basis to a prediction that identifies chemical and morphological features in the tumor microenvironment that is independent of conventional clinical information, opening the door to similar advances in other solid tumors."

In contrast to magnetic resonance spectroscopy, where the MRI technique was rapidly commercialized and adopted in hospitals worldwide within 10 years of demonstrating its feasibility in the laboratory, the uptake of vibrational spectroscopic techniques for medical diagnosis has been slow. Nonetheless, I believe that, within the next decade, the techniques described in this paper could displace current staining techniques for histopathological analysis – or, at the very least, I expect that they will be used alongside them.

Peter's Landmark Paper

JT Kwak et al., "Improving prediction of prostate cancer recurrence using chemical imaging", Scientific Reports 5, Article number: 8758 (2015). PMID: 25737022.

Practically and Fundamentally Excellent

By Peter Schoenmakers, Education Director COAST; Editor, Journal of Chromatography A; Professor, Analytical Chemistry/ Forensic Science, University of Amsterdam, The Netherlands

A recent paper by Fabrice Gritti, Thomas McDonald and Martin Gilar struck me for several reasons. Firstly, it is an immensely important publication for liquid chromatographers. Such steadfast folk tend to strive for high efficiencies and excellent separations by making or using better columns with very small and uniform particles, perfectly homogeneous monoliths, and so on. But, unfortunately, they forget that long or poorly designed connectors and extra-column apparatus cause them to lose what they aim to gain – a problem the paper attempts to correct. In addition, the paper describes ingenious and original methods. It is a practical liquid chromatography (LC) paper, with theory supporting the experiments that aim to capture the causes of "misdemeanors" by short pieces of connection tubing. The results from the tedious experiments are consistent and rather surprising, the latter being mandatory for an interesting paper.

The paper contains some recommendations that will not surprise you, such as using the narrowest possible tubing and elevated temperatures, and voices a suggestion that we hear with increasing frequency, "Shouldn't we all convert to supercritical-fluid chromatography (SFC)?" When we replace conventional water-acetonitrile mixtures in LC with mixtures of carbon dioxide and methanol for SFC, it is possible to perform faster separations (which we already know) and extra-column band broadening may be less of a threat (which is more remarkable than it seems – see SFC Star). Therefore, it will be interesting to see whether SFC will begin to catch up with LC. SFC has fundamental properties on its side (faster diffusion, lower viscosity), but aqueous-organic mixtures have proven to be fantastic eluents for all kinds of samples.

There is another reason why this paper is important for the entire chromatographic community. Something – or rather somebody – is missing. Fabrice Gritti published more than 200 papers together with Georges Guiochon in just over a decade. We do miss Georges (who passed away in 2014), but his absence is not felt when reading this paper because it contains excellent science – something we need to see more regularly. Indeed, I shall try to motivate my students to embark on these kind of studies. I suggest that you take note of the experiments shown in Figures 2 and 3, which take between a few seconds and six minutes.

There are many experiments in this study (30 or more different flow rates, quintuple injections and several different configurations), but the total net measuring time is just a few days. One instrument could produce a hundred papers per year, which seems like a dream to academicians like me – but a nightmare for the authors' employers! However, it needs a very special kind of instrument. When someone submits a manuscript to Journal of Chromatography A describing the use of "a virtually dispersion-less instrument", we ask the authors to control their fantasies. Unless it is Fabrice Gritti! His approach to science – after serving an exceptional apprenticeship – is virtually flawless.

"Fabrice Gritti's approach to science – after serving an exceptional apprenticeship – is virtually flawless."

Peter's Landmark Paper

F Gritti et al., "Accurate measurement of dispersion data through short and narrow tubes used in very high-pressure liquid chromatography", J Chromatogr A, 1410, pp. 118–128 (2015). PMID: 26255113.

Go with the Flow (Field)

By Pat Sandra, Emeritus Professor, Organic Chemistry, Ghent University; Founder and President, Research Institute for Chromatography, Kortrijk, Belgium.

Selecting "the paper of the year" is by definition subjective and very personal. Moreover, it depends on your own fields of interest in a given period and on the journals that you have consulted. This year, I have read many good publications particularly those on chromatographic and mass spectrometric topics. A couple of papers related to my first love – capillary gas chromatography (CGC) – struck me the most. CGC is a mature technique and, in recent years, the developments have been linked to pre- or post- column hyphenation, for example, automated sample preparation and high-end mass spectrometers, respectively.

Had I been asked to name "the paper of the year" before the 2015 summer holiday period, my answer would have been undoubtedly: "Evaluation of conditions of comprehensive two-dimensional gas chromatography that yield a near-theoretical maximum in peak capacity gain" by Matthew Klee et al. (1). The paper combines a very sound theoretical basis with a high practical impact for GC×GC users. However, in August (with a gin and tonic in my hand… I'm an emeritus professor after all), I read: "Flow field thermal gradient gas chromatography" by Peter Boeker and Jan Leppert. As is typical for me when reading articles, my attention was drawn by the chromatograms with peak capacities over 200 in less than one minute.

For more than 60 years, analytical scientists have known that a negative temperature gradient in the direction of the gaseous mobile phase can offer interesting features. The suppression of chromatographic dilution or solute focusing – resulting in increased sensitivity for trace analysis – was mentioned by Schuchowitzkij and Turbeltaub in 1957 (2, the original publication was in Russian and also featured a book: "Some Developments in Gas Chromatography in the USSR", Butterworths, London, 1960). Over the years, several well-known researchers were active in this field; John Phillips (the inventor of GC×GC) and Leonid Blumberg (the "Giddings" of CGC theory), for example, and it's quite interesting to read their controversial discussions. More recently, the Lee group (Brigham Young University, Provo, Utah, USA) developed different technical approaches to perform negative thermal gradient CGC and, through many lectures at international symposia, rekindled interest in the subject.

The ingenuity of Boeker and Leppert's concept lies in correctly estimating the importance of heat loss due to convection. Flow field thermal gradient gas chromatography (FF-TG-GC) is based on the precise control of the convective heat loss from a heated capillary. They demonstrated their concept by producing a temperature gradient using a cooling gradient flow field generated by a pressure drop inside a tube with a helical channel (in which the separation column is positioned and resistively heated) packed with porous material. The pressurized air in the tube flows through the porous material and exits through the helical channel. The flow decreases as the flow path increases, resulting in a smooth decreasing flow from the bottom (detector side) to the top (injector side). This results in a negative temperature gradient with a direction from injector to detector, which can be adjusted by varying the gradient fan. Without activating the gradient fan, a conventional temperature programmed analysis is obtained. For readers interested in the fundamentals we refer to the original paper (2). Now, I'd like to emphasize some features and future perspectives of FF-TG-GC.

The first observation is that all separations shown, with or without off-TG, are excellent and illustrate clearly the present state-of-the-art in CGC. Peak capacities around 200, with peak production rates (peak/min) of ca. 400 (heating rate 70°C/s) are obtained with the set-up described in the article. The gain in peak capacity and in peak sharpness (focusing) with FF-TG is only around 5 percent, but the same solute elutes at a substantially lower temperature in FF-TG. As an example, for C28 the reduction is 45°C or when translated to C24 and C30 the solutes elute at the same temperature without FF-TG and with FF-TG, respectively. This offers possibilities for high-temperature GC (for example,

"The ingenuity of Boeker and Leppert's concept lies in correctly estimating the importance of heat loss due to convection. Flow field thermal gradient gas chromatography (FF-TG-GC) is based on the precise control of the convective heat loss from a heated capillary."

simulated distillation) or separation at much lower temperatures for thermolabile compounds. In the paper, this is shown for explosives and natural pyrethrins. The authors mention that future improvements will include using hydrogen as the carrier gas, working in the constant flow mode, injection via a programmed temperature vaporizer, and so on. Application as second dimension column in GC×GC is another possibility. In addition, I can see features for sequential GC or continuous heart-cutting GC. This should create peak capacities that are impossible to obtain in conventional GC×GC! Taking the optimized, first dimension column (60 m x 0.25 mm), and temperature programming time (80 min) described by Klee (1), together with 30s sequential heart-cutting using a FF-TG set-up with high peak production rate, it should give a much higher total peak capacity compared with the 4500 in optimized GC×GC – and in the same amount of time. I think it is very worthwhile following this development.

Pat's Landmark Paper

P Boeker and J Leppert, "Flow field thermal gradient gas chromatography", Anal Chem 87, 9033–9041, (2015). PMID: 26235451.

References

1. MS Klee et al., "Evaluation of conditions of comprehensive two-dimensional gas chromatography that yield a near-theoretical maximum in peak capacity gain", J Chromatogr A, 1383, 151–159 (2015). PMID: 25627969.
2. AA Schuchowitzkij and NM Turkeltaub, Dokl. Akad. Nauk SSSR116, 986 (1957).

The modern evaporator to make you smile

The **Genevac EZ-2 personal solvent evaporator**, the first of its kind and market leader ever since, provides high performance and versatility to cover the widest range of **applications***.

- **Pre-programmed methods save you time**
- **Dri-Pure bumping protection ensures sample integrity**
- **Unsupervised operation including auto-stop increases productivity**

Simply load your samples, set, start and walk away…
…you couldn't be happier.

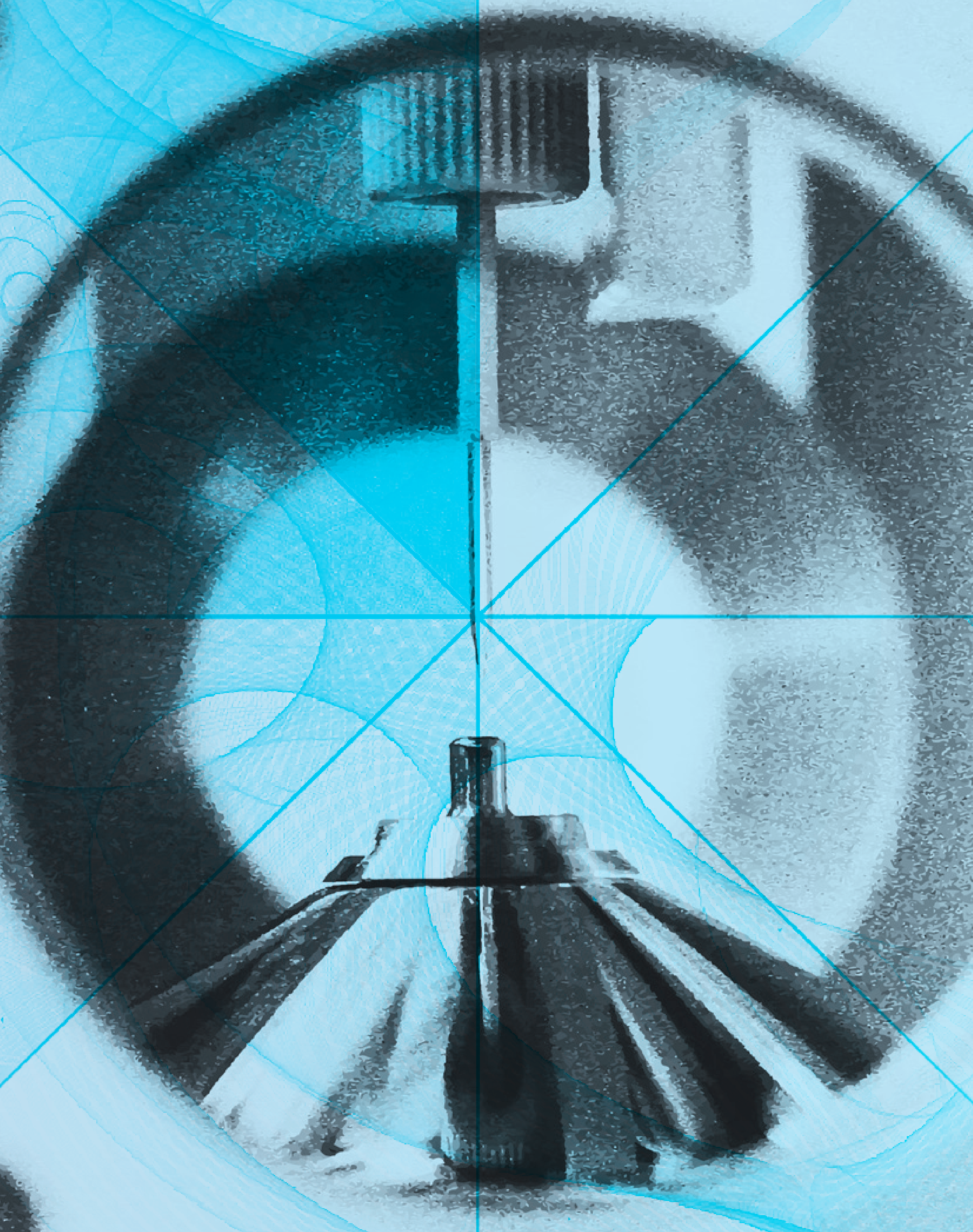
www.spscientific.com/Genevac

Genevac
SP SCIENTIFIC
Making Time for Science

* Applications

* Further information

Genevac Ltd, Farthing Road, Ipswich, Suffolk, IP1 5AP, UK
Tel: +44 (0)1473 240000 Email: salesinfo@genevac.co.uk



Gurus of CE-MS

Capillary electrophoresis-mass spectrometry (CE-MS) appears to be caught in a dichotomy – proven success in some fields, skepticism in others. Here, Norman (Norm) Dovichi, Michael Knierman, Rob Haselberg, Tomoyoshi Soga, and Christian Wenz discuss the road ahead for a hyphenated technique that appears to be on the up.

Where does capillary electrophoresis (CE) stand today?

Norm Dovichi: CE has had one overwhelming success – the sequencing of the human genome (1)! And though I believe the technique has matured in terms of theory and technology, detector sensitivity continues to be its main weakness.

Michael Knierman: For the biopharmaceutical industry, CE is an excellent substitute for polyacrylamide gel electrophoresis (PAGE) based techniques in protein size and isoelectric point determinations. Such applications are robust and are available in kits from vendors. But despite the many publications on coatings and separation methods for CE analysis, it is still not widely used by analytical laboratories.

Rob Haselberg: It is well established in many fields: Norm has already mentioned genomics, for example, and then there's biopharmaceutical characterization (see Figure 1). CE is also established in glycan and ion analysis, and chiral separations. In the early days of CE, Stellan Hjertén and later Jim Jorgenson played an important role in its development. Since then, many other people have contributed to its success – Dick Smith, Barry Karger, Andras Guttman, Norm (of course), and Jonathan Sweedler to name but a few. They're all responsible for pushing the technology into different fields. The main challenge for CE has (unfairly) been the high expectations set in 1980s and 1990s, which it didn't live up to; today, the technology still struggles to shake off the negativity because of those early failings.

Tomoyoshi Soga: Back in the late 1990s, I worked as an application chemist at Hewlett-Packard where I developed several CE applications, such as a simultaneous anion analysis method. Many Japanese electroplating bath companies bought CE systems and kits for quality control. Also, food companies used the method. Nevertheless, sensitivity, identification capability and migration time stability were – and remain – problems for the technology.

Christian Wenz: CE is now vital in biopharmaceutical R&D and production. Over recent years, methods such as CZE (capillary zone electrophoresis), cIEF (capillary isoelectric focusing), CE-SDS (CE- sodium dodecyl sulfate) have been adopted as specification methods for the majority of newly marketed biotechnology protein products. Many biopharmaceutical companies – of all sizes – have implemented these methods. Regulatory agencies have also recognized the value of CE methods and now encourage the shift from slab gel-based methods to CE. As well for other high molecular weight biomolecules like DNA or RNA, CE (using capillaries or microchips) is now a routine method, for example for sample quality control in next generation sequencing workflows. For the analysis of low-molecular-weight pharmaceuticals, CE has evolved into a prime separation tool; for example, for separating enantiomers, many other ionizable molecules of high biological relevance, and for ion analysis. Implementing robust methodologies, using rigorous protocols for capillary preparation and pre-conditioning, and automatic buffer replenishment has played an important part in developing CE. And, of course, conferences have helped educate people about its utility, encourage its use, and drive development. Close to maturity is UV-Vis detection in CE, which can achieve sensitivities close to theoretical limits and provide a large linear dynamic range by using extended light path capillaries or Z-shaped flow detection cells. However, CE is inherently a micro-separation technique that can't be scaled up to larger dimensions.

Michael Knierman

Michael is currently a senior research scientist with Eli Lilly and Co, Indianapolis, Indiana, USA). His laboratory works on top down proteomics with high resolution mass spectrometry, informatics for proteomics, identification of protein modifications, protein chemistry, and biomarker, and drug target discovery and validation. He currently has three CE-MS systems in his lab and is working on improvements for the technology.

Norman (Norm) Dovichi

Norm is the Grace-Rupley Professor of Chemistry and Biochemistry, at the University of Notre Dame, Indiana, USA. His postdoctoral fellowship at Los Alamos Scientific Lab introduced the concept of single molecule detection, leading to the development of a capillary array DNA sequencer that became the workhorse tool used in the human genome project.

Rob Haselberg

"Although I apply several other technologies in my research, CE-MS is my main approach. It often provides information that cannot be obtained with any other technology," says Rob, senior post-doctoral researcher at the VU University Amsterdam, where his research is directed at characterizing biomacromolecular compounds. CE-MS has undergone positive development over the last few years, he explains. "CE-MS was mainly used by academics, but today I am in regular contact with companies that are starting to use (or are planning to use) CE-MS in their laboratories. I am able to share my knowledge with them and in return I get a good insight of the research questions that drive them."

Tomoyoshi Soga

Tomoyoshi majored in applied chemistry and graduated from Keio University, Tokyo, Japan. He then spent 17 years as an application chemist for HPLC and CE at Yokogawa Corp. and Yokogawa Analytical Systems Inc. He is a pioneer in the development of CE-MS based metabolome analysis technologies. His research interests include development of CE-MS methods for metabolome analysis, understanding underlying mechanisms for the regulations of cancer cell metabolism, and biomarker discovery.

Christian Wenz

Christian is an R&D scientist for Agilent Technologies in Waldbronn, Germany. He joined Agilent Technologies in 2002, where he became heavily involved in instrument and application development for automated electrophoresis systems (for example, the 2100 Bioanalyzer and the 2200 TapeStation). Since 2009, he has worked with the 7100 CE system and has authored several application notes and other technical literature for the instrument.

What does hyphenation with MS add to CE?

RH: As with any separation technology hyphenated to MS, CE and MS is an extremely strong combination. Separation of mixtures decreases complexity prior to detection, so more (mainly low-abundant) compounds can be detected, and the coverage of sample constituents is much higher. CE is an orthogonal technique (compared with LC) when it comes to its separation mechanism. Different compound classes or types of modifications can, therefore, be separated, which will provide other important insights. Moreover, MS can provide the identification, sensitivity and selectivity often needed in contemporary analysis.

ND: Mass spectrometry coupled with CZE (note the Z) is now generating interesting applications in both proteomic and metabolomics analysis. These applications arise from improved interfaces and mass spectrometers, which are producing quite high detection sensitivity.

MK: Today, CE is in a similar place to where HPLC was before it was linked to a mass spectrometer, where retention time was the only measure of peak identity compared to a standard. When an HPLC system was eventually connected to a mass spectrometer, peak identity was determined by retention time, mass, and fragmentation. Crucially, the sensitivity of modern mass spectrometers can overcome the main limitation of low injection volumes on CE separation, allowing for sufficient MS signal acquisition. Now, CE peaks can have a true physical mass measurement and an additional orthogonal separation for coeluting species when connected to a mass spectrometer.

CW: Hyphenation enables compound confirmation and identification with high specificity. CE-MS has proved to be excellent for protein and peptides (for example, hydrophilic peptides and glycopeptides). Also, native and intact proteins, or conjugates, can be analyzed with virtually no upper molecular weight size limit, a task that is difficult to accomplish with LC-MS.

How important is CE-MS in research?

RH: In research, CE-MS is definitely an established technology. I am aware of labs, such as those of Tomoyoshi and Harald Mischak, where CE-MS is routinely used in bioanalysis of metabolites and peptides. Clearly, this is possible when proper training is provided. In other industries, it is used increasingly in R&D. However, there is not much use of MS in general by QC-type laboratories – those people prefer optical detection. Therefore, it is not surprising that in a QC environment you won't see much use of CE-MS.

TS: In 2003, I first developed a metabolome analysis method based on CE-MS, which enabled the simultaneous analysis of several thousand charged metabolites by cationic and anionic methods (for examples, see Figure 2, 3 and 4). Since then, I believe that CE-MS has become one of the standard methods in bioscience research.

MK: Currently, research laboratories are working out the sample handling and robustness issues. And, it is in use in metabolomics laboratories. However, it is a challenge to find CE methods that are compatible with mass spectrometry. I don't think that CE-MS is a mature standard platform for analysis, but it is an area that is ready for growth as current challenges are overcome.

ND: CZE-MS is awaking from a 20-year slumber and it is attracting modest interest from the research community. Its main applications are found in biopharma for analyzing recombinant therapeutics and characterization of glycans. Secondary applications may come in clinical analysis of peptides.

CW: Besides GC-MS and LC-MS, CE-MS is a key technology in metabolomics. And in proteomics, it is not only a complementary technique, it is also a true alternative to complex nano LC-MS. For biopharmaceuticals, the determination of charge heterogeneity and glycosylation by CE-MS has the highest potential and it is getting a lot of use for those kinds of applications.

What opportunities are there for CE-MS in industry?

TS: I think there are many opportunities. For example, I founded a bio-venture, Human Metabolome Technologies (HMT), which utilizes CE-MS extensively and has been listed on the stock market since 2013. We perform contract metabolomics analysis for university and industry customers.

ND: The biopharma industry is beginning to pay attention to CZE. This interest is driven by regulatory agencies, who demand careful understanding of the structure of recombinant therapeutics and identification of host-cell (and other) impurities present in those therapeutics.

RH: I think industry is beginning to recognize the benefits of CE-MS. For example, many companies are interested in having CE-MS available in their research labs – mainly those involved in protein analysis, as CE can provide separations that are not easily achieved with any other technology.

MK: I would say there's an opportunity for developing applications with the increase in sensitivity and robustness of new CE-MS interfaces – and I think industry is ready to accept it if it proves to be robust and commercially available. However, it must perform better than other existing methods if it is to gain wider acceptance.

CW: Equipment suppliers should look more into providing solutions rather than what users perceive to be black box technologies and methods. Another opportunity is electrokinetic sample preparation methods in CE as opposed to SPE in HPLC, which should be interesting, for example for the food industry.

Which application areas provide the best opportunities for CE-MS?

RH: CE-MS is strong in areas where other technologies do not provide optimal separations. Protein analysis is something that can be done with CE-MS quite easily these days. Also, the analysis of small polar compounds is easier with CE-MS compared with other methodologies. It is in these areas where investing in CE-MS is well worth doing.

MK: CE is an easy way to separate proteins and analyze them in a mass spectrometer. So, intact protein analysis, top down proteomics applications and immunoprecipitation MS-based assays will benefit from it. It is the methods for CE-MS that deliver the superior separation ability that will drive the applications.

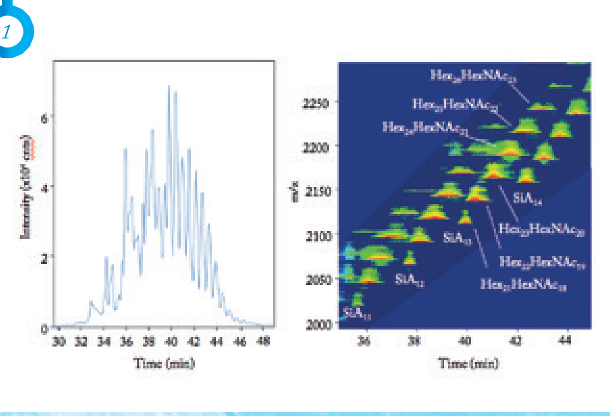
CW: The highest growth potential is in the biopharmaceutical industry, metabolomics, and proteomics.

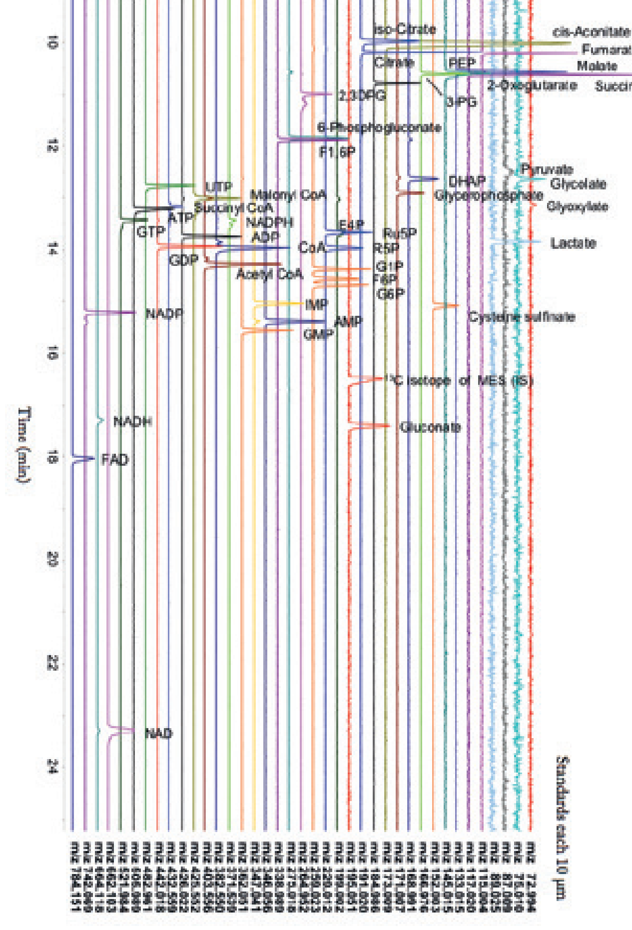
ND: Again, CZE is well suited to characterization of recombinant therapeutics. And there have been interesting applications of CZE for characterization of urinary and spinal fluid peptidomes in clinical studies.

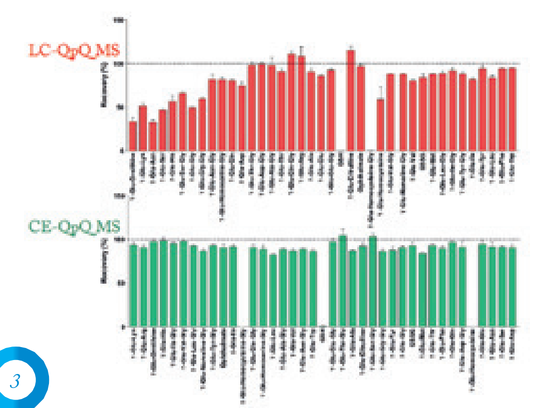
TS: Compounds that are low molecular weight charged species are well suited to CE-MS analysis and ideal application areas are bioscience, clinical, food and fermentation.

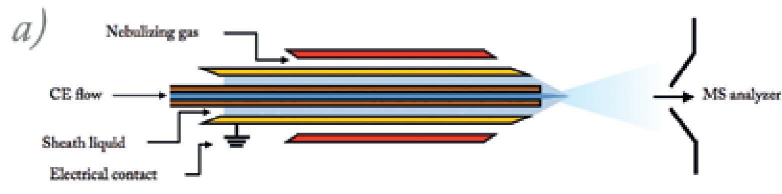
Do you expect CE-MS to breakthrough into routine and high-throughput applications soon?

MK: Yes, I do. The critical parameters of stability, sensitivity, ease of use, and robustness needed are being addressed so that it is finding its place; for example, CE-MS has changed the way our laboratory approaches protein analysis, because of the speed, low sample requirements, sensitivity, minimal carryover, and robustness. But commercial development is critical, as most users are not going to develop their own systems.









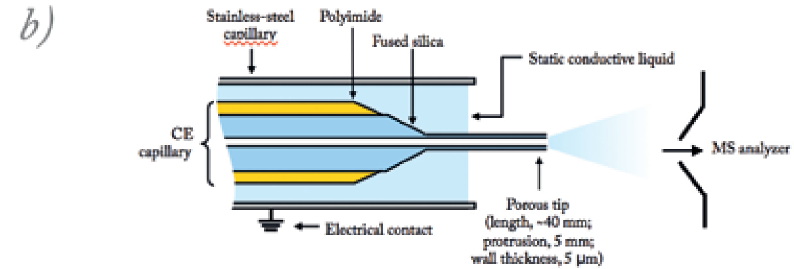


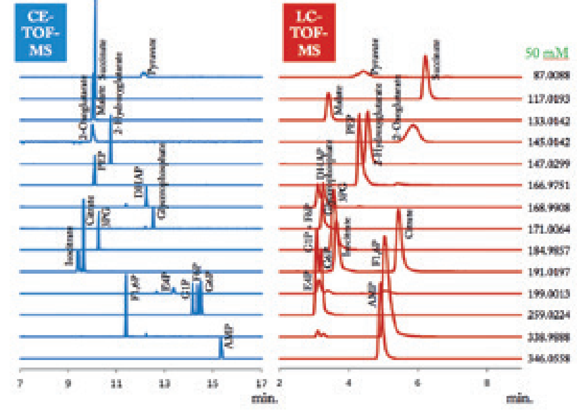
Figure 1. CE-MS analysis of human recombinant erythropoietin. Analysis of this glycoprotein results in a 20-min separation window (left panel) in which more than 250 protein isoforms could be identified. Notably, this is considered a pure protein! In the right panel, the elegant separation of CE is shown. Differences in charged residues (sialic acids, in this case) result in a significant time shift, whereas the addition of neutral residues (HexHexNAc units) yield an additional shift in migration time (4)

Figure 2. Analysis of anionic metabolites by CE-TOF-MS (2)

Figure 3. Ion suppression effect in LC-MS and CE-MS in the analysis of oligopeptides in human serum in a spike and recovery test (3).

Figure 4. Separations of anionic metabolites by CE-MS and LC-MS.

Figure 5. Schematic representation of the sheath liquid (A) and porous tip sheathless (B) CE-MS interfaces.



4

ND: There are three issues. First, the review community at the US National Institutes of Health (NIH) is loath to support research on CZE-MS. Until the community supports investment in the field, progress will be slow. Second, the instrumentation industry is conservative in its investment in the field. Third (and related to the first), the pitiful investment in academic CZE research is leading to a very small base of trained researchers. As a result, the pharmaceutical industry finds it difficult to recruit scientists to develop high-throughput applications.

MK: Commercialized advances, awareness of CE advantages, robust CE capillary coatings, and reliability have hindered its acceptance. Current sample volume requirements on commercial instruments must improve for sensitive analysis too. If the disparity in vial volume to loading amount onto the CE is resolved, together with sample preparation and handling of small volumes, it should lead to a substantial improvement in sensitivity for CE-MS. Sample preparation methods also need to be developed in parallel to instrument development.

CW: CE will continue to evolve because there is a constant and sincere drive to meet unmet analytical needs especially in R&D and production of new biopharmaceutical drugs. This is where CE-MS will have a lot of impact.

TS: In Japan, CE-MS based metabolomics is used widely not only in universities but in various types of industries. The greatest advantage of CE-MS is high quantification accuracy and it is hardly affected by matrix effects, such as ion suppression and enhancement, unlike LC-MS. In fact, if CE-MS sensitivity is improved, it might replace LC-MS!

RH: In some cases, CE-MS is already used routinely and it often provides faster separations than other methods. In protein characterization (this includes intact protein analysis, peptide mapping and glycan profiling, for example), I think it will become a more routine method, adding more selectivity to the analytical toolbox.

ND: This is an interesting question. I suspect that CZE will find a role in routine characterization of recombinant therapeutics within the next five years.

What do you think held it back in the past?

CW: The basis for CE method development is a good understanding of CE separation fundamentals as well as knowledge of substance properties and previous experience – and this lack of knowledge has presented obstacles. Today's students are not trained extensively in separation science (both for HPLC and electrophoresis), which hampers the knowledge transfer from academia to industry. In addition, more officially approved CE methods (United States Pharmacopeia, European Pharmacopoeia, US Food and Drug Administration, etc.,) would be very helpful for a wider distribution of this technology.

RH: For a long time, the lack of reproducibility has been an issue that has stigmatized CE-MS. However, I think that many recent studies prove reproducibility is not an issue. In addition, the lack of good commercial solutions for interfacing CE and MS has also contributed to the negativity, but this is also changing.

TS: Sensitivity, stability in analysis and correction software for migration time shift have all contributed to slow uptake.

What about the CE-MS interface?

RH: I am aware of two commercial approaches for CE-MS interfacing (see Figure 5). The Agilent interface has been on the market for about 20 years now and definitely is considered a robust way of interfacing. Unfortunately, it is only compatible with Agilent (Santa Clara, California, USA) and Bruker (Bremen, Germany) MS systems. Beckman Coulter (Brea, California, USA [now Sciex Separations]) recently introduced a new interface that is compatible with more MS brands. In addition, it shows improved sensitivity compared with others, but as it is new it needs to build a track record – and I am confident that it will. In addition, it is nice to see that academia is showing an increased interest in interfacing. For example, Norm and Dick Smith are both developing improved CE-MS interfaces and showing their applicability. Let's hope we see more commercial solutions in the future. In my opinion, every MS vendor should have a suitable CE-MS solution – it would make the market more competitive and the technology would benefit.

CE(-MS) Through the Years

Rob Haselberg highlights the milestones that have led to a renewed interest in CE-MS.

1930 Tiselius describes moving boundary electrophoresis.

1948 Tiselius receives Nobel Prize in Chemistry.

1960-1980 Hjerten, Virtanen, and Mikkers et al. perform narrow-bore electrophoresis experiments.

1981 Jorgenson and Lukacs perform first experiments in ≤100 μm ID capillaries. Modern-day CE is born.

1987-1988 Richard D. Smith publishes first papers on CE-ESI-MS.

1988 First commercial CE instrument is marketed.

1991 First commercial CE instrument is coupled to ESI-MS via a sheath liquid interface

1995 Sheath liquid interface is commercialized by Hewlett Packard (now Agilent Technologies).

1996 CE-MS makes it into Science for attomole protein characterization.

1990s–2000s Several CE-MS interface designs are introduced, briefly applied and subsequently dismissed – often due to lack of long-term stability Simultaneously, several fields of application of CE-MS are further explored.

2007 Moini shows potential of porous tip sheathless CE-MS interfacing.

2010–today Renewed interest in CE-MS interfacing. Amongst others, Chen, Dovichi and Smith introduce improved interfacing technologies.

2014 Porous tip sheathless interface is commercialized by Sciex Separations.

2015 First interlaboratory study (a collaboration between academia and industry) on CE-MS is published.

MK: The sheathless capillary electrophoresis-electrospray ionization (CESI) interface is a promising solution for high sensitivity analysis but it is integral to the capillary. Also, the triple tube interface is stable, easy to setup, accepts any capillary, but is not as sensitive as other interfaces. However, the nanospray electroosmotic flow (EOF) driven sheath interface appears robust, sensitive and accepts any capillary.

ND: My group has developed a very sensitive and robust CZE-nanospray interface and licensed it to CMP Scientific (New York, USA), a small startup that collaborates with Prince Technologies (Emmen, The Netherlands) and others for distribution. The interface appears to be successful in a number of applications.

TS: Most vendors – except for Agilent and Bruker – do not earth (ground) their CE-MS probes, which can cause extrusion of injected samples from the inlet capillary in both cationic and anionic modes. Today, a CE-MS sheath flow interface is commonly used, which enables very stable CE-MS analysis, but with decreased sensitivity. It's also a complicated system (you need both a sheath liquid and a pump). I think that the next generation of this technology will be sheathless CE-MS, which may much improve sensitivity.

CW: Today, commercially available sheath liquid interfaces for CE-MS are robust, versatile and have sub-ppm detection limits. Standard dimension capillaries (off the roll) can be used with this technology. Commercially available sheathless interfaces, on the other hand, provide high sensitivity but these need non-standard, dedicated, and very expensive capillaries. We still need a CE-MS interface that combines high sensitivity with versatility and robustness at reasonable cost.

Where will CE-MS be in five years from now?

MK: To drive it forward over the next five years, we need more people working with CE-MS. It already has its place in intact proteins, protein complexes, and complex protein separations; the separation of intact proteins has driven me to look into the technique. I think that the issues of stability, sensitivity, ease of use, and robustness are being overcome, so they are much less of a problem and obstacle for the technique. The speed and low sample consumption of CE-MS analysis will increase uptake and I think cycle times of five minutes between injections are achievable. But in five years, don't expect that CE peaks will be identified just by migration time.

ND: As the famous "philosopher" Yogi Berra once said, "It

is tough to make predictions, especially about the future." If investments are available, then CZE will find increasing application in industry. It is reasonable to expect CZE to find routine use in characterization of recombinant therapeutics, and to find growing acceptance in both top-down and bottom-up proteomic analysis.

TS: Let's see more availability and use of sheathless CE-MS to improve sensitivity – and we need to develop (and make available) very stable and easy-to-use methods.

CW: I'm convinced that in the near future CE-MS will play a much more prominent role in many application areas. However, this technology will have its greatest impact on the characterization of biopharmaceutical drugs – an area where already today CE has a strong position.

RH: Good question! The current momentum is definitely in favor of CE-MS. So, the analytical chemistry community should keep it going. Obviously, we shouldn't put unrealistic expectations on the technique as then it may result in disappointment (again). It has proven, and will continue to prove, very useful for certain applications and it should be used accordingly. If we do that, I think CE-MS will finally become the routine technique I already know it can be.

Reference

1. *N J Dovichi and J Zhang, "How capillary electrophoresis sequenced the human genome", Angew Chem Int Ed Engl., Dec 15, 39(24), 4463–4468 (2000). PMID: 11169637.*
2. *T Soga et al., "Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption", J Biol Chem, 281(24), 16768–16776 (2006). PMID: 16608839.*
3. *A Hirayama et al., "Development of quantitative method for determination of γ-glutamyl peptides by capillary electrophoresis tandem mass spectrometry: an efficient approach avoiding matrix effect", J Chromatogr A, 1369, 161–169 (2014). PMID: 25441083.)*
4. *R Haselberg, GJ de Jong and GW Somsen, "Low-flow sheathless capillary electrophoresis-mass spectrometry for sensitive glycoform profiling of intact pharmaceutical proteins", Anal Chem, 85, 2289–2296 (2013).*

The Never-Ending Challenge of Pesticide Analysis

A growing target list, increasingly complex matrices, and the need for low limits of detection can make our field seem like an uphill treadmill. Here, I share my thoughts on some of the major challenges – and consider how new technology might help us push through the pain barrier.

By Kate Mastovska, Associate Scientific Director, Nutritional Chemistry and Food Safety, Covance Laboratories, Madison, WI, USA.

Earlier this year, I had the pleasure of delivering the plenary lecture at the 1st International Symposium on Recent Developments in Pesticide Analysis in Prague, Czech Republic (watch the presentation online at: http://tas.txp.to/1115/Mastovska).

I wanted to provoke discussion, and so decided on a bold (perhaps even intimidating) title: "New and Never-Ending Challenges for Pesticide Routine Testing Laboratories." Why do the challenges feel never-ending? Firstly, pesticide residue analysis must constantly react to three (ever-changing) compounding factors: large numbers of analytes, low limits of detection, and a diversity of matrices.

Moreover, the increasingly global nature of trade in the food industry adds to the mix. Wider sourcing of raw materials (and distribution of products), unknown pesticide use in certain regions, and different regional regulatory landscapes all add extra complexity and scope. At Covance, we are well aware of the global nature of the challenge and are focused on global harmonization. That means using the same robust methods, the same SOPs and quality systems – even the same laboratory information management systems – across the company, which is no mean feat.

From a regulatory point of view, even more challenges emerge. We know that there are different maximum residue limits and different compounds in use around the world, but pesticide residue analysis is more than just meeting the appropriate regional regulations. Global companies – and our clients – are increasingly interested in measuring everything, in everything, from everywhere – setting global specifications based on the strictest requirements in each case. Our target lists are growing...

"Sample number 1037593: dark green, sticky, strange scent."

For regulatory and contract labs, strange (and sometimes unknown matrices) are a regular occurrence – especially when it comes to botanicals and other supplements. And though analyzing an unknown sample for (known or unknown) pesticides is clearly an extreme case, it does highlight a challenge that will not go away: the matrix. Perhaps more importantly, it also highlights a trend; gone are the days when cereals, fruits and vegetables were the mainstay of analysis. The matrix challenge appears to be an ever-increasing circle that began with produce, grains and oils, and then expanded to include specialized matrices, such as spices, tea, cocoa, and so on. Today, the circle has grown bigger still, with herbal drug mixtures, dietary supplements... The list continues – as does the complexity.

Maintaining quality in the mayhem

In our labs, we use the SANCO guidelines for pesticides analysis both for validation and routine quality control as a minimum. The importance of quality control, particularly for difficult matrices, cannot be understated. In these difficult matrices, quantitation accuracy can represent a significant challenge, because unknown matrix effects can potentially affect sample preparation (recovery) and quantification (signal suppression/enhancement).

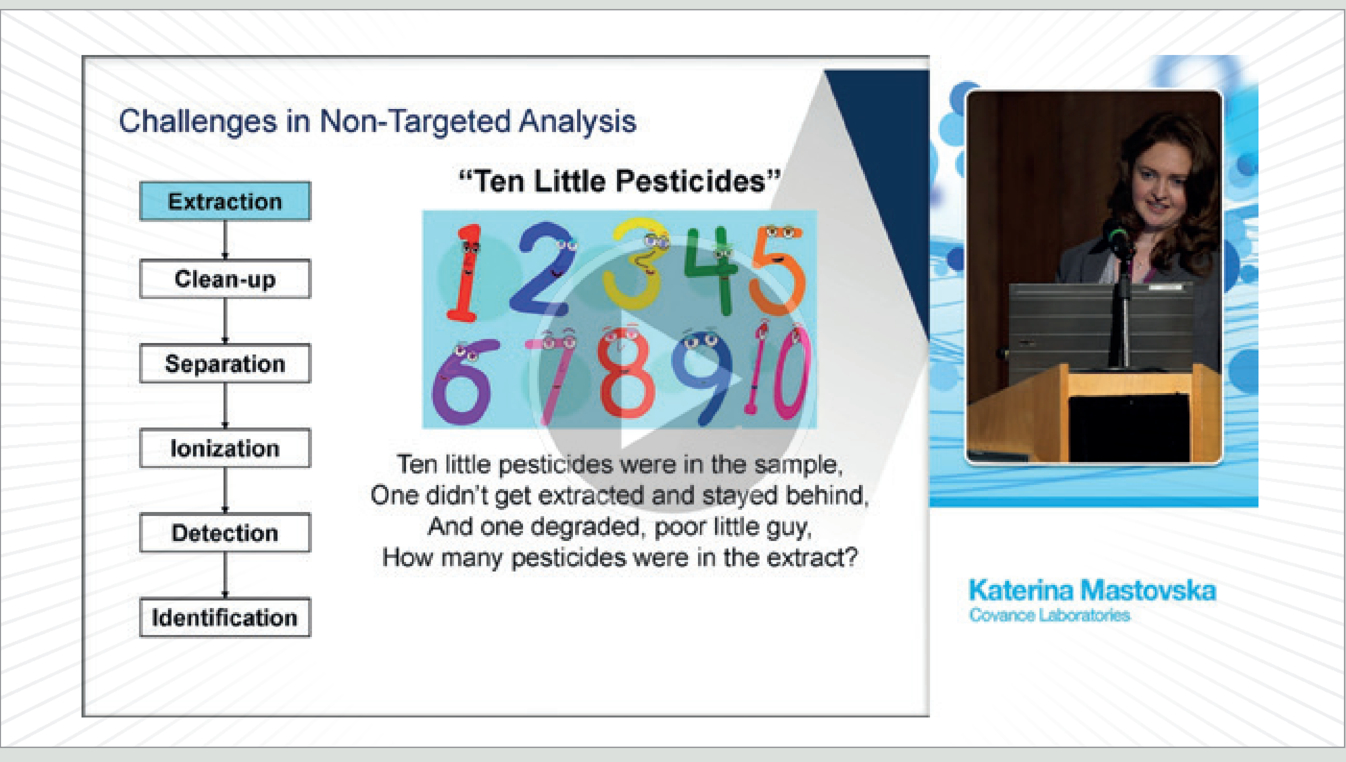
Clearly, in all walks of analytical life, identification of contaminants is of paramount importance. Just the presence of certain unexpected contaminants could have huge economic implications (and actually make quantification unnecessary in some cases). Conversely, the quantification of a wrongly-identified compound is entirely pointless.

In short, we need very high confidence in our results. For identification with MS/MS, SANCO/12571/2013 states that the minimum should be:

- ≥ 2 product ions
- ± 30 percent maximum relative tolerance for ion ratios.

But are we satisfied with minimum confidence? Notably, improved selectivity and identification confidence can be gained by developing methods that fully exploit the significant analyte overlap between GC-MS/MS and LC-MS/MS, using orthogonal selectivity as a means of confirmation. Another way of improving confidence in challenging matrices is by developing methods that closely evaluate multiple MS/MS transitions – not just the ones that offer sensitivity, but rather those that confer better selectivity.

Last but not least, the use of high-



Watch Kate Mastovska's presentation at the 1st International Symposium on Recent Developments in Pesticide Analysis in Prague, Czech Republic: http://tas.txp.to/1115/Mastovska)

resolution accurate-mass (HRAM)-MS instruments, such as the Q Exactive™ systems, can increase confidence in compound identification by providing additional accurate mass information and thus increasing selectivity. And though right now we don't use such technology routinely for pesticide analysis, in difficult cases (where other techniques have failed to give us the confidence we need), we have found the selectivity of HRAM-MS analysis very useful. In other applications areas, for example, non-targeted analysis of adulterants, full-scan, accurate-mass, high-resolution data really comes into its own.

When we consider our ever-expanding list of compounds in our target list (right now, we are currently validating a method that looks at over 500 compounds), the ability of HRAM-MS systems to perform non-targeted analysis starts to look increasingly attractive.

What do targeted and non-targeted really mean?

There appears to be a slight lack of consensus on the meaning of targeted and non-targeted – at least in my experience. From a holistic standpoint, you can consider the difference as two simple questions:

- Targeted: is compound X in the sample?
- Non-targeted: what is in the sample?

The reality is, of course, much more complex – and I believe that it is important to consider both data acquisition and data processing. If you are using analyte-specific conditions, then your data acquisition is targeted (for example, multiple/single reaction monitoring, selected ion monitoring). If not, you are acquiring data through non-targeted means (for example, full-scan MS, all-ion fragmentation, data-independent MS/MS). However, when it comes to data processing, the complexity increases; after all, can't we process non-targeted data in a very targeted way? At this point, Rumsfeldian analogies are inevitable:

- Known knowns: targeted processing of targeted – or non-targeted – acquisition data, using analyte-specific conditions (retention time, MRM or selected ions) in the data processing method created with reference standards.
- Known unknowns: (non-)targeted processing of non-targeted acquisition data, using database/library search (fragment match, structure correlation, accurate mass) to get presumptive identification.
- Unknown unknowns: non-targeted

The never-ending challenges in pesticide analysis are driven by a growing target list and a growing number of increasingly complex matrices.

processing of non-targeted acquisition data, using chemometric (differential or statistical) analysis, followed by identification of compounds of interest. A little like trying to find a needle in the haystack.

The realities of non-targeted analysis

Having defined non-targeted analysis, we are now in a position to consider the challenges, which I hinted at earlier with the term "analyte-specific conditions." When we think about non-targeted analysis, we typically focus on the mass spectrometry aspect. But in my presentation in Prague, I told the sad (but poetic) story of "Ten Little Pesticides," where only one lonely pesticide was identified in non-targeted analysis. My point was: how do we know that all analytes of interest even make it to the data processing step? In other words, all steps of the analytical workflow (extraction, cleanup, separation, ionization, detection, identification) could lead to loss of analytes of interest. The real challenge here? Optimizing non-targeted methods and establishing adequate quality control for those methods.

Despite that warning about non-targeted approaches, let us not be too quick to dismiss the power of HRAM-MS in addressing some of the broader challenges in pesticide analysis. HRAM-MS has utility across the full spectrum of users, which includes academia, pesticide R&D labs, government, the food industry, and contract testing laboratories. We can break that down more simply into two areas: research and routine.

"I can see a point in the future where we can conduct both targeted analysis and non-targeted screening on a single platform."

In research, HRAM-MS is clearly useful for discovery and identification of new metabolites, for fate studies for new pesticides, or for the identification of unexpected/illegal pesticides. For routine use, I believe HRAM-MS is well suited as a complementary tool to targeted analysis of pesticides for comprehensive testing or – especially in the commercial world – for the development of risk-based target lists for customized food-safety testing programs. Indeed, we are launching two non-targeted methods that we feel meet our clients' needs.

What is potentially powerful in both areas is the ability to retrospectively interrogate data, which could be particularly interesting when considering emerging contaminants or investigating whether a new problem is in fact a new problem at all.

A single platform?

As the sensitivity of HRAM-MS instruments increases, I can see a point in the future where we can conduct both targeted analysis and non-targeted screening on a single platform – a very attractive proposition. In fact, for less complex matrices, we are probably pretty close to that point already. But...

Implementing new technology involves a great deal of effort for accredited routine labs (new method development, validation of all aspects), so I suspect that many laboratories will continue to use triple-quad instruments for quite some time. Nevertheless, there's certainly a real buzz about non-targeted analysis at conferences – the introduction of GC to the Orbitrap™ portfolio will probably add to that buzz. Right now, I get the sense that non-targeted data acquisition (with its potential to speed up method development) followed by streamlined and targeted processing of that data is a good midpoint between the old and the new for routine labs (we don't need or want every sample to be a research project!). Data processing is an ongoing challenge, but it seems that the software is fast catching up with the hardware.

In five or ten years' time, who knows how far we will have traveled on our treadmill?

HUMANITY IN SCIENCE AWARD

phenomenex ...breaking with tradition

& the Analytical Scientist

2015 Winners
Andreas Seidel-Morgenstern (left)
and Peter H. Seeberger (right),

Who will be the winner in 2016?

Nominations for the 2016 Humanity in Science Award are now open

Analytical science has the power to change human lives for the better, but rarely receives the same fanfare as other scientific disciplines. The Humanity in Science Award was launched to recognize and reward a recent breakthrough in analytical science that has truly made the world a better place.

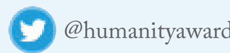
The 2016 award will be presented on May 10 in Munich, Germany.

Could the grand winner be someone you know? Nominate an analytical science project with a humanitarian impact now at www.humanityinscienceaward.com

Why enter?

- *$25,000 grand prize*
- *All-expenses paid trip to Analytica 2016*
- *Opportunity to tour Phenomenex headquarters in Torrance, California*

Nominations close on November 27, 2015 - *Good luck!*



Solutions

Real analytical problems
Collaborative expertise
Novel applications

Finding a Piece of the Protein Characterization Puzzle

Coupling whole column imaging detection to a mass spectrometer for protein structure characterization is fundamentally challenging. Could a relatively simple capillary cartridge system unlock the potential?

By Tiemin Huang, CEO, Advanced Electrophoresis Solutions (AES) Ltd, Cambridge, Ontario, Canada.

The Problem

Whole column imaging detection (WCID) has revolutionized charge-based protein separation with capillary isoelectric focusing (CIEF) providing rapid method development, high resolution, reduced sample volume, and high-throughput analyses. But how can these advances be reliably coupled to mass spectrometric detection?

Background

Biopharma and life science protein researchers need high-resolution, high-sensitivity, and high-throughput protein separation tools for product development and quality control; they need to know the nature of their target isomers and they may need to provide structural information. Mass spectrometry (MS) is the best approach for protein structure characterization because it facilitates understanding of the nature of protein isomers, posttranslational modifications, and product degradation pathways.

I became passionate about WCID with CIEF while I was a graduate student at the University of Waterloo in Canada; it was here where the concept of WCID was conceived and developed. That was in 1998, when we had to build a WCID CE system with a high voltage power supply, a CCD camera, and whatever cartridge we could assemble. Though this may seem simple to do, it was very challenging because we wanted to generate high quality, reproducible results. I found, as other researchers did, that there were many fundamental things that needed to be addressed before CIEF could be used as a reliable and robust analytical tool for protein separation and characterization. After graduating, I joined Convergent Bioscience Ltd (Toronto, Canada), a startup company that was a pioneer in commercializing WCID CE technology. I wanted to improve the detection sensitivity of WCID, to develop more (and improved) reagents and test kits so that WCID could have broader applications.

However, at the time WCID technology could not provide efficient fraction collection for MS protein isomer characterization nor could it be coupled directly to a MS. The problem was that the inner and outer diameter (ID; OD) of the separation and transfer capillary that connect through a small section of membrane capillary were the same. The ID of the membrane capillary, however, was larger than the OD of the separation and transfer capillary – this gap caused a loss in resolution when forcing the focused proteins from the separation capillary into the transfer capillary. Also, separated proteins remixed in the large ID section of the hollow capillary membrane. So, researchers had to use other fractionation methods such as liquid-phase isoelectric focusing to isolate protein isomers before they could be characterized.

The Solution

We wanted a solution, and none seemed forthcoming, so it was time to take matters into our own hands. But developing a state-of-the-art analytical instrument is not simple; you need highly qualified mechanical, electrical,

software, and production engineers. AES really all started from trust, a willingness to collaborate closely, and an eye for evaluation. And it took us several years to select and form such a team with the necessary diversity in background. I firmly believe that the combination of great chemistry, openness, and trust among team members has been the foundation for our solution.

In brief, we have developed what we believe to be the world's first high-resolution iCIEF fractionation and direct-coupling iCIEF to MS solution, which comprises the CEInfinite WCID CE instrument, MS compatible capillary cartridges, and related test kits. We have also developed our own carrier ampholytes and stable capillary coatings, which are essential for protein CIEF. Because we develop and manufacture all the key components in house, we can customize reagents to better suit diverse protein sample separation challenges.

The CEInfinite system is based on WCID and uses a small length of separation capillary (from 2 to 10 cm) in a column-diameter transformation cartridge (patent pending), which is monitored by an imaging sensor (see Figure 1). Detection is provided by a series of snapshots of the whole separation capillary. The advantage of WCID over conventional single point detection is real-time monitoring, which allows high throughput CE separation and study of dynamic separation processes.

In fact, the cartridge is at the heart of the system and comprises a large ID separation capillary, and a small ID transfer capillary. The larger ID of the separation capillary improves the detection sensitivity, but once the proteins are separated, they move into the transfer capillary with minimal peak remixing ahead of preparative fractionation or direct coupling to the MS system (see Figure 2).

Though there has been an increasing demand for high sensitivity detection and CIEF-MS for many years, no one had really been able to find an answer. Indeed, it took a great deal of research and tests back and forth, before we found the answer in unconventional column-diameters. But the solution isn't an easy one; the manufacturing of such unconventional cartridges with small capillaries of different IDs is very difficult. Nevertheless, our chemical engineering team has worked hard to create a proper formulation for the membrane, and our production team is now able to consistently manufacture cartridges according to our stringent criteria.

And so more recently, we have been very focused on demonstrating the high-resolution protein isomer fractionation of our cartridges, and in the process, we've seen unexpected CIEF separation improvements for some complicated fusion proteins when using our larger ID (180 or 200 µm) cartridges. Notably, the carrier ampholytes (Aeslytes) also play an important role by helping to improve resolving power for other fusion proteins and antibody drug conjugates. From a practical point of view, the cartridges also facilitate sample injection and reduce capillary clogging.

In summary, a number of features have been implemented together to create our solution for CIEF-MS:

- The ID of the separation capillary is much larger than that of the transfer capillary; therefore, remixing of separated protein during transfer after CIEF is minimized.
- MS compatible cartridges allow protein fractionation or coupling to MS after CIEF, which enables protein isomer structure characterization with WCID, something that has never been achieved before.
- MS compatible carrier ampholytes (Aeslytes) minimize interference of ionization of protein, during mass spectrometric detection.
- Small molecule pI markers consist of non-peptide synthetic molecules that cover the full pH range from 2.5 to 10.5, allowing convenient

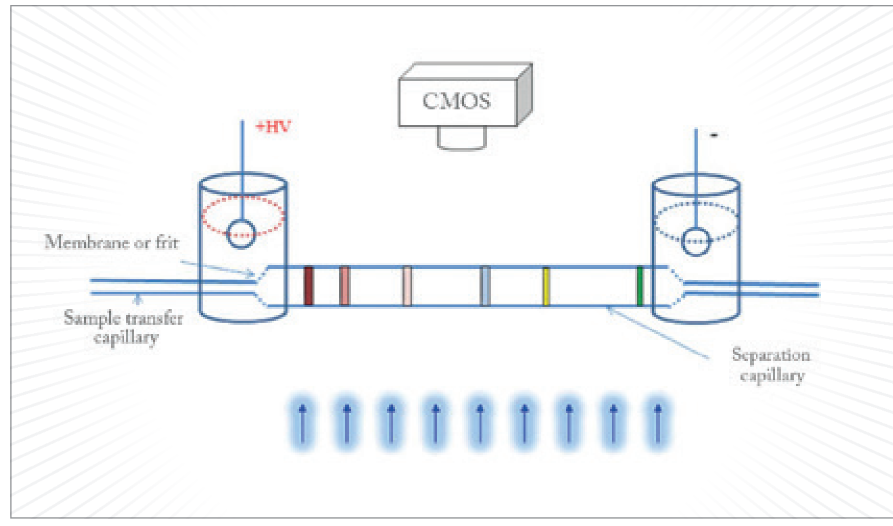


Figure 1. High-sensitivity imaging CIEF (iCIEF) is achieved by recording and processing the profile of the line light intensity through the entire separation capillary with a high resolution CMOS camera.

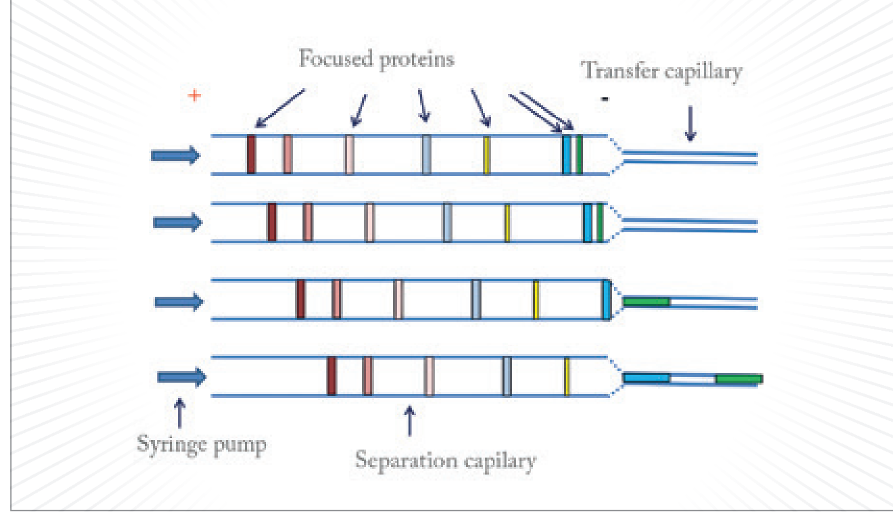


Figure 2. Schematic of column diameter transformation imaging cartridge, showing controlled focusing and mobilization.

application of pI markers even in the presence of enzymes, such as carboxypeptidase B.

- The ID of the separation capillary is a major factor which determines the detection sensitivity for WCID CIEF, which is why our cartridge technology is so important.

Beyond the Solution

So far, we have sold systems and a variety of CIEF related products to biopharmaceutical companies, research institutions, and universities in North America and Asia. We have also just launched our full product line in Europe. In the next few years, we aim to become a leading total solution provider for high-throughput and high-resolution protein separation and characterization. To do this, we are working closely with researchers in the biopharma industry and life science research institutions to pinpoint their needs and provide further solutions. I foresee that our products will be desirable in therapeutic protein development and quality control in the biopharma industry, as well as in clinical laboratories for protein disease marker analysis – so we are keen to develop strong collaborative relationships in these areas.

As with any new system, support is important, and our team of experts can aid in method development (which can take only a couple hours for simple protein and up to a week or so for complicated samples). Our ultimate hope is that by applying our technology, with its high analytical throughput and capability to perform multiple assays (for instance, protein identity, protein binding, and protein concentration), our clients can speed up the drug development process – and we are passionate about trying to help our customers in this endeavor.

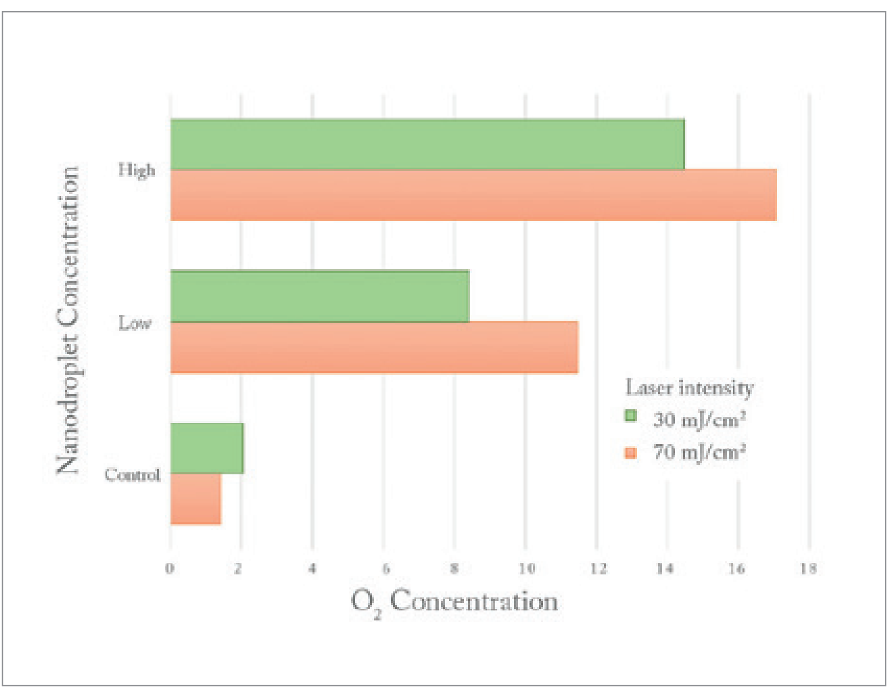
I have to say that a personal highlight for me was when we received an evaluation from a major potential customer, with a glowing report of our CEInfinite system in routine sample analysis. In the extensive four-month validation process, many therapeutic proteins were analyzed, and we were informed that our CE system provided better reliability and reproducibility, with separation and quantitation results comparable to other CE instruments that they had used. Our CE system also provided better resolution for some monoclonal antibodies and fusion proteins. And of course, we have found the missing link between iCIEF and mass spectrometry. We are proud of what we have achieved up to this point – and we look forward to developing our WCID technology further.

Bringing New Life to Old Arteries with Oxygen

Optimizing oxygen delivery improves stem cell treatment of peripheral artery disease.

By Cicely Rathmell, M.Sc.

One in three people over the age of 70 suffers from peripheral artery disease (PAD), a narrowing of arteries that occurs most often in the legs. Plaque, a substance composed of cholesterol, calcium and fibrous tissue, builds up in the arteries and hardens over time, reducing blood flow. Symptoms range from pain to difficulty fighting infection and in severe cases, tissue death. Treatment with adipose-derived stem cells offers great promise for restoring blood flow, but the inherently oxygen-deprived environment causes the majority of stem cells to die before they can be of regenerative benefit.



Effect of nanodroplet concentration and laser energy on oxygen release as compared to a control sample.

Experimental Conditions

Researchers at the University of Texas at Austin are working on a controlled oxygen delivery system to improve the survival of stem cells during treatment and thereby promote growth of new blood vessels. The teams are planning to add oxygen-saturated perfluorocarbon (PFC) nanodroplets to the hydrogel stem cell carrier destined for implantation at the treatment site. Oxygen would then be released from the nanodroplets into the surrounding tissue through passive diffusion at a rate that depends on the composition of the nanodroplet shell and the implantation environment.

Additionally, a more dramatic oxygen release can be accomplished via laser "activation" of the nanodroplets. Irradiation at specific wavelengths, dependent upon absorbers encapsulated within the PFC nanodroplets, causes a rapid phase-change into a microbubble, facilitating oxygen release. This allows the location and timing of oxygen delivery to the treatment area to be controlled and tunable on a per patient basis.

Laser activation can be used to control the release of oxygen from the nanodroplets into surrounding tissue. As a first step, the researchers looked at oxygen release in solution. Galvanic oxygen probes proved unsuitable, being too bulky and prone to interference from the bubbles that occurred during activation, resulting in inaccurate measurements. Instead the team chose our NeoFox optical oxygen sensor based on phase fluorimetry. NeoFox is immune to most environmental effects and can be configured with a probe that is <1 mm in diameter for use in small samples.

Results

In testing, a marked increase in oxygen levels was seen for the nanodroplet-containing solutions as compared with the control, scaling with concentration. Use of higher activating laser intensity also increased oxygen release. Most importantly, the oxygen release achieved appears to be sufficient to boost oxygenation to levels that will increase implanted stem cell survival and lead to improved therapeutic outcomes for PAD patients.

Conclusions

The next steps for the group will be to repeat these experiments in a hydrogel format, followed by in situ tissue studies. The small size of an optical oxygen probe and its ability to work in different media will allow the team to use a single system for the full scope of research, and speed their progress in improving the effectiveness of a promising treatment for peripheral artery disease.

Swiss Precision Research

Sitting Down With… Detlef Günther, VP Research and Corporate Relations, and Professor for Trace Element and Micro Analysis, ETH Zürich, Switzerland.

How did your additional VP role come about?

Firstly, I was asked to take the job – but additionally, I've had such a great time with my own research at ETH that I wanted to give something back. I now have a broad role with my staff – guiding research, internal research funding, establishing platforms; developing own competence centers and national competency centers; and managing ETH's connection with the Swiss National Science Foundation (SNSF) and the European Research Council. And I'm the direct contact for all professors with respect to research.

Sounds like a lot to organize...

The major part of my work is focused on supporting research (which is predominantly fundamental or basic science). We have strategic research projects (in the energy, climate, and food sectors, to name just a few). Essentially, we look at research initiatives and consider how we can support the research groups and connect professors from different disciplines and fields. Another aspect is technology transfer, which includes everything related to patents, licensing, spinoffs, and industrial relations in general.

What is the advantage of your approach?

People now talk to each other much more than they did. They are also more aware of new research projects – moreover, their interaction is enabling technology transfer into many more areas of the school; for example, applying Big Data Science to other research areas, e.g. to personalized medicine. My role is to ensure that relevant researcher meet each other to share their thoughts and expertise, at which point we can begin to form competence centers to develop a sound research direction from bottom up.

It can take a bit of effort and encouragement to bring different departments and disciplines together. However, most researchers are keen to bring about cultural change and happy to work with other departments. After all, opening up your own world to other ideas and expertise can accelerate your own progress and greatly increase the impact of your work.

Where does analytical science slot into the puzzle?

Analytical science is very important. For example, it has a major impact on developing new materials because it's essential for characterization. It's also clear that mass spectrometry (MS) can have a huge impact on personalized medicine. In fact, all the analytical techniques are important and they are moving into new areas. Another example is the close connection between earth science and analytical science – we no longer just provide a lab service to our colleagues, we work alongside them. The increased interaction allows to get serious about improving analytical techniques for a greater impact. Just look at imaging; it now connects with all fields of medicine to help provide new prognostic and diagnostic monitoring tools. Clearly, there's a steep learning curve from a scientific language point of view, but at least we no longer need to force people to talk to each other – they already understand the benefits of broader interaction.

How do you measure success?

It would be very easy just to highlight the list of Nobel Prize winners from ETH – it's a very impressive list. However, there are other important criteria. For example, we are active in climate model development studies and our earth science department is one of the highest ranking in the world. But I consider the excellent educated students who are taking leading positions in academia and industry as one of the most significant successes of ETH.

We are celebrating our 160th birthday, and right from the beginning we've always welcomed talent from all over the world; Switzerland is simply too small to provide all talents needed. We are also very successful in securing basic funding for each professor, which allows them to follow up their ideas and begin research immediately – if they have a great idea today, they can start work on it tomorrow.

What about your own success?

I am very fortunate to have a professorship and a fantastic research group at ETH. Success will be if we succeed in encouraging greater interaction between departments and professors from different research fields. We want this to happen throughout the school so that others are more inclined to work together and share intellectual property rather than simply focus on building their own reputations. If we could transfer our technical potential into medical research, I would consider that a great achievement.

My ambition is to change some funding schemes. Instead of demanding extensive CVs and publication lists, and established reputations, I want to make it easier for young talented people to pursue their ideas. I think we have to trust young talents and give them the support they need to pursue innovative science. So, it's really not about me. My goal is to achieve success for the school and I can tell you it is a very exciting pursuit.

Before my new role, I only saw ETH from the perspective of my own department and professorship. In just nine months, it feels like I am flying above and gainin a broader view of the school every day. I've even spotted five or six new research topics I'd like to pursue myself...

SHIMADZU
Excellence in Science

Nexera-e provides a wider range of applications in any research field

Exponential chromatography

The new *Nexera*-e comprehensive LCxLC system specializes in the analysis of natural products and other complex mixtures with many different compounds and chemical classes. *Nexera*-e targets the food and chemical industries as well as polymer and pharmaceutical industries. The "e" implies exponentially better chromatography due to an exponential increase in peak capacity and resolution.

- **Identification of multiple compounds** in a single chromatogram through higher peak resolution and peak capacity
- **Highest efficiency** achieved with just one injection instead of multiple approaches
- **Reliable analysis of targeted compounds** provided by best-in-class LC/MS/MS and PDA detectors
- **Connecting to LabSolutions software** for method set-up
- **Powerful ChromSquare software** for data processing, evaluation and presentation

www.shimadzu.eu