"Advances in Separation Science" The Chromatographic Society Spring Symposium 2011

Held at Novartis Institute for Biomedical Research, Horsham, West Sussex, Wednesday and Thursday 11th-12th May 2011
Review by Pauk Ferguson and John Lough

As delegates weaved their way through leafy West Sussex on the morning of 11th May it was a bright sunny day. These were gloomy times though for R&D in major UK pharma but to all intents and purposes it was just like any other ChromSoc Spring Symposium at a major pharmaceutical site. There was a buzz in the air with just over 100 delegates attending and lively networking taking place in the exhibition area (15 exhibitors and/or sponsors: Waters (Gold Sponsors), Agilent Technologies, ARC Sciences, Chemputeam SA, Chiral Technologies, Crawford Scientific, Dionex (UK), Dynamic Extractions, Gilson Scientific, Hichrom, International Labmate, Sigma Aldrich, Thermo Fisher Scientific, TTP LabTech, VWR International) as everyone eagerly anticipated the next day-and-a-half's proceedings. The theme was "Advances in Separation Science" but given the nature of the venue the emphasis was on advances in separation science apertaining to early Drug Discovery.

The show began with the heavy artillery up front. ChromSoc Martin Medallist, Professor Wolfgang Lindner (University of Vienna, Austria) gave the opening lecture on "Enantiomer Separations with Chiral Ion Exchangers, a Unique Class of Chiral Columns". Professor Lindner began with an elegant philosophical discourse on enantiomer separations, covering multiple-site interactions, modelling and the free energy differences needed to bring about some enantioselectivity and the need to strike a balance so that stereospecific interactions were not swamped by non-stereospecific ones. He continued with a justification for the use of quinine/quinidine basis for the range of niche' chiral anion-exchangers that his group has produced over the years (commercially available e.g. as QD-AX from Chiral Technologies). These show broad enantioselectivity for chiral organic acids. Buffer salts in the mobile phase act as competing ions and accordingly do not affect enantioselectivity but can be used to moderate retention. In general, if an optimised chiral separation is obtained for one organic acid, these conditions tend to be less suitable for other organic acids. He described how, since carbon dioxide acts as a weak acid, some of these phases, such as WAX QD-1, can be used for chiral SFC without any modifiers in the mobile phase. He then went on to describe how his group had used the principle of microscopic reciprocity to develop chiral cation exchange phases. These have been shown to be useful for amino-alcohols such as clenbuterol and for some very polar chiral compounds. In the final phase of the presentation, Professor Lindner described the most recent of his groups chiral stationary phases (CSP) that had been commercialised i.e. zwitterionic phases containing a sulphonic acid group as well as a quinine/quinidine moiety. These work for acids, bases and zwitterions, albeit the cation exchange phases work better for bases. The zwitterionic CSP have been used for screening amino acids and give particularly good enantioseparations for derivatised amino acids. When the sulphonic acid group is on an extended chain, chiral separation may be had for peptides and peptidomimetics where the chiral centres are quite far apart. A final application described was the use of the zwitterionic phases for the study of conformational isomerism.

Novartis, Horsham

Also in the opening session, Professor Jeremy Glennon (University College Cork, Eire) presented on " Recent Progress in Supercritical Functionalisation and

Superficially Porous Particles" utilising a very striking video of the supercritical fluid. This solvent-free form of functionalisation had been used to make, for example, fluorinated phases. In a wide-ranging talk covering the work of his group since ~2002, Professor Glennon covered green issues, functionalisation of silica hydrides, the use of mercaptopropyl silica, the importance of uniform surface coatings, the supercritical fluid preparation of a quinine CSP and the construction of nanostructures. The group have now moved on to prepare fused silica particles by building up layer by layer using seeded growth. This product has been named "EireShell"!

Continuing on the SFC theme, Dr Andy Aubin (Waters, USA) described " Harnessing the Power of Sub-2 µm Chromatographic Particles in Supercritical Fluid Chromatography". Waters had taken over the Thar Technologies SFC operation but evaluating the Thar instruments in 2009 the true benefits of sub-2 µm particles could not be observed because of the system volumes involved. Waters therefore set about designing a whole new SFC system based on similar concepts that had lead previously to the ground-breaking Acquity UPLC system. This new Waters UPSFC system can fully utilise the benefits of sub-2 µm particles (although it was noted that this does not apply fully to chiral phases) and is being promoted as an alternative to NPLC. Aubin noted that increasing pressure from increasing flow rate has an effect on mobile phase (CO_2) solvating strength, even under constant back-pressure conditions (isobaric). This implies that if a van Deemter plot is constructed for a certain column and the flow rate is varied (retention factor is usually held constant by adjusting the % organic in the mobile phase), measurement of peak efficiency and thus H may not be representative. Waters suggested that a more appropriate measure might be to use isopycnic (constant mobile phase density) conditions to mitigate this effect.

Other presentations on SFC were from Dr Pilar Franco (Chiral Technologies, France) on "Super Critical Fluid Chromatography Screening Strategies for Chiral Separations" and from Dr Denis Berger (Chemputeam, Switzerland) on "Aurora SFC". Dr Franco proposed that a primary chiral screen should utilise the IA, IB, IC and ID columns (all immobilised polysaccharide selectors) which are compatible with a much greater range of solvents than the older non-covalently bonded materials. A secondary screen would include AD-H, AS-H, AY-H, AZ-H and the 'O' equivalents. Butylamine was proposed as an alternative to diethylamine (DEA) for optimisation of chiral amine separations. (Work on a similar theme to that of Dr Pilar's talk is described in the paper "Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical discovery environment" (L. Miller and M. Potter J. Chromatogr. B 875 (2008) 230-236). Dr Berger indicated that for most SFC systems, pumping less than 8% organic is still difficult for current SFC pumping technology (possibly also the case for the Aurora system). He highlighted 'SuperSep' instrumentation for recycling carbon dioxide in preparative scale separations.

Post-dinner the chromatographic conversations flowed into the night

Before the afternoon tea break Tony Taylor (Crawford Scientific, UK) provided a brief respite from SFC. Tony described the latest in stationary phase developments from Agilent in a talk entitled "Agilent Technologies Poroshell 120: Superficially Porous Particle Technology for Increased Sample Throughput and Resolution in a Diverse Range of Applications". One of his principle themes was that the assessment of efficiency per unit pressure was a good way to evaluate columns. He highlighted this by presenting examples with coupled columns.

After the break it was back to SFC but Dr John Langley (University of Southampton, UK) "Achiral SFC-MS and Small Molecules – An Analyst's View" did provide something a bit different, as his title suggests. John discussed the need to use a T-piece in a make up flow of liquid prior to the source capillary to prevent freezing of the capillary due to carbon dioxide expansion. His group typically uses 5 mM ammonium acetate for the make-up flow (see J.D. Pinkston et al. J. Sep. Sci. 27 (2004) 115). He described phenomena whereby ionisation of analytes was observed with the MS CV turned off in both APCI and ESI modes, which is thought to be due to a 'sonic spray' process.

The final talk of the afternoon, "Remote Open Access – The Lab2Lab™ Advantage" was given by Dr Brian Everatt of the host company, Novartis, UK. Brian described an automated sample delivery and analysis

Delegates at symposium dinner

L to R: Speakers McCalley, Lindner and (seated) ChromSoc Vice-President, Paul Ferguson

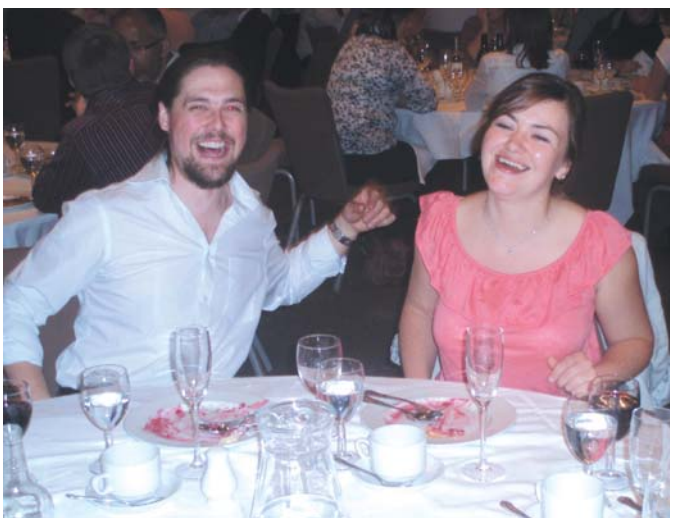
sytem for open-access chemist use. Samples are input with a small amount of data on a terminal linked to the L2L system. The sample vial is placed in a holder and placed into the loading unit which is moved through tubing via compressed air to a router. The router is intelligent enough to know which systems are idle and send the sample to an appropriate instrument for analysis. This system is scalable to any lab size. In tours the following day, delegates were able to view this system in operation.

In the evening the majority of the delegates attended a dinner at the nearby Ghyll Manor Hotel. A convivial time was had by all. While this year the assembled throng was spared an after-dinner speech, ChromSoc Vice-President, Paul Ferguson, addressed to group to say a little about the Society and to thank the speakers and sponsors.

The opening sessin of Day 2 was a tribute to the late Dr Uwe Neue and featured speakers who had worked closely with him over recent years before his untimely death in December, 2010. Uwe's colleague Pam Iraneta (Waters, Chemistry Operations, USA), "Eulogy to Uwe Neue - Key chromatographic contributions" highlighted the contributions of Uwe Neue to chromatography and in particular to the Waters business.

Professor Peter Myers (University of Liverpool, UK) also talked fondly of his associations with Uwe Neue and in the spirit of their mutual love for innovation gave a talk on "HPLC on a Compact Disc". Channels are etched into a compact disc and capillary tubes packed with silica are placed into the grooves. The sample is placed in a chamber and is held behind a break valve which is melted by the standard CD player laser. The sample is moved through the column using centripetal force and is detected using the standard CD laser detection which identifies compounds leaving the column as 'errors'. These error counts can be converted into something like a chromatogram through digitisation.

James Heaton (Kings College London, UK) is a final year research student in Norman Smith's Waters-supported group at Kings and, as such, had frequently been mentored by Uwe Neue. In his talk "Investigation into the effect and utility of temperature in ultra high performance liquid chromatography" James described the importance of temperature on the 'C' term of the van Deemter equation (mass transfer kinetics). He suggested that the volume of the Polaratherm pre-heater is too large for efficient mobile phase heating. For efficient heat transfer in/out of the column, a large surface area to column volume ratio is required which is achieved by decreasing the column i.d.. When moving from a 0.084" (standard) to 0.026" column wall thickness an increase in retention was observed for some molecules with increasing temperature which was attributed to a change in the partial molar



Symposium dinner was notable for good attendance of student delegates

volume of the analyte (essentially desolvation of the molecule making it more hydrophobic).

Dr David McCalley (University of the West of England, Bristol, UK) gave a moving eulogy to Uwe Neue and in his talk on "Are superficially porous particles a viable alternative to sub-2 µm particles for fast, efficient analysis in HPLC?" frequently cross-referenced to his discussions on separation science with Uwe. He discussed the effect of increasing flow (and pressure as a consequence) on the retention of analytes. For neutral molecules a 2-12% increase in the retention factor (k) was observed when the pressure increased by 500 bar. Charged basic analytes (e.g. nortriptyline) exhibited a 35-51% increase in k with the same pressure change. The greater effect on the charged analytes is thought to be due to the more extensive hydration of these analytes compared to neutral molecules. This observation may have an impact when moving from HPLC to UHPLC methods. In the second part of his presentation he discussed the significant loss in efficiency when 0.21 and 0.46 mm i.d. columns are used on standard HPLC instruments compared to UHPLC instruments. For a 0.46 mm id column an efficiency loss of around 6% was noted, but for a 0.21 mm column, this was nearer 20%. This was due to extra column band broadening effects form the HPLC system. He rounded out his presentation by discussing fused core particles and demonstrated that 0.21 mm i.d. Kinetex columns had a worse hmin than 0.46 mm columns. He also demonstrated that Kinetex columns could be easily overloaded at sample concentrations as low as 1000 ppm. Details of this work may be found in: D.V. McCalley et al J. Chromatogr. A (2008) 1209 95; D.V. McCalley J. Chromatogr. A (2010) 1217 276; D.V. McCalley et al J. Chromatogr. A (2007) 1169 125; D.V. McCalley J. Chromatogr. A (2011) 1218 2887

The session before lunch was given over to vendor presentations. Dr David Keay (Dynamic Extractions, UK) , "Maximising both purity and recovery in preparative liquid chromatography" discussed high performance counter current chromatography (HPCCC). The technique can be thought of performing multiple, high-speed liquid-liquid extractions where the aim is to separate compounds by varying the selectivity of the liquid mobile and stationary phases rather than efficiency. Dr Ken Cook (Dionex, UK) presented in his usual lively style on "Automated, High Resolution Therapeutic Protein Analysis" , inevitably touching on ion-exchange separations, an area of some expertise for Dionex. Simon Lambert (ARC Sciences, UK) discussed Scherzo multi-mode LC phases both extolling the virtues of multi-mode operation and highlighting the impressively high efficiency e.g. Imtakt C18 (3 µm) – 200,000 N/m. In the final talk before lunch Stephan Altmaier (Merck Millipore, Germany) described the latest developments in his company's work on monolithic silica HPLC columns (Chromolith 2nd generation and Chromolith capillaries).

Suitably invigorated by lunch, networking, viewing exhibitor stands and, not least, the fascinating ChromSoc AGM, delegates settled down to the final session which consisted of presentations by speakers from Discovery phase scientists from major UK pharma companies i.e. GSK and Novartis on the theme of purification strategies. Dr Bob Boughtflower (GSK, UK) discussed GSK's long-standing collaboration with Shimadzu to develop an automated purification system where crude Discovery compound are input and >99% pure material is output in a short time period. This had moved on considerably since Bob first described this initiative at a ChromSoc Prep meeting some years ago. It is now well established and has led to some useful developments in retentive stationary phases. Dr Jenny Kingston (Novartis, UK) discussed Novartis (Horsham's) strategies to support their medicinal chemists. This includes principally RPLC, SFC and HPCCC platforms. In their experience the Phenomenex diol HILIC phase performs better than ethylpyridine phases, but the latter does offer complementary selectivity. In another contribution from GSK, the use of counter-current chromatography in Development as opposed to Discovery was described.

In his closing address ChromSoc President Alan Handley reflected upon yet another highly successful Spring Symposium held at a major UK pharmaceutical R&D site. Let's hope that it is not the last!

New Aromatics and Saturates Analyser

Separation Systems, Inc is proud to introduce a powerful new analytical system to simultaneously determine both the physical and chemical characteristics of petroleum streams with a boiling point range of 200 - 1100°F (121 – 593°C). The system utilises a proprietary programmable sample inlet, GC/FID and GC/MS along with the company's industry leading SimDis Expert® (v.9) simulated distillation analysis software. Samples are introduced to the inlet and then homogeneously passed onto a high performance capillary column. The sample components eluting from the column are then precisely split utilising novel micro-fluidic technology and directed to the FID and MS detectors; simultaneously. Through the use of the ASA Expert (Aromatic Saturate Analysis) functionality embedded in the SimDis Expert® software, sample data is obtained from both the GC/FID and GC/MS data systems and combined into a single 'environment' that dramatically simplifies data acquisition, analysis, review, and report generation. Compositional results are characterised and include: 4 saturates classses, 12 aromatic classes, 3 thiophene classes and 6 aromatic species. The system eliminates the need to perform any physical separation of the hydrocarbon fractions (ASTM D2549, D2786 and D3239) saving both time and money. Critical cut points and subtle changes in stream composition are quickly determined for faster, better decisions. Separation Systems, Inc is a highly experienced provider of both turnkey and customised GC and GC/MS analysis systems for the biofuels, fossil fuels, petroleum, petrochemical, chemical, industrial gases, and power industries.

For more information visit: www.separationsystems.com