Advances in Stationary Phases for Liquid Chromatography

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This year, the Chromatographic Society Spring meeting and AGM was held at the impressive Madejski Stadium, home to Reading Football and London Irish Rugby clubs, from the 21st – 22nd May 2008. The adjacent conference and hotel facilities, providing both comfort and convenience, made for an ideal venue.

Figure 1. Madejski Stadium, Reading – The venue for the Chromatographic Society Spring Meeting and AGM.

The presentations, stretching over two information packed days, included sessions on 'Current state-of-the-art and future directions', 'Stationary Phase characterisation', 'Industrial perspectives' and 'Novel supports and stationary phase'. These were interspersed by three scientific vendor presentations. Whilst sub 2 µm silica phases were well featured in a number of talks, it was the performance of the superficially porous particles and the potential of monoliths which stole the show.

The principal sponsors of the event were Agilent Technologies (Gold sponsor) and significant sponsorship was also provided by Merck Sharpe & Dohme (Harlow).

Many of these presentations are available for view on the members section of the Chromatographic Society web site (www.chromsoc.com).

Mini-Exhibition

As an integral part of the symposium, 18 companies had tabletop displays of their latest product offerings. In addition there were three vendor presentation sessions interspersed with those of the invited scientific speakers.

Company	Key Products On Display	Contact
Agilent Technologies	Agilent 1200 Series LC systems and Poroshell and Zorbax Columns	www.chem.agilent.com Tel: +44 (0)845 712 592
AECS	QuickPrep™ range of chiral and non-chiral columns	www.chiral-hplc.com +44 (0)1656 782 985
Chiral Technologies Europe	DAICEL range of chiral columns	www.chiral.fr +33 (0)388 79 52 00
Crawford Scientific	YMC range of LC columns and chromatographic training services	www.crawfordscientific.com +44 (0)1357 522961
Dionex	Ultimate® 3000 Rapid Separation LC (RSLC) and Acclaim® range of LC columns	www.dionex.com Tel: +44 (0)1276 691722
ESA	HPLC detectors, complete systems and columns	www.esainc.com Tel: +44 (0)18442 239381
Kromasil	Kromasil LC columns	www.kromasil.com Tel: +44 (0)1442 233 555

Grace	VisionHT UPLC columns	www.discoverysciences.com Tel: +44 (0)1480 32 4430
Hichrom	Hypersil Gold, Nucleodur and Zorbax RRHT UPLC columns and chromatographic training services	www.hichrom.co.uk Tel: +44 (0)118 930 3660
Phenomenex	Gemini-NX advanced pH silica and Onyx 2mm ID monolithic columns	www.phenomenex.com Tel: +44 (0)1625 501367
Polymer Laboratories	A range of rigid polymeric media reversed phase and ion-exchange LC columns	www.polymerlabs.com Tel: +44 (0)1694 723581
Selectscience.net	A product and application resource featuring the latest news and user's views for laboratory scientists.	www.selectscience.net Tel: +44 (0)1225 874666
SeQuant	ZIC®-HILIC LC columns	www.sequant.com Tel: +46-90-154880
Shimadzu	Recent Developments in Column and Instrument technologies	www.shimadzu.co.uk Tel: +44 (0)8708 375209
Supelco Analytical	Ascentis™ LC Columns	www.sigma-aldrich.com Tel: 0800 717181
Thermo Fisher Scientific	Hypersil Gold UPLC columns	www.thermo.com Tel: +44 (0)1442 233 555
Varian	HPLC systems and a wide range of column types and packing materials including Pursuit® and Polaris® LC Columns	www.varianinc.com Tel: +44 (0)1865 291 500
Waters	Acquity UPLC™ hardware and columns	www.waters.com Tel: +44 (0)208 238 6100

Summary of vendor presentations

Presenter	Vendor	Title Of Presentation
Dr Adam Woodhouse	Agilent technologies	Back to the Future – Method development for high resolution Liquid Chromatography
Dr Denise Wallworth	Sigma Aldrich	Suitability of Ascentis Express Fused Core™ Columns for Rapid Screening of Pharmaceuticals
Dr Przemek Stasica	Shimadzu	Solid Phase Trapping as a Generic Compound Isolation Step in the Chromatographic
Dr Brian Freer	Chiral Technologies	More New Chiral Phases from Daicel – Immobilised for Preparative Productivity and Low Particle Size for Screening Resolution and Speed
Dr Doug McCabe	Waters	Small Particles - The Big Picture Explained
Dr Monica Dolci	Thermo Fisher Scientific	Exploring the extremes: LC separations with 10mm and 200mm Length Columns packed with 1.9 µm Particles
Dr Einar Pontén	Sequant	Fast Hydrophilic Interaction Liquid Chromatographic Separations on Bonded Zwitterionic Stationary Phases
Dr Xiaodong Liu	Dionex Corporation	Mixed-Mode Stationary Phases and their Applications

The Scientific Program

Dr John Lough opened the spring symposium with a welcome address. He commented on the wide range of topics covered in the programme with something to interest everyone. He also gave particular thanks to the vendors for their financial support of the meeting, Agilent in particular, and Hichrom for helping to organise the arrangement at the venue. John then went on to introduce the first keynote speaker, Ron Majors, who had recently been awarded the Chromatographic Society "Martin" medal award for his contribution to separation science.

Session 1- Current state-of-the art and the future

Keynote Lecture 1: Dr Ron Majors (Agilent Technologies, USA) spoke on the subject of 'Considerations in High-Throughput and High Resolution HPLC'. First he outlined the drivers for improvements in column technologies which included the need for productivity gains from faster separations and rapid method development, improved quality of analysis (more reproducible columns giving better peak shapes) and the challenge of biomolecules. His suggested approach to increasing throughput included the use of small sub 2 µm particle porous silica, superficially porous (pellicular) phases, monoliths and the use of parallel LC systems. He took the audience through the theory of chromatographic resolution and showed how the separation could be maintained using shorter columns with the use of smaller stationary phase particles. However, he also explained that whilst column efficiency is inversely proportional to the particle size, the pressure drop across the column is inversely proportional to the square of the particle size. Therefore the practical limitation of reducing porous silica particle size is the increased pressure drop across the column.

The core of Dr Major's presentation was on the application of porous shell pellicular packings, firstly using a practical example of the separation of four proteins using a Poroshell 300SB-C18 5 µm column. The column was able to sustain both efficiency and resolution when the mobile phase was increased from 0.5-3.0 mL/min. His explanation for the flattening of the Van

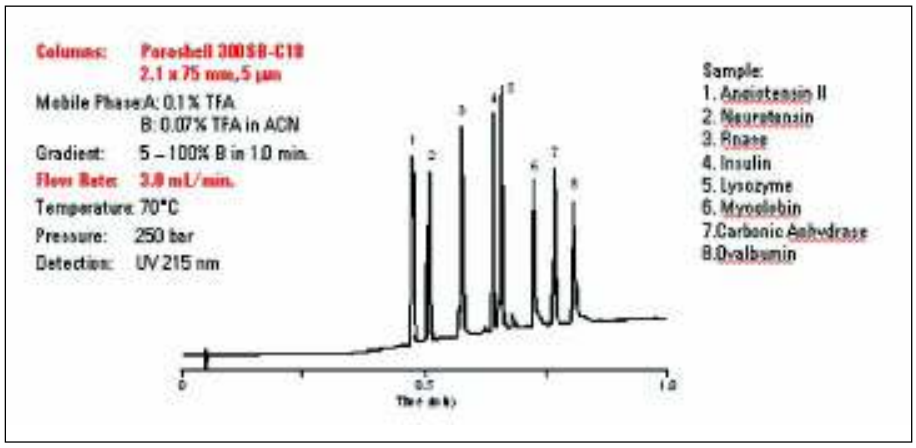

Figure 2. Example of high flow rates achievable on an Agilent Poroshell C18 column for high resolution separation of proteins from Dr Major's presentation.

Deemter equation was that, by restricting the diffusion of the analytes into the stationary phase, the C-term of the equation is reduced allowing efficiency to be maintained at higher flow rates. Thus, for large molecules, the use of the 300 angstrom porous shell material, with elevated column temperatures to increase the diffusivity of the anaylte and flow-rates of up to 3 mL/min, fast efficient separations are possible. However, the benefits of superficially porous particles are not restricted to large molecules. The application of a Poroshell 120 2.72 µm column to the separation of small molecules was shown. The column was capable of similar efficiencies as the sub 2 µm particle porous silica with a 40-50% reduction in the back pressure.

In the next section of the talk, Dr Majors described the characteristics of silica-based monolith phases relative to packed microparticulates columns. He showed a SEM picture of a PEEK encapsulated monolith illustrating very elegantly how this process has overcome the problem of column wall effects. The final part of the talk covered the use of multiple channel HPLC systems allowing rapid column screening in method development and high-throughput sample analysis.

Keynote Lecture 2: Prof. Gert Desmet (Brussels Free University) spoke on the subject of 'Support morphology and chromatographic performance'. A discussion of the general rules governing stationary phase shape and structure and its performance was provided and exemplified with illustrations and experimental data. He then went on to paint a picture of where he saw the future development of stationary phases heading.

Prof. Desmet began with a discussion around the characteristics of particle size and shape and also emphasised how the permeability of a column drops as an inverse of the square of particle size. He described the use of kinetic plots and showed that there is still a place for large particles (5 µm) where a separation requires a large number of plates. However, column theory predicts that using perfectly ordered systems, e.g. 3-dimensional ordered monoliths have the potential to improve efficiency. He went on to discuss porous shell particles. His explanation for the higher efficiency separations relative to similar sized porous particles is the narrower size distribution. This allows more uniform packing of porous shell columns with a subsequent reduction in the A (multiple flow path eddy dispersion) term of the Van Deemter equation. He also noted that although there is a benefit from utilising fused core particles in terms of mass transfer (a reduction in the C term in the Van Deemter equation), this only accounted for approximately a 10% reduction in the plate height. He commented that, for small molecules, he felt that this has more impact on column efficiency than the resistance to mass transfer, which was the explanation used by most of the manufacturers.

His vision of future developments was for chemically anodised etched pillar array channels with frits that aid radial diffusion of analytes onto the head of the column. This he illustrated with some fascinating examples captured on digital film. This also demonstrated a minor limitation of the pillar array which exhibits faster flow velocity at the walls of the separation channel compared to the centre which can have a minor effect on band broadening. He concluded the talk commenting that the optimisation of support morphology still holds high expectations for improvements with pillar arrays having the potential to eliminate eddy dispersion. The 2.5 µm porous shell particles are as efficient as <2 µm porous particles when used in optimal column lengths and that there was little advantage in driving particle size below the 1.7-1.8 µm already achieved.

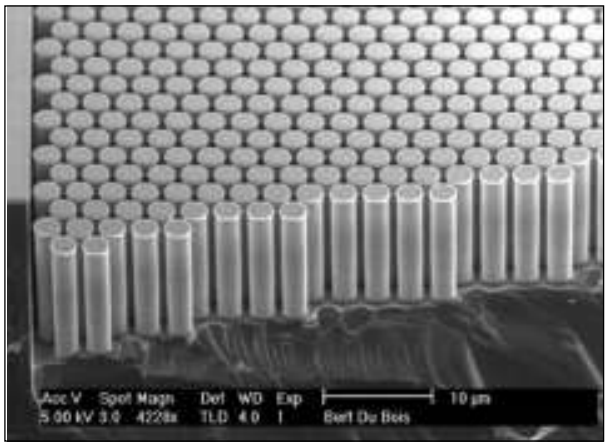

Figure 3. Photograph of 3D channel pillar array from Prof. Desmet's presentation.

Session 2: Stationary Phase Characterisation

Prof. Mel Euerby (Hichrom, UK) – LC Stationary Phase Characterisation for "Dummies!" Prof Euerby gave an overview of the different stationary phase parameters stressing the importance of stationary phase characterisation when there are 65 classes of columns and about 750 reverse phase materials worldwide. He then presented the Euerby & Peterson protocol for characterising columns which is a modification of the Tanaka protocol. The column probes are used to determine stationary phase variables of surface coverage, hydrophobicity, steric selectivity, hydrogen bonding capacity and ion exchange capacity at pH <3 & >7. Principle component analysis is then used to plot the column variables and hence characterise the column. By using the associated loading

plots, it was possible to relate column characteristics to analyte functionality e.g. polar embedded columns were shown to be the most shape selective of the tested columns. He concluded by saying that phase characterisation is an important tool as it enables analysts to select the best column for a separation, allows rapid method development, indicates an equivalent phase for method development, assesses column ageing and enables column manufactures to design new and novel phases.

Prof. Roman Kaliszan (University of Gdansk, Poland) – Molecular mechanism of HPLC retention and column classification in view of QSRR. Prof. Kaliszan concluded the session with a presentation showing the theory behind an alternative approach to column characterisation using Quantitative Structure Retention Relationships, Multi Linear Regression and a different set of probe analytes but with a similarly powerful endpoint.

Session 3: Industrial Perspective

Dr Melissa Hanna-Brown (Pfizer, UK) – 'The Pursuit of Ultra High Resolution in Pharmaceutical Development'. Dr Hanna-Brown's presentation included a discussion on the importance of resolution versus speed, column efficiency, the impact of UHPLC in pharmaceutical analysis and the role of selectivity in column resolution. Through a comprehensive explanation and use of kinetic plots, she presented the theory, illustrated with practical examples, on the impact of stationary phase particle size, column temperature and pressure on efficiency and speed of analysis. She concluded in saying that UHPLC makes high speed separations easily achievable, and when used in combination with higher temperature, is a powerful tool for achieving high resolution. Whilst UHPLC method development strategy is no different to HPLC it does make it faster when used in combination with orthogonal screening methods and optimisation software. She also reminded people that, despite all the advances in column efficiency, selectivity still remains a critical factor in achieving high resolution separations.

Dr Tony Edge (Astra-Zeneca, UK) – 'Stationary Phase Selectivity in High Temperature UPLC'. Dr Edge's presentation centred on the use of temperature to effect column selectivity and the use of hot water (>100oC) as a mobile phase with small particle columns. He illustrated this technique with some impressive practical examples of separations using isothermal, thermal and isobaric thermal gradients.

Prof. Peter Myers (University of Liverpool, UK) – 'Alternative Stationary phase Supports – Why Use Silica?' Prof Meyers started his presentation by listing the inadequacies of silica as the stationary phase support, then suggesting that graphitic carbon and alumina can offer specific advantages. He then went on to describe Dynamic Field Gradient Focusing Chromatography which requires no stationary phase at all. In this technique an electric field gradient is established in a narrow channel with buffer pumped through the chamber at a constant rate separated by a porous membrane. Analyte selectivity is then achieved by a combination of interaction with the hydrodynamic flow in the first dimension and the electrostatic field in the second dimension.

Dr Frederic Lynen (University of Ghent, Belgium) – 'Thermally Responsive Polymers'. Dr Lynen gave a thought provoking talk on a class of stimuli (temperature) responsive polymers that demonstrate changes in polarity with temperature. As the temperature of a separation is increased, through a combination of solubility, hydrophobicity and swelling changes in the polyacrylamide polymer, reversed-phase retention is increased. Similarly, the addition of salt (NaCl) to the mobile phase also increased analyte retention. A number of practical examples were shown to illustrate the phenomena and also how these phases could be utilised as a 'green' solid phase extraction media..

Dr Richard Ansell (Leeds University, UK) – 'Customised stationary phases prepared by molecular imprinting'. Dr Ansell began his talk with an elegant explanation of the production of a molecular imprinted polymer (MIP) using the template (target analyte) and a monomer. After the polymerisation process, which encapsulates the analyte, the template is washed out of the polymer leaving a 3 dimensional site with selectivity for the analyte molecule. Although the polymer efficiency is low, it is the selectivity which gives the potential for use not only in chromatographic applications but also for SPE, sensors, catalysts, drug delivery and antibody mimics in competitive binding assays. He went on to describe how, by modifying the polymerisation recipe, it is

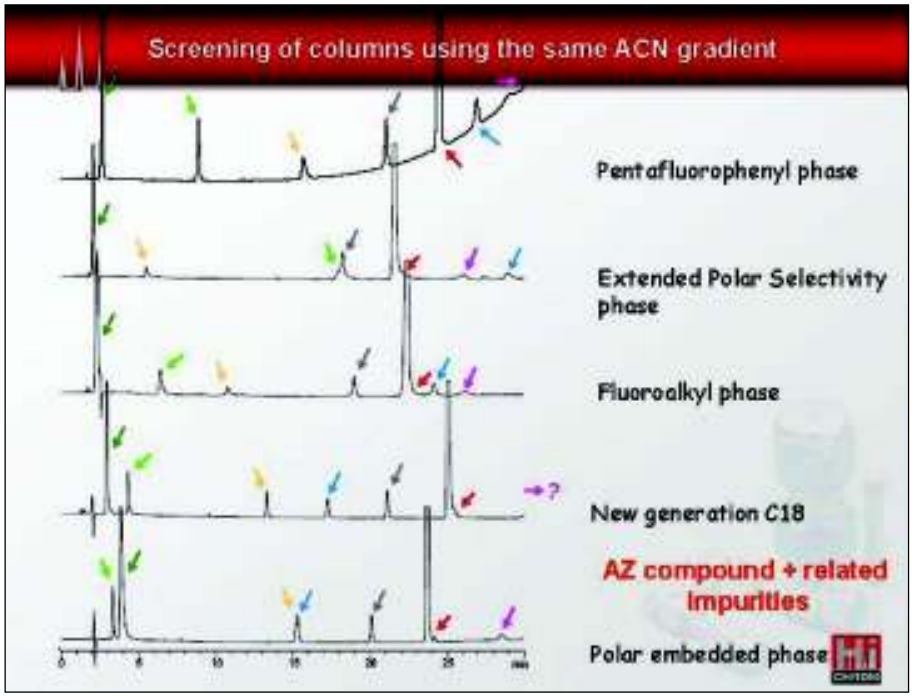

Figure 4. Illustration of the effect of changing column chemistry on selectivity of a pharmaceutical API and its related impurities taken from Prof. Euerby's presentation.

possible to influence the strength and homogeneity of binding sites. SFC results in better resolution using MIPs compared to HPLC, as the CO2 'mobile phase' offers greater permeability, reaching the binding sites other mobile phases can't. With >500 papers published/year over a number of application areas in recent times, this is clearly a technique with enormous potential.

Dr Zhengjin Jiang (Novartis, UK) – 'Monoliths for small molecule separations'. Dr Jiang described how the structure of large through pores and small mesopores provides fast mass transfer characteristics, resulting in high permeability without sacrificing column efficiency. After describing how columns are prepared, he went on to illustrate their use with a number of applications in the areas of vitamins, carbohydrates, phenols, nucleotides, chiral molecules, amino acids, PAHs and basic compounds. He concluded by saying that competition between monoliths and conventional particle columns will continue to drive the development of both phases at pace.

Advances in Stationary Phases for Liquid Chromatography – closing comments

The most enduring image that most people will take from the meeting was that of a glimpse on how the future of chromatography might look with Prof. Desmet's pictures of perfectly ordered, micro-machined pillar arrays offering the potential to produce chromatographic supports with an optimal compromise between minimal diffusion distances and maximal bed permeability. Whilst that might be the future, there were plenty of impressive applications of what can be achieved today with both small porous and superficially porous particles and monolithic columns elegantly explained with a multitude of Van Deemter and kinetic plots. John Lough's opening comments were very adequately met with a wide range of topics covered, with some excellent presentations, and something to interest everyone.