

A Century of Separation Science — Celebrating the Past: Predicting the Future



1–2 April 2004, Royal York Hotel Conference Centre, York, UK.

This meeting was conceived to honour 100 years of chromatography as we have come to know it today. Credit must go to the RSC C&E Group Committee under the chairmanship of Tom Lynch, for providing the organizational powerhouse and also the idea for a retrospective/prospective approach to help ensure those twin essentials: sponsorship and delegates. In the event there were some 130 delegates at all career stages, two major sponsors (Agilent and Waters), and 12 other sponsors (The Chromatographic Society, Advanstar Communications (*LC•GC Europe*), Anatune (ATAS), Cohesive Technologies, Dionex, Greyhound Chromatography, Polymer Laboratories, SciMed, Siemens Applied Automation, Sigma Aldrich, Thermo Electron and Varian). As the President of the Chromatographic Society, Dr Chris Bevan (GSK), generously acknowledged when summing up the meeting, to his delight, had been a great success on every level. There were many calls for the formula to be repeated in the years to come.

Dr Leslie Ettre was to have given the opening talk — Separation science in the first half of the 20th century — but he was unable to be present because of illness and Ted Adlard ably stepped into the breach. After paying tribute to Tswett (1903), who had introduced the use of particles packed in pressurized, small diameter columns, the work of Leroy Palmer in the USA and Edgar Lederer who worked in Heidelberg in the 1930s on the separation of pigments in egg yolk was reviewed. The seminal paper of Martin & Synge (1941), who propounded the use of small particles as well as introducing the concept of height equivalent to a theoretical plate (HETP) and predicted the development of gas chromatography (GC), was then discussed. In the 1940s a considerable amount of work was performed on gas-solid chromatography but it was not until the work of James and Martin on gas-liquid chromatography in the early 1950s that the technique then revolutionized and the age of instrumentation was introduced. Ted's account of the development of GC was published recently in *Chromatographia*.¹

Professor John Knox (University of Edinburgh) then gave an erudite account of the development of high performance liquid chromatography (HPLC) interspersed with details of his own contributions. The highlights are well known: The work of Martin & Synge (1941); the publication of the van Deemter equation (1955); the introduction of HPLC (Huber & Horváth, 1965, 1966); microparticulate packings (Kirkland, 1972) and finally the era of electrochromatography and miniaturization ushered in by Jorgenson (1981) at the "coffin slot" at the Avignon HPLC

meeting (to your reviewer's everlasting shame he confesses to enjoying Muscat de Beaumes de Venise in the main square in Avignon with a group of Denis Desty's BP colleagues at the time). As a PhD student at Cambridge in 1951, John had been asked to look at the separation of aldehydes and acids. Having read a paper by Howard Purnell (1952) on gas chromatography, Knox and Purnell became friends, but a paper they wrote in 1953 on GC separation of butane isomers using temperature programming was rejected as too speculative! Knox began publishing his series of papers on the mathematical basis of liquid chromatography in the late 1960s (Knox & Pachett, 1969; Knox & Saleen, 1969), culminating in the publication of the famous "Knox equation" (Knox & Laird, 1975). The introduction of carbon-based HPLC packings was another notable achievement (Ross & Knox, 1997). In discussion, Ted Adlard pointed out that A.J.P. Martin had attended a lecture at Cambridge by Kuhn in the 1930s so it is probable that Tswett's work became known to him at that time. A further comment elicited the response that 4.6 mm i.d. stainless steel tubing (as used in standard HPLC columns) just happened to be 0.25 in. o.d., the standard US central heating tubing.

Future trends in micro LC and capillary electrochromatography (CEC) were reviewed by Professor Peter Myers (X-tec). As a later speaker emphasized "it's difficult to make predictions, especially about the future" (the audience were divided as to the correct attribution of this quotation, but many favoured Homer Simpson). Nevertheless, Peter gave a lucid presentation of work in progress. Much time was given to the hybrid Si-C bonding technology used in the Waters Acquity stationary phase (2004) that uses 1.7 μm particles (Ultra-performance liquid chromatography, UPLC) to give higher efficiencies at high flow-rates (4 mm s^{-1} linear velocity), but with the penalty of increased back pressure. The Acquity system, which won the Gold Award for best new technology at Pittcon this year, was the subject of a presentation by Dr Jeff Mazzeo of Waters later in the day. The polyethoxysilane (Si-C) technology used in making the stationary phase was accompanied by re-engineered instrumentation designed to cope adequately with fast, high efficiency separations. It was observed, in passing, that the Swagelock hydraulics connection system dated from 1947 hence was somewhat outdated. Peter also emphasized that spherical particles may not be ideal as packing materials, an oval rugby-ball shaped particle having better packing characteristics. It was speculated that the Vydac material might have such a shape.

Professor David Perrett (St Bartholomew's Hospital Medical School, London) and Dr Kevin Altria (GSK) gave talks on classical

and capillary electrophoresis, respectively. David noted that electrophoresis was the senior science as compared with chromatography, the first description of the separation of colloids in an electric field being that of Reuss (1807) whilst the terms anode and cathode were coined by William Whewell in the 1820s. The term electrophoresis was first used by Michaelis (1909). In the 1930s Arne Tiselius working at the Karolinska Institute exerted a major influence on the development of the technique. He received the 1948 Nobel Prize for Chemistry in recognition of his work. David then listed subsequent developments in media, detectors and instrument manufacturers. The introduction of polyacrylamide gels (PAGE) by Raymond & Weintraub (1959) and the use of the SDS/PAGE technique to ascertain the formula mass of proteins (Shapiro et al., 1967) were important landmarks. Nowadays electrophoresis remains important in clinical analysis and also in proteomics and genomics, as many as 10 000 proteins being identifiable on two-dimensional PAGE systems.

Kevin spoke of his early days in capillary electrophoresis (1985/6) when he used the same power supply as used in the Harrier jump jet to power its radio! From some 10 publications in 1985 CE has expanded enormously, the human genome project (87% of the analyses performed using CE) being a major force driving development of the technique. UV and mass spectrometers were the detectors of choice. CE applications had been quick to develop. Nowadays CE/CEC was used widely with chiral separations using additives such as cyclodextrins being an

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important area. MEEKC (micellar encapsulated electrokinetic chromatography) applications were also widely used. Arrays with 96 capillaries were available. Nevertheless, because of the very small sample capacity of CE, concerns to enhance sensitivity remain; loop injectors and injector focusing systems seeming capable of 10 000-fold sensitivity enhancement for certain analytes. Further miniaturization was another goal.

To add to the diversity of the first day, Rob Sample (Agilent) gave an instrument manufacturer's view. The (renovated) garage where Bill Hewlett and Don Packard had begun to make instruments in 1939 (367 Addison Avenue, Palo Alto) was now a Californian Historical Landmark and looked on as the origin of Silicon Valley. HP had acquired the GC manufacturer F+M Scientific Corporation in 1965. In 1970 the 5930A dodecapole MS was introduced and LC manufacturers Hupe & Busch (founded 1963) were acquired in 1973. In 1979 the first diode array HPLC detector (8450A) and the first commercially available fused silica capillary GC columns were introduced. Instruments for SFC and CE/CEC followed in 1993. Recent emphasis had been on modular systems. A Zorbax 1.8 μm HPLC packing has been introduced this year. Future plans include cartridge-based "chip" HPLC systems using technology developed for ink-jet printers.

The garage also received a mention in the talk on thin layer chromatography (TLC) given by Peter Wall (Merck) — there are still those, it seems, who practise the art at home! Although the origins of TLC can be traced to the 1930s, it was not until the late 40s/early 50s that the technique became widely used. The term TLC was coined by Stahl (1956), who used a gypsum binder to give mechanical strength to silica layers (13% calcium sulphate - Silica

gel G). The term HPTLC was introduced by Halpop (1973). TLC retains the advantage that aggressive developing solvents and visualization reagents can be used that would never be allowed near HPLC columns. Bonded phases including some chiral phases, are now available and automated spotting and development equipment can be purchased. There are a wide range of operating modes (over-pressure, saturated/unsaturated tanks etc.) and an almost infinite variety of eluent combinations.

By way of contrast, Paul Humphrey (Thermo) discussed GC-MS. MS (Thompson, 1910) and electron ionization (EI) (Dempster, 1918) both antedated GC, but GC-MS (Gohlke, 1957) followed quickly from the description of GC, which was long regarded as the ideal sample introduction system for the mass spectrometer. The quadrupole MS was described at this time (Paul, 1954), with the ion-trap (Paul, 1956 — "Quistor") soon following. CI was reported a few years later (Munsen & Field, 1966). Paul & Romsey were awarded the 1989 Nobel Prize in Chemistry for their work on MS detectors. Although quadrupole (66% of sales) and ion-trap (25%) with magnetic sector instruments (5%) now dominate the market, Paul Humphrey was firmly of the opinion that time-of-flight (ToF) MS was the technique of the future. ToF was introduced by Stephens (1947) of Penn State University as the "Velocitron". It was first coupled to GC in the early 1960s (Bendix ToF) but suffered from severe mass discrimination. TOF instruments are, in principle, very simple in that all that is measured is ion velocity. Advanced timing electronics are now available as are benchtop instruments and coupled with very fast temperature programmes such instruments can give extremely short analysis times.

Comprehensive GC was discussed by Dr Ally Lewis (University of York). The concept of multidimensional GC was developed in the early 1990s by John Phillips and the technique expanded rapidly — peak capacities of 5000 were achievable, although figures of 20 000 and even 100 000 were perhaps attainable. Ally gave a number of examples of the use of the technique from his own research area of environmental toxicology, although in discussion Ted Adlard made the point that our ability to detect, identify and measure compounds in the environment has outstripped our ability to interpret the results in terms of health effects and other aspects of possible impact on the biosphere.

Professor David Goodall (University of York) next discussed enantiomer separations by LC and CE. Developments in this field have been especially important to the pharmaceutical industry. The work of Pirkle on chiral stationary phases (CSPs) was followed by the use of chiral additives, such as cyclodextrins and macrocyclic antibiotics, as exemplified by vancomycin. CSPs were established and gave high sensitivity and could be used in preparative mode, but selectivity might only be moderate. Nevertheless, mobile phase additives were very versatile and relatively inexpensive to use, but could not be used in preparative work.

Supercritical fluid chromatography was reviewed by Dr Lester Dolak (Thar Inc., USA). Lester carried on the theme of chiral separations in introducing his talk. There was much emphasis in Pfizer in reducing the time and cost of chiral separations: 600 enantiomer/diastereoisomer separations had been performed in 2002, many using SFC. Carbon dioxide had many advantages over other organic solvents, being safe, cheap and easy to purify/recover/remove/dispose of. It was now widely used in dry cleaning in the US. As compared to HPLC, SFC had the advantages of reduced organic solvent consumption, higher throughput, fast equilibration and was gentler on (expensive) CSPs. In addition higher efficiencies could be attained (50 000 plates can be

obtained from a 25 cm column).

Other presentations in the morning sessions included Innovations in new geometry LC–MS for proteomics (Dr M. Harrison, Thermo), Turbulent flow LC (Dr H. Quinn, Cohesive Technologies), Large-scale LC (Dr O. Ludeman-Hombourger, Novasep SAS), Inverse GC (Dr F. Thielmann, Surface Measurement Systems) and Size-exclusion chromatography (Dr G. Saunders, Polymer Laboratories).

In opening the final session, Professor Walt Jennings (Consultant to Agilent, USA) reviewed the early days of GC as regards capillary columns. Having paid due tribute to the early development of the technique in the UK, Walt reviewed the work of Denis Desty in producing coiled glass capillary columns and his own work with viscous polysiloxanes such as OV-101 as stationary phases. These columns were efficient, long-lived, relatively inert, reproducible, selective and had low bleed. However, surface deactivation to permit the analysis of polar compounds remained a problem — injections of extracts of bovine excreta were even tried! The problems were because of metallic impurities and surface silanols — acid washing was used to remove metals and silanization to deactivate silanols. Serendipity took a hand in 1979 — a GC heater malfunctioned when testing a column coated with SE54 stationary phase (1% vinyl) and the resulting high temperature led to a very stable column — vinyl cross-linking had been discovered. The downside from the manufacturer's point of view was that columns lasted too long! When fused silica capillaries came along (very inert columns) then the cross-linking technology really came into its own.

After Walt's retrospective, Tom Lynch (BP Chemicals) gave a high-speed tour of future trends in GC. Largely based on fused silica columns with electronic pressure/flow control, fast GC was now commonplace. High throughput, on-line analysis in process control was an important area that had been leading developments in the area of GC for the last few years. Recent innovations included multicapillary columns, vacuum outlet GC, flash GC, multidimensional GC, parallel chromatography and miniaturization. The Siemens Microsam process GC was a major advance using micro-machined detectors and "live" column switching and injection technologies to provide unrivalled performance — and all done using less power than a 40 W light bulb! There were now some 1500 process GCs on-line in BP Chemicals plants around the world.

Keeping to the same theme, high throughput LC–MS was discussed by Dr Keith Brinded (GSK). His group now maintained 11 LC–MS systems on user open-access. LC–MS had largely replaced TLC in reaction monitoring with generic methods having an average 6.6 min cycle time. He was allowed a 5 min response time in the event of instrument problems — it was possible to "repair" instruments from home via a datalink. There were some 200 users with 30 new users annually. The systems produced 0.5 terrabytes of data each year. The average waiting time for samples to be run was 15 min — 165 000 samples annually analysed. The HPLC columns were changed after every 1000 (now 500) samples. Use of preparative systems was also discussed — 30 000 preparative analyses had been performed in 2003. An analysis of the GSK compound databank showed that 1.2 million compounds were analysed in 18 months! GSK has now retention information on 500 000 compounds and aims to use the data to predict retention of future compounds. Breathtaking figures delivered at breakneck pace but what of the rest of the world that still struggles along with UV detectors, autosamplers etc.?

The pace then slackened with a presentation on current and

future trends in ion chromatography by Dr Chris Pohl (Dionex). After paying due tribute to the work of Hamish Small (1975), which was notable for the introduction of two innovations at once (suppressed detection and the accompanying low-capacity ion-exchange columns), Dr Pohl discussed future ideas. Polymer encapsulated cation exchange materials were designed to overcome the defects of silica and were finding applications in the analysis of methylamine and ethylamine, for example. Overall the aim was to develop IC that would be continuous with minimal eluent consumption, small hence portable, self-calibrating when not in use, automatically identify common analytes, assay compounds based on conductivity and use narrow zone high permeability media for high sample throughput without high eluent consumption. In discussion John Knox asked why Dionex had not tried graphite packings; Dr Pohl said he would do this.

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The last speaker was Dr Bob Boughtflower (GSK) who discussed the future of chromatography in the pharmaceutical industry, probably the single largest user of the technique in all its forms. The main desire was to see the "industrialization" of HPLC to make it less expert dependent/more robust. This required, in the short term, the introduction of generic methods, instrumentation, and column technologies. The next generation HPLCs need to be self-starting, self-checking and self-authorizing to produce calibrated data. In-built diagnostics should be able to certify an instrument as fit for purpose and to predict possible failure. Column technology could also be improved by a greater understanding of the processes occurring during a separation and this was illustrated by magnetic resonance imaging video footage of separations occurring inside a column and highlighting column defects. The use of a more suitable test mix to better characterize columns was also proposed. In the longer term there was a need for reduced method cycle times and increased peak capacities. This might be achieved by miniaturization and parallel operation and by real time monitoring of processes with feedback control.

There were other highlights. Dr Ian Wilson (Astra-Zeneca) kindly brought along some of his collection of old GC and HPLC apparatus and this provided a talking point for many. At the conference dinner Ted Adlard was presented with a life-time achievement award by Dr Chris Bevan. Last but by no means least, Dr Steve Haswell of Hull University held the audience enthralled with his after-dinner entertainment. The search is on for another anniversary to justify the next meeting...

Bob Flanagan

References

1. E.R. Adlard, 50 Years of Gas Chromatography, (Suppl): S-13AS-18, *Chromatographia*, 57; 2003.