Chromatography Today talks to David McCalley, Chromatographic Society Jubilee Medallist 2008

Following on from last year's interview with Keith Bartle, for the second in a series of interviews with Chromatographic Society medal winners Chromatography Today talked to David McCalley, University of the West of England who was presented with the Society's Jubilee Medal at HPLC 2008 in Baltimore .

How did you become interested in chromatography?

I took an MSc course in Analytical Chemistry at Bristol University after my first degree. Somehow it felt better learning about things that had an immediate and obvious application. Such feelings were reinforced with my project at one of the Bristol Hospitals, where I was using gas chromatography to develop methods used for the diagnosis of metabolic diseases in young babies. I left Bristol for a year to become a research assistant at Imperial College, using atomic absorption and plasma emission spectroscopy to look at metal pollution around the country as a result of natural geology and mining and smelting activities. It was great fun collecting the samples, but compared with the mysteries and uncertainties of the "black art" of chromatography, metals analysis seemed very straightforward. Developments seemed mostly governed by engineering advances by instrument companies. So I returned to Bristol to do a PhD, mostly using GC and GCMS to analyse sterols as tracers for sewage pollution. At the time, there was much interest in column production methods, and applications of the (relatively new) capillary columns. I still got to collect some samples, but not always from as scenic places as previously!

David (centre) in regal company, Dr Ron Majors (left) and Prof. Georges Giochon (right) at HPLC 2008.

You have worked on some of the fundamentals of chromatography, following some of the great UK researchers. Does this work interest you more than work on applications?

I was always interested in understanding why things happen and in trying to rationalise experimental results. Such work is more appealing to me than for example analysing a series of valuable samples by a routine method (even if they were Martian soil samples!). I am certainly not a theoretician and I envy people who are as comfortable thinking in equations as I am reading The Beano. However, it is important to understand the basic principles and predictions of theory, in order to rationalise experimental work. Chromatography has traditionally been an "inject and see what happens" technique - probably because it is such a complex subject. However, with knowledge of theory, it is possible to restrict considerably the number of such experiments, predict what is likely to happen and constructively optimise a separation.

How significant is the work that you have done on the effect of pressure on retention? Is this something the average chromatographer needs to be worried about?

The pressures available from some commercial instruments for work with small particle columns is up to three times higher than with conventional instruments. Clearly, any effects of pressure are going to be considerably magnified. It has been known for some time that within the pressure range of conventional HPLC, retention factors (*k*) could increase by a factor of 2 for proteins, but the effects were very small for the low MW non-polar species that were mostly studied. However, we were able to show a 50% increase in k for some ionised pharmaceuticals as a result of a pressure increase of 500 bar. We believe the increases are due to partial loss of the hydration layer as polar or ionised compounds enter the hydrophobic stationary phase in RP-LC. If such solutes are present in mixtures with small non-polar solutes, considerable changes in selectivity may result. Such selectivity changes may affect transfer of methods from larger to small particle columns. Our experiments studied pressure effects largely in the absence of frictional heating. In practical situations, this effect, which usually acts in the opposite direction to decrease retention, may moderate the effects of pressure alone.

You have done a lot of work concerning the poor peak shapes that are obtained with many pharmaceuticals and biomedically important compounds, which are basic compounds. Has this problem gone away with modern RP columns?

Older (Type A) silica RP columns possess strongly acidic silanols (due to the presence of metal impurities) that are ionised under typical RP mobile phase conditions. These groups can undergo detrimental ionic interactions with basic compounds that are also ionised, giving poor, often exponentially tailing peak shapes. Modern (Type B) columns are made from very pure silica which contains much more weakly acidic silanols, whose ionisation can be largely suppressed by working at acidic pH. These columns give far superior results to Type A columns, when small amounts of solute are injected. However, Type B columns also suffer from overloading when ionised solutes are introduced, often giving right angled triangle peak shapes. This overloading can occur when > 0.5µg of solute (equivalent to 10µl of a 50 ppm solution) is introduced on to 0.46mm ID columns with typical RP buffers. With low ionic strength buffers like formic acid, the problem is usually even worse, and occurs even with the most inert high purity phases. The problem could be due to mutual repulsion and ionic exclusion of ionised solutes, leading to a much lower surface area available for solute adsorption. However, an alternative is that sites of different energy exist even on these highly inert columns at low pH, and that the overloading of strong sites may be responsible for the poor peak shape. Certainly, interest in this area has stimulated work which has also informed our general ideas on the mechanism of RP separations.

You have done some work on fused core column - what is your opinion of them?

Fused core column have a porous shell surrounding an impervious centre. Unlike the earlier pellicular phases, the porous fraction is the majority of the particle volume, so these columns should not suffer seriously from reduced sample capacity, like their predecessors. These columns have back pressures similar to 3µm particle size phases, but higher efficiency, that appears to result more from their narrower particle size distribution and better packing, than improved mass transfer into the thinner stationary phase layer. Some reports suggest that these columns can produce 80% of the efficiency of sub-2µm particles with half the back pressure, although some of the latest columns give even higher efficiency. There certainly seems some truth in the claims that these columns can deliver UHPLC –type

Addressing delegates at HPLC 2008, Baltimore

performance on conventional instruments (using pressures up to 400 bar). Nevertheless, developments are coming extremely rapidly, with improvements also in sub-2µm phases recently being made. Even the sub-2µm columns, and instruments for their use, have been developed only in the last few years and the characteristics of both types of column have not been fully evaluated.

Do you think HILIC is going to become a more important technique - or is this just a passing fad? Is it really any different from normal phase (NP) chromatography?

My view is that Martin and James did the first HILIC-type separations in the 1940s - so this separation mechanism has been around for a long time! HILIC really is distinct from NP in that it uses a considerable concentration of water in the mobile phase - typically from 2.5% to 40%. In this way, the problems of the presence of very small variable amounts of water, that may be present in HPLC solvents or in the injected sample, are avoided. Water is a very strong solvent in NP and therefore these variable amounts produce irreproducible retention. The mechanism of HILIC seems more complex than NP, and may involve partition of solutes between a surface layer of water and the bulk mobile phase, ion exchange, adsorption on polar column groups, and even under some conditions, RP interactions. HILIC has particular advantages in the increasingly important technique of LC-ESI mass spectrometry, as the low viscosity solvents used lead to better liquid spraying and desolvation of the droplets. Low viscosity solvents also facilitate the use of long columns, the use of smaller particles and fast analysis (enhanced solute diffusion plays a part here). A big advantage of HILIC is the strong retention of polar and ionised solutes, which are difficult to retain in RP. However, while solutes tend to elute in the reverse order to that in RP separations, the order of elution can show no correlation in the two techniques, rather than a simple inverse correlation - producing so-called "orthogonal" selectivity. While HILIC is not as versatile as RP (for example there is little retention of non-polar solutes), I think its use is set to increase. A particular area of increased application might be in the quantitation of polar drug metabolites.

You attended the "Separation Science - State of the Nation" conference organised by the Chromatographic Society last year. What is your view on the state of our subject in the UK?

Firstly, this was a well-balanced and organised meeting. It was well attended, but I couldn't help noticing that only one representative from the major Pharma companies was present-and he was there mainly as a representative of the Chromatographic Society. Neither were there many university academics in Separation Science present, but as Peter Myers pointed out, there are few of us left! Altogether I left the meeting feeling pessimistic. You know, I was invited to speak at the AFSEP (Francophone association for Separation Sciences) conference in Marseilles last December. There were about 90 mostly excellent lectures in all aspects of Separation Science from delegates representing universities, industrial companies and research institutes. There were only two UK speakers - I guess this was understandable this time (it is usually the same even at Anglophone conferences - take a look at the programme for HPLC 2010 in Boston!!) as the whole conference was in French (my talk was in bad French!), with the delegates coming mostly from France, Belgium and Switzerland. However, I could not help feeling that it would be impossible to organise such a conference with entirely UK speakers - we no longer have the depth and breadth of research necessary, despite our population being not too different in size from that of the Francophone group. Continuing re-organisation, outsourcing of analytical work and rationalisation in the Pharmaceutical Industry has not helped matters.

What about the situation in our universities?

We now have only a handful of university research groups whose interests lie in separations *per se*, rather than their applications - even though the latter group are performing really important work. I believe the problem lies mostly with funding. University research is subject to periodic review through the Research Assessment Exercise, that has been conducted in 1992, 1996, 2001 and 2008. The next review (now called the Research Excellence Framework) will occur in the next year or two. At each successive exercise, the number of university chemistry departments choosing to enter the

David McCalley off-duty

contest has reduced, the number of new universities (which traditionally have incorporated significant analytical chemistry sections) entering the contest has also reduced (only 1 entered in 2008) and the number of universities that have significant numbers of Faculty members involved in analytical chemistry (let alone Separation Science) has reduced. The reasons for this are debatable, but the most recent assessment panel included only 1 member who is a recognised analytical chemist, the rest being from physical, theoretical, inorganic and organic chemistry. A poor performance (or "no-show") in the RAE inevitably affects recruitment to degree courses, and a university's position in the inevitable subject "league tables" that are published unofficially, but widely read. My own university gave up offering a degree purely in chemistry a long time ago, although we have a degree in forensic chemistry, and other degrees that have a significant chemistry content.

Clearly, an individual's success in obtaining Research Council funding can be influenced by an RAE rating, and the composition of the panels who make the final judgments on applications (although proposals are initially sent out to specialists) again tends to reflect the dominance of the traditional areas of chemistry. I always get the feeling that any type of analytical chemistry (including Separation Science) is regarded as applied science, that should be funded by industry rather than the Research Councils.

So how has your research survived?

I have been fortunate in obtaining funding from the Pharmaceutical Industry, although for the reasons described previously, such funding has almost completely dried up. Pfizer have given generous funding to the excellent research group of Pat Sandra, but this is a Belgian group.

Recently, I have benefitted from the excellent performance of the University of the West of England in the 2008 RAE – but in Biomedical Science, not chemistry. Our 6th position (above many in the "Old University" sector) in the aforementioned League Tables resulted in significant funding. I have benefitted from this, being part of the submission due to my involvement in biomedical and pharmaceutical applications of Separation Science. I have also received support from instrument companies and column manufacturers, who have generously given me equipment and consumables without any pre-condition or strings attached to the gifts. It is a credit to these companies that they incorporate at least some employees who value scientific knowledge above marketing opportunities.

Sabbatical visits by university lecturers to eminent researchers' laboratories seem to be a thing of the past. However, have you gained inspiration by any collaborations or communication with any such researchers?

You are right- I cannot imagine who would undertake my teaching and administrative duties in Bristol during a sabbatical period- I have never had the good fortune to be able to make such a visit!

However, communication in the modern age is much simpler than it was, and I have benefitted enormously from interactions with (in alphabetical order) Peter Carr, Georges Guiochon, Jack Kirkland, Uwe Neue, Lloyd Snyder and many others. These distinguished researchers have always been generous in their readiness to discuss the latest work in our subject with me, and our debates have led to joint publications with three of the above. These interactions are facilitated by face to face discussions at the annual international HPLC conferences, which I have been lucky to attend for almost 15 years. One thing that has amazed me is the complete absence of a one-sided "scientific geek" character in all of them. Our discussions have included debates on Linguistics, the British Constitution, Literature (outside science!) , Music and History. One of them in another life would have made an excellent tourist guide or writer of travel books, and another is extensively involved in charity work.

You mention interests outside science-one of our previous interviewees, Professor Keith Bartle turned out to be a cricket groundsman! Do you have any interests outside science?

I have always been interested in music, and once considered it as a career-but that was a long time ago! I practice the organ at one of the Bristol Cathedrals every week, and occasionally play for services, usually at Christmas or Easter when the professionals are overloaded with commitments. The organ I play was built in 1973 in the neo-Baroque style and is thus ideally suited to Bach's greatest works. I am sure that due to the complexity of his Fugues, Bach must have been an excellent mathematician! As a contrast, I also like to play jazz piano music at home. However, my repeated futile attempts to emulate the genius of the great American pianist of the 1930s-1950s, Art Tatum, are not always popular with my family or my neighbours. One of my greatest ambitions in life has been to live in a detached house, but I am never likely to achieve this with prices in Bristol and with what I get paid!

Thank you very much for your time, David. We wish you well. Hopefully house prices will slump in Bristol and you will get a whopping great pay rise!

In a darkened corner of a restaurant by Baltimore Harbor during a symposium social event and look who's tickling the ivories!

Readers interested in some of the work David talked about in the interview should check out the following:

The challenges of the analysis of basic compounds by high performance liquid chromatography: Some possible approaches for improved separations

McCalley, DV

J Chromatogr A 2010 1217 858-880

Further investigations of the effect of pressure on retention in ultra-high-pressure liquid chromatography

Fallas, MM; Neue, UD; Hadley, MR, McCalley, DV

J Chromatogr A 2010 1217 276-284

Study of the selectivity, retention mechanisms and performance of alternative silica-based stationary phases for separation of ionised solutes in hydrophilic interaction chromatography.

McCalley, D V

J Chromatogr A 2010 1217 3408-17

Evaluation of the properties of a superficially porous silica stationary phase in hydrophilic interaction chromatography

McCalley, DV

J Chromatogr A 2008 1193 85-91