Cover Story

2  An Easy Way to a Fast Universal Method for Surfactant Analysis
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Features

13  An Age of Discovery
The Column spoke to Martin Giera of Leiden University Medical Centre,
Leiden, The Netherlands, about developments in biomarker discovery.

Regulars

10  News
Nanoparticle measuring, the recipient of a Thought Leader award, deceptive
orchids and a food safety training laboratory are featured this week.

12  Market Trends & Analysis
Market Profile: High Content Screening
Glenn Cudiamat, Strategic Directions International Inc., Los Angeles,
California, USA.

18  CHROMacademy
Update on what’s new on the professional site for chromatographers.

19  Training Courses and Events
An Easy Way to a Fast Universal Method for Surfactant Analysis

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Surfactants present a real challenge to the analytical chemist as they are a very complex class of chemical compounds. As well as having widespread use in household cleaners, personal care products and cosmetics, they are also found in photochemicals, oil field chemicals, construction materials, adhesives, lubricants, metalworking, mining, pulp and paper and pharmaceutical products. The term “surfactant” is derived from surface-active agent. Surfactants contain two characteristic parts: an oil-soluble hydrocarbon chain and a water-soluble ionic or polar hydrophilic group. When added to water or solvents, they reduce surface tension for the purposes of wetting, emulsifying, dispersing, foaming, scouring or lubricity. As emulsifiers, they are used in agriculture to treat hydrophobic soils, and in the oil industry they are used as complex dispersant mixtures to treat oil spills. In analytical chemistry, they are used as ion-pairing agents in chromatography and they form the basis of micellar electrokinetic chromatography (MEKC), a capillary electrophoresis variant to separate neutral substances in an electrical field. Surfactants can be categorized based upon their structure and include nonionic, anionic, cationic and amphoteric (zwitterionic) classes. According to market reviews, anionic and nonionic surfactants make up 85% of the global surfactant demand and the trend clearly points to nonionic variants.¹

Many commercially available surfactants are mixtures containing complex homologous members or ethylene glycol oligomers and polymers. They pose analytical detection challenges, especially when different surfactant classes are combined and the chain length distributions may overlap during the chromatographic separation. Chromatographic approaches can separate the molecules on the basis of carbon chain length, chain branching,
Oxyethylene chain length or positional isomer distribution, but they also need to discriminate between the different types of functional groups. With the exception of alkyl benzene sulphonates (ABS), surfactants typically do not contain a chromophore which makes them invisible for UV–vis detection: the most common LC detector. They are usually measured using reversed-phase (RP)-HPLC with non-suppressed or suppressed mode conductivity. In ion-exchange chromatography they can be detected indirectly using photometry and a UV-active mobile phase co-ion. This detection principle is commonly applied in capillary electrophoresis, in the aqueous mode and non-aqueous variant, a powerful method to analyse complex surfactant mixtures.

Coupling liquid chromatography (LC) to mass spectrometry (MS) provides both versatile detection and peak identification capabilities and has been summarized in a review by Petrovic and Barcelo. Despite countless publications on surfactant analysis with modern methods, two main challenges remain: separation of related complex mixtures and quantitative detection of surfactants lacking a chromophore. It has been a primary goal to find a universal detector that can quantify analytes by mass, when no specific standards for calibration are present. Charged aerosol detection (CAD) is the only LC detection technique that is capable of this. It can measure any non-volatile,
and many semi-volatile compounds, usually to low nanogram (ng) levels. Typical characteristics of chromatography with charged aerosol detection include over four orders of magnitude of dynamic range and high-precision results, less than two percent relative standard deviation (RSD) for peak area. Analyte response is also largely independent of chemical structure, providing clear relationships among different analytes in a sample, and is therefore ideal for measuring surfactant species.

**Figure 2:** HPLC with charged aerosol detection chromatogram of a surfactant mix in acetonitrile/water (1:1). Single-pump eluent gradient conditions are in black, and dual-pump inverse gradient conditions in blue.

- 1. Xylene sulphonates.
- 2. Laurylpyridinium.
- 3. Laurylidimethylbenzyl ammonium.
- 4. Triton X-100.
- 5. Cetyl betaine.
- 6. Decyl sulphate.
- 8. Linear alkylbenzene sulphonate (LAS).
The way in which charged aerosol detection works is described in Figure 1. This sensitive, mass-based detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube leaving analyte particles, which become charged in the mixing chamber. This technology has greater sensitivity and precision than evaporative light scattering detectors (ELSD), and it is simpler to operate than a mass spectrometer.

To separate mixtures of ionic and non-ionic compounds by LC, mixed-mode stationary phases that combine hydrophobic moieties with ion-exchange functionality prove to be the method of choice. Multimode phases are an effective way to separate surfactants. The silica-based speciality column applied in this work is a dedicated column for surfactant analysis and combines hydrophobic and anion-exchange moieties. It provided ideal selectivity for simultaneous separation of surfactants.

**Figure 3:** (a) Overlaid chromatograms of TWEEN 80 (black) and TWEEN 85 (blue); 20 mg/mL in isopropanol using single gradient conditions. (b) HPLC-charged aerosol detection chromatogram overlays of Span 80, -83 and -85 at 20 mg/mL in isopropanol.

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anionic, nonionic and amphoteric surfactants and is well suited for the determination of cationic surfactants in the same run. Moreover, it was excellent in resolving ethoxylated surfactants and capable of retaining highly hydrophilic compounds, such as hydrotropes. It is optimized for very low column bleed and is suitable for trace level quantitation with charged aerosol detection, MS, suppressed conductivity detection (SCD) and UV–vis detection (UV).

**Experimental**

**Sample Preparation:** Samples were dissolved in water, isopropanol or isopropanol/water (1:1) to various concentrations, typically between 10 to 50 mg/mL. After an optimized solvent was used, the samples were centrifuged if insoluble material remained. The Spans and TWEENs were dissolved in isopropanol/water (1:1), and the laundry detergent was dissolved in water.

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**Figure 4:** Chromatogram of a laundry detergent.

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Liquid Chromatography and Charged Aerosol Detection: The UHPLC system used was a Thermo Scientific Dionex UltiMate 3000 ×2 Dual RSLC system, consisting of a dual-gradient pump (DGP-3600RS) with respective degasser (SRD-3600) that can simultaneously generate two independent ternary gradients, a thermostatted well-plate compatible autosampler (WPS-3000TRS), a thermostatted column compartment (TCC-3000RS) and a Corona ultra RS charged aerosol detector. The analysis was performed using a Thermo Scientific Acclaim Surfactant Plus, 3 μm, 4.6 × 250 mm, designed especially for this class of compounds. Chromatographic conditions were 1 mL/min flow-rate, mobile phase A was 100 mM ammonium acetate at pH 5.4 and mobile phase B was acetonitrile. For the gradient details, see Table 1.

For inverse-gradient mode, the streams of both pumps are combined in a T-connector after the separation column. Dilution from charged aerosol detector is a mass-based, rather than a concentration-based, detector. Inverse gradient was used to overcome changes in nebulization efficiency resulting from mobile phase composition and viscosity by ensuring that a constant mobile phase composition entered the detector (here, 51% acetonitrile). This approach has substantial benefits for all nebulizer-based detectors.

Injection volume was 5 μL, column temperature 30 °C and the settings for the charged aerosol detector were ambient nebulizer temperature and a filter setting of 0.

Data Analysis: All UHPLC chromatograms were obtained and compiled using Thermo.

### Table 1: Timetables for elution gradient (right pump in DPG) and inverse gradient (left pump in DGP).

<table>
<thead>
<tr>
<th>Elution Gradient</th>
<th>Inverse Gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td>Flow rate (mL/min)</td>
</tr>
<tr>
<td>-5</td>
<td>1.0</td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
</tr>
<tr>
<td>20</td>
<td>1.0</td>
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Results and Discussion

Analysis of complex surfactant samples was possible without significant method development or sample preparation effort. A mixture of eight surfactants – four anionic, two nonionic, one cationic and one zwitterionic surfactant – were analysed using the single-pump gradient elution and inverse-gradient programmes for comparison. As shown in Figure 2, the column clearly separated the different surfactant classes, including separating the components within more complex surfactants. The use of an inverse gradient provides two benefits: a flatter baseline is observed and possible elevation in the relative response factors associated with increasing mobile phase organic solvent was minimized. Even when greater amounts of organic solvent are present in the eluent stream, the response factors for analytes remains fairly consistent and yield chromatograms that were easily and accurately integrated. More importantly, relative response factors across the gradient were more consistent allowing for improved results on mass-percent values in the sample, even with a lack of standards for individual calibration.

In another experiment, two surfactants, TWEEN 80 (polyoxyethylene-20-sorbitan monooleate) and TWEEN 85 (polyoxyethylene-20-sorbitan trioleate) were dissolved in isopropanol and analysed using the gradient-elution conditions described earlier. The two chromatograms were overlaid as shown in Figure 3(a). Note that not only were the sub-components of each TWEEN distinguished, but TWEEN 85 also eluted later than TWEEN 80 due to the greater amount of oleate moieties contained within the polymer.

Three Span surfactants were analysed using the single-pump gradient elution parameters shown above. Span-80* (sorbitan monooleate), -83 (sorbitan sesquioleate) and -85 (sorbitan trioleate) were dissolved in isopropanol at a concentration of 20 mg/mL. The similarity between the chromatograms reflects the similarity in composition: Span 83 is similar to Span 80 except that it contains 50% more oleate. This may be reflected in the slight increase of the later-eluting portions of the Span 83 chromatogram. In addition, the trolein form, Span 85, contains the greatest amount of later-eluting, hydrophobic material relative to the other two, which is clearly seen in the chromatogram overlays in Figure 3(b).
The method was finally evaluated for the analysis of an off-the-shelf laundry detergent. This product was diluted in water at a concentration of 50 mg/mL and analysed using the conditions described in the experimental section. As seen in Figure 4, the detergent sample appeared to contain alcohol ethoxylates, two main varieties of sulphonates, and a variety of more hydrophilic materials that eluted before 3 min. This shows that a finished consumer product can be taken directly from the container and analysed for the type and quantity of surfactants without any sample preparation beyond dilution.

Conclusion
Combining the use of a specialty column for surfactant analysis with the universality, reproducibility and sensitivity of the charged aerosol detector enables a simplified approach to chromatographic analysis of surfactants. Method development was straightforward, as very complex mixtures could be separated with simple generic aqueous ammonium acetate to acetonitrile gradients. Detection did not require further optimization as the charged aerosol detector has few operation variables and was operated at ambient nebulizer temperature. The method shown was capable of separating and quantifying all classes of surfactants, from simple to complex, with analysis times of less than 21 min. The use of the inverse-gradient, in combination with charged aerosol detection, enabled more consistent response throughout the gradient and based on this a quantification of unknown peaks in the chromatogram was possible. Other surfactants have been successfully analysed with the presented methodology, including Aerosol OT (docusate sodium) as a single peak, as well as Span 20, Span 60 and Pluronic F68 (polyoxyethylene-polyoxypolyethylene block copolymer), a common surfactant in pharmaceutical/biotechnology products.

References
1. Market Study: Surfactants, Ceresana Research: Germany, February 2012.

Frank Steiner, PhD, is manager of small molecule solutions for Thermo Scientific UHPLC+ products, Germering, Germany. Prior to Thermo Fisher Scientific, he worked for many years for Dionex Corporation in various HPLC product marketing roles. He currently manages marketing strategies and solution development for small molecule UHPLC analysis and is coordinating scientific collaborations with partners from academia.

Marc Plante has over 10 years of experience developing methods in chromatography, including eight years working in small pharmaceutical laboratories developing analytical HPLC methods. Marc joined ESA Biosciences (now a part of Thermo Fisher Scientific) in 2007, where he developed applications for electrochemical and charged aerosol detectors. Much of his efforts are now focused on the chromatography of lipids, including lipid profiling and quantitation, biodiesel analysis and surfactants.

Bruce Bailey has worked in the applications department of ESA Biosciences, Dionex Corporation and now Thermo Fisher Scientific for the past 23 years and is currently the Product Applications Manager. He has over 34 years experience using HPLC, has co-authored more than 30 publications and helped develop many commercial HPLC applications using electrochemical and charged aerosol detectors.

Ian N. Acworth is Director Customer and Application Support at Thermo Fisher Scientific, formerly Dionex Corporation, and an Adjunct Associate Professor of Pharmacology, at the Massachusetts College of Pharmacy and Health Sciences, Massachusetts, USA. Prior to working with Dionex, he was vice president of applications and customer support at ESA Biosciences for more than twenty years for electrochemical and charged aerosol detectors. He was co-recipient of an NIH roadmap grant for the development of metabolic approaches using both EC and mass spec.
Thought leader recipient

Nanoparticle measuring

A team of scientists from Taiwan has developed a nanoparticle/virus mass spectrometry technique to make rapid and accurate mass and mass distribution measurements of nanoparticles and viruses. The team had previously developed a technique that allowed them to measure the mass of a cell or a microparticle. The method used was laser-induced acoustic desorption (LIAD) and the team was able to take measurements within 1 min.

The team concluded that their research has potential for the quality control of nanoparticle production, where differences in size can affect the properties, and the identification of various viruses. The method could be used in the future to monitor drug delivery when nanoparticles are used as carriers. It could also be possible to measure the degree of infection by measuring the number of viruses in specific cells or in plasma.


Agilent Technologies (Santa Clara, California, USA) has announced the recipients of its latest Thought Leader award: Dr Jens Frisvad and the centre for microbial biotechnology in the department of systems biology at the Technical University of Denmark.

The centre is working to create a targeted metabolomics approach for thousands of compounds produced by aspergillus, penicillium and fusarium – filamentous moulds that frequently contaminate food and can lead to illness. Scientists will use mutable internal standards to calibrate changes in both chromatographic retention and mass-spec sensitivity in order to compare extracts over many years.

The award will provide the lab with use of a LC-QTOF system for discovery metabolomics, as well as a LC-QQQ system for target screening. Agilent will thereby be supporting research in food safety, specifically the metabolomics of mycotoxins (toxins made by mould).

Dr Frisvad commented, “Our research is aimed at showing the impact that analysis of LC-QTOF and LC-QQQ data can have on understanding the metabolomics of mycotoxins. We are extremely pleased that Agilent is helping us continue this important food-safety research.”

Mike McMullen, president of Agilent’s chemical analysis group, said, “Our support of the work being done at the centre for microbial biotechnology closely aligns with a major initiative at Agilent: providing tools and methods that help test the global food supply. By providing instruments to Dr Frisvad and his team, we continue Agilent’s long history of helping create innovative ways to improve food safety.”

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Deceptive orchids

A team of researchers from Australia has conducted a study into the way in which Drakaea livida (Orchidaceae) is pollinated.1 They discovered that the orchid specie deceives the wasp Zaspilothynnus nigripes (Thynnidae) by emitting the same compound, 2-hydroxymethyl-3-(3-methylbutyl)-5-methylpyrazine, that females emit when searching for mates. Gas chromatography–electroantennographic detection (GC–EAD) and gas chromatography–mass spectrometry (GC–MS) were used to isolate this novel pyrazine. The main chemical compounds were separated and identified in the mixture.

The team concluded that this compound may represent the first known case of pyrazines as sex pheromones in Hymenoptera insects.

Market Profile: High Content Screening

High content screening (HCS) is a high-throughput method which enables the functional analysis of cells. HCS plays a vital role for drug discovery applications, including drug targets verification and pathway detection, in both the pharmaceutical and biotechnology industries. Often, the technology is used in neuroscience research, especially in drug development for Alzheimer’s disease, Parkinson’s disease and other neurodegenerative diseases.

The first step in performing a HCS experiment is to label the cell of interest with a fluorescence biomarker. After the cells are loaded into the wells and placed into the instrument, a particular wavelength of light is shined onto the cells, which illuminates the labeled cells and gives the embedded camera the opportunity to take photos.

One drawback of HCS is the difficulty of analysing the vast amount of data obtained. Both HCS instrument manufacturers and third party software vendors have been allocating significant resources into developing more accommodative software to address this need. Another obstacle of HCS is its lacklustre image resolution. However, the development of higher resolution digital cameras, faster shutters, automated microscopes, quantum dots and new fluorophores has dramatically improved the resolution.

Combined, pharmaceutical, biotechnology, academia, CRO and government research industries account for 90% of the global high content screening demand. Other industries include agriculture/food, hospitals/clinical labs, government testing and environmental labs.

The foregoing data was extracted and adapted from SDI’s A Complete Life Science Perspective report. For more information, contact Glenn Cudiamat, VP of Research Services, Strategic Directions International, Inc., 6242 Westchester Parkway, Suite 100, Los Angeles, California 90045, USA, +1 310 641-4982, fax: +1 310 641-8851, e-mail: cudiamat@strategic-directions.com
An Age of Discovery

The Column spoke to Martin Giera of Leiden University Medical Centre, Leiden, The Netherlands, about developments in biomarker discovery.

Q: Your group is actively involved in biomarker discovery. When did you become involved in this field and how has it evolved since you first started?
A: I got involved in biomarker discovery around two years ago. During this time I would say that speed is becoming more and more important, especially because studies are becoming larger and larger, which naturally involves increased sample numbers.

Q: What are the main chromatographic techniques that you use and what are the biggest challenges you face in biomarker research generally?
A: Our group uses different separation technologies, ranging from well-established, robust methods such as gas chromatography (GC) to a relatively experimental set-up based on capillary electrophoresis. However, HPLC — either in a capillary set-up or in combination with 2-mm core-shell columns — remains our main chromatographic technique. Core shell columns have proved to be very useful because they provide close to UHPLC performance, but we can also use our standard LC equipment.

Q: Your group has focused on derivatization strategies in biomarker discovery. Why is derivatization still important in this field and is this approach time-consuming?
A: Most biomarker studies today are performed with LC–MS platforms operated with a RP-18 or HILIC column. Of course, this will restrict the analytes that can possibly be detected to what is retained on the incorporated columns. Many analytes also show restricted ionization capabilities that makes their detection even more difficult. Hence we asked the question if it would be better to derivatize certain analyte subclasses for better chromatographic separation and enhanced detection. Even though this might lead to more bias in the analysis it might allow the detection of metabolites that would otherwise be overlooked.

One example where we enhanced the detection limits by a factor of almost ten with a derivatization strategy was for the analysis of small carboxylic acids, including the intermediates of the tri-carboxylic acid cycle. In addition to this, alternate isotope-coded derivatization (AIDA) and group specific internal standard technology (GSIST) approaches can provide convenient quantification in combination with derivatization strategies.

Another important field of derivatization is the selective derivatization of established biomarkers. The main goal here is the highly sensitive and fast determination of such substances. In this field we recently conducted a study aimed at the determination of malondialdehyde: an important reactive carbonyl, a biomarker of oxidative stress and a side-product of thromboxane synthesis. With the developed labeling strategy we were able to detect malondialdehyde down to concentrations of around 2 nM in urine samples while the chromatographic run times were shorter than five min. Crucial for the separation of highly fluorescent urinary components and our derivatized analyte was the use of a cyano-column which we used in the reversed-phase mode.
Q: You are involved in some interesting work relating to human infectious diseases such as urinary tract infections and tuberculosis. How does this work benefit patients and what was the biggest challenge you had to overcome in terms of chromatography?

A: Our project on Urinary Tract Infections is part of a collaboration between our group and the department of Infectious Diseases at the LUMC (headed by Professor Jaap van Dissel). We are aiming to find markers for the identification of the causative bacteria. A current “golden standard” of pathogen identification is bacterial culture, which takes 24 h to 48 h. If we could develop a method that gives us an answer within, for example an hour, it could lead to a much more tailored treatment strategy and, most importantly, reduced risk for the development of bacterial resistance to the applied antibiotics.

The project about tuberculosis biomarkers is a result of our collaboration with the KIT (Royal Dutch Tropical Institute, particularly Alice den Hertog and Richard...

![Figure 1: Derivatized TCA cycle intermediates separated on a C-18 core shell column.](image)
Anthony). The goal of this study is to evaluate a recently proposed “treat-to-test strategy” where the treatment and consecutive response are used as the diagnostic tools. Well, it remains to be seen whether the “treat-to-test strategy” will work or not; our role in this project, however, is to identify a time window after the treatment when the pathogen-related substances (metabolites) appear and provide as detailed as possible characterization of these substances.

In terms of chromatography: it is frequently about making sacrifices. If you are facing several hundred samples your primary goal is usually speed. This is why we tend to use core-shell particle columns. With respect to the column chemistry one has to decide whether to use HILIC or RP: both chemistries have their benefits and drawbacks. However, the well-established robustness of RP separations usually makes us use this column chemistry. For the future it will be interesting to see how C30 columns or mixed-mode phases might influence the field.

**Figure 2:** Comparison between urine samples spiked with different amounts of MDA 0 (black), 50 (yellow), 200 (green) and 400 nM (red).
Q: What other projects are you excited about at the moment?
A: A very recent study which we performed in co-operation with Professor Charles N. Serhan from Harvard Medical School and the department of rheumatology at the LUMC was aimed at the analysis of lipid mediators in arthritis patients. In addition to the analysis of the lipid mediator class we also performed a general lipid profiling of the patient material. At present we are very excited about the results and are now planning to investigate the biological roles of the lipid mediator class in different biological assays further.

Q: How do you see this work evolving in the future?
A: I am pretty sure that sample numbers will keep getting larger, which will demand faster separations and extremely robust analysis platforms. For the biomarkers themselves I believe that their thorough analysis platforms. For the biomarkers themselves I believe that their thorough
validation is key. Additionally, it will also be crucial to develop, as far as possible, simple antibody- or derivatization-based tests that can easily be applied in the clinic by the doctors or nurses.

Q: Anything else you would like to add?  
A: I’d like to thank all people involved in my past and present work, especially Charles N. Serhan from HMS, Andre M. Deelder, Oleg A. Mayboroda, Andreea Ioan-Facsinay and Rene Toes from the LUMC and my former colleagues at the VU University Amsterdam, Wilfried MA Niessen and Henk Lingeman.

Martin Giera studied pharmacy at the universities of Heidelberg and Munich, Germany. After a 7 month stay in the analytical department of Boehringer Ingelheim (Biberach Riss, Germany) he joined the group of Professor Bracher at the LMU Munich for his doctoral studies in pharmaceutical chemistry. He received his PhD in late 2007. In 2008 he joined the group of Professor Hubertus Irth at the VU University Amsterdam, The Netherlands, as a postdoc. In 2009 he was appointed assistant professor in the same group. Since May 2011 he is mainly responsible for the lipidomics activities in the biomolecular mass spectrometry unit of Professor Andre M. Deelder/Associate Professor Oleg A. Mayboroda at the Leiden University Medical Centre (LUMC).
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