

## Meeting Report - Separation Science: Applications in Clinical Biochemistry

### *A Meeting to Mark the Official Retirement of Dr CK Lim*

**A joint meeting of the Royal Society of Chemistry, Separation Science Group and the Association for Clinical Biochemistry Southern Region**

**Friday 13 March 2009**

**Robens Suite, Guy's Hospital, London**

Chang Kee Lim, latterly of Birkbeck College, University of London, has been a leader in the application of separation science in biochemistry for many years. A capacity audience at Guy's met to pay tribute to him and his achievements, and to discuss current trends in the use of separation science in clinical chemistry. Sponsorship from ABS, Agilent, ESA, Jasco, Jaytee Biosciences, Thermo Scientific, and Waters is gratefully acknowledged.

In opening the meeting, David Perrett (Queen Mary, University of London) recounted some milestones in Dr Lim's career. A BSc in Chemistry and Zoology was followed by a PhD from Westfield College, University of London. In 1973 Chang Kee went to work with Professor Charles H. Gray at King's College Hospital Medical School. There he began to work on porphyrin metabolism, which he continued at the Clinical Research Centre (CRC), Northwick Park, 1976-1991. With the closure of the CRC, Chang Kee moved to the MRC Toxicology Unit, firstly at Carshalton and then in Leicester.

In 2000 Chang Kee moved back to London, still under the auspices of the MRC, to establish a BioAnalytical Science Unit at Birkbeck College. Although retired, Chang Kee continues to edit the journal *Biomedical Chromatography* that he helped establish in 1986. Prof. Perrett also recalled the meeting 'High Pressure Liquid Chromatography in Clinical Chemistry' Chang Kee organised at King's College Hospital in 1975, and the ensuing book 'HPLC of Small Molecules' (IRL Press, 1986) that was a classic in its day.

### *Porphyryns 40 Years On*

The session Chair, Dennis Wright (Northwick Park Hospital) recounted the valediction 'look at the chemistry' that guided him in his early PhD studies on porphyrins with Chang Kee. Porphyrins have been Chang Kee's abiding interest, and in the opening lecture he emphasised the contribution of HPLC and of LC-MS/MS to the study of porphyrins and the porphyrias.

Two topics stood out. Firstly, it had been claimed in a paper published in the Proceedings of the National Academy of Sciences, USA, in 2007 that a porphomethene inhibitor of uroporphyrinogen decarboxylase, namely uroporphomethene ( $m/z$  835), was the cause of *porphyria cutanea tarda*. However, not only was the LC retention of 'uroporphomethene' incompatible with what would be expected of a porphomethene, but also fragmentation characteristic of polyethylene glycol ( $m/z$  835 with product ions at 791, 747, 703 and 659, i.e. successive loss of  $\text{OCH}_2\text{CH}_2$ ) was present. This was wrongly interpreted as loss of  $\text{CO}_2$  from the porphyrin carboxyl groups.

On a related theme, 2-vinyl-4,6,7-tripropionic acid porphyrin (harderoporphyrin) had been reported in the Harderian glands of rodents. However, work using a neutral extraction procedure and LC-MS/MS showed that 'harderoporphyrin' was protoporphyrin monoxyside, protoporphyrin itself and protoporphyrin monoglucoside being minor components of the gland. Again, understanding the underlying chemistry of the analytical procedure was vital to a proper understanding of the natural products under study.

On a lighter note Chang Kee outlined work undertaken early in his 'retirement' in conjunction with the Centre for Ornithology, Birmingham and the Natural History Museum on the origin, evolution and functions of eggshell colours and patterning. Porphyrins again – it seems that the colouration of sparrowhawk and emu eggshells, for example, is due to the presence of biliverdin and protoporphyrin in different proportions.

### *Vitamin D, Purines, and Drugs of Abuse*

Sandra Rainbow (Northwick Park Hospital) pointed out that vitamin D research was very active, with some 1,000 papers being published in 2007 alone. LC-MS/MS was rapidly becoming the method of choice for clinical 25-OH-D<sub>2</sub> and 25-OH-D<sub>3</sub> analysis. Derivatisation was not required and there were advantages of selectivity over immunoassays. However, an internationally agreed reference material was lacking and measurement of 1,25-dihydroxyvitamin D remained challenging. Sandra also drew attention to work using chiral LC-MS/MS that demonstrated the presence of both 25-OH-D<sub>3</sub> and of epi-25-OH-D<sub>3</sub> in patient samples.

Tony Marinaki (GSTS Pathology) outlined work in screening for inborn errors of purine and pyrimidine metabolism. For purine analysis a 250 x 3 mm i.d. Waters Spherisorb ODS1 column with gradient elution gave a 33 min analysis time. Use of a bridged ethyl hybrid (BEH) C18 column (Waters Acquity) (1.7 µm aps) gave improved speed of analysis, resolution, and sensitivity, but with a higher column back pressure. However, more recent work had suggested that Supelco Ascentis Express Halo columns (2.7 µm particles) could give analogous or better results at lower back pressures.

Finally, Richard Evers (King's College Hospital) discussed problems and pitfalls in clinical drugs of abuse screening. LC-MS/MS offered lowered consumables costs and the ability to identify unequivocally a range of underderivatised analytes. However, sequential analysis limited sample throughput, the potential for ion suppression necessitated great care in method validation, and sample hydrolysis was still needed in the event that sensitivity towards morphine and buprenorphine glucuronides was poor or non-existent. The potential for use of ion trap and accurate mass technology in overcoming these problems was just beginning to be developed.

#### *From Tswett to Metabonomics*

After lunch, David Perrett began by paying tribute to another pioneer in the application of analytical methods to clinical problems, Professor Charles Enrique Dent (1911–1976). Using the then new technique of 2-dimensional paper chromatography, David felt that Dent had probably discovered/developed major insights into more diseases (including Fanconi syndrome, Hartnup disease, argininosuccinic aciduria, homocystinuria, cystinuria, and xanthinuria) than anyone else. David then discussed his own recent research using capillary electrophoresis with low-wavelength UV detection in which some 80 compounds can be detected in urine in 10 minutes. He also took the prize for the oldest sample discussed during the day with a slide of the CE-DAD analysis of organic compounds in an extract of an Egyptian mummy (site of sampling not stated)!

David's talk neatly picked up the theme initiated by Chang Kee Lim earlier in the day – it is not simply 'hypothesis driven' research (with which funding bodies seem sometimes to be obsessed) that advances knowledge, but more often than not it is either the application of new techniques to old problems, or the critical appraisal of existing data that leads to key developments.

Having discussed aspects of ICP-MS operation, including GC- and LC-ICP-MS, Andrew Taylor (Royal Surrey County Hospital) gave a brief overview of metallomics (chemical speciation, dynamics and kinetics of trace elements in biological systems). Applications discussed included isotope measurement to help identify sources of lead exposure, the use of <sup>65</sup>Cu administration as an additional diagnostic test in Wilson's disease, differentiation of arsenic species in urine, and recent work aimed at unravelling the complexities of selenium metabolism. An interesting aspect was the use of selenium isotope patterns to help identify selenium-containing species in complex chromatograms. Potential pitfalls such as 'polyatomic' (e.g. <sup>40</sup>Ar<sub>2</sub><sup>+</sup> on <sup>80</sup>Se<sup>+</sup>) and 'isobaric' (e.g. <sup>64</sup>Ni<sup>+</sup> on <sup>64</sup>Zn<sup>+</sup>) interferences were given due prominence. A clinical problem of gadolinium used as contrast medium was discussed – <sup>156</sup>Gd<sup>2+</sup> has the same *m/z* as <sup>78</sup>Se<sup>+</sup>.

Edwin Carr (St Helier Hospital, Carshalton) next discussed catecholamine measurement using HPLC with electrochemical detection (ED). Nowadays the use of ED in clinical chemistry is virtually confined to diagnosis of pheochromocytoma, with urine being the specimen of choice unless the patient has renal failure. There is almost universal acceptance that, at present, metanephrines analysis is superior to other means of diagnosing this condition, yet the evidence for this is still ignored by many.

The final talk returned to the 'omics' theme with Paul Thomas (University of Loughborough) discussing volatile profiling using GC-MS and other techniques. After describing investigations into breath volatiles in, for example, chronic obstructive pulmonary disease, Paul outlined work to develop a hand-held 'sniffer' device based on ion mobility MS to seek out people trapped as a result of an earthquake, for example. It seems that not only do specially trained sniffer dogs have a very short working period (20 min or so) before needing prolonged rest, but also suffer high mortality (up to 70 % was quoted), hence there is a real need for such a device.

All-in-all the day was a fitting tribute to Chang Kee Lim and his contribution to the application of separation science in biochemistry.

Bob Flanagan

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