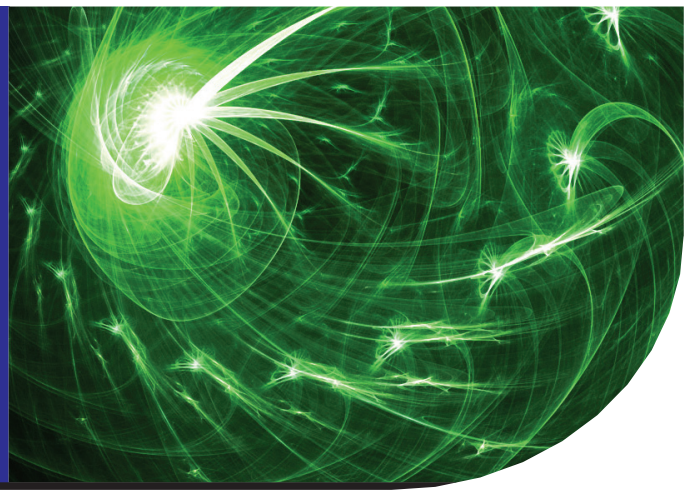
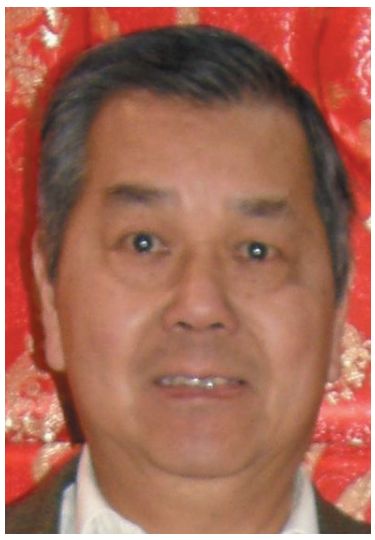


Electronic Interaction Chromatography (EIC) - A Much Underutilised Tool.

One of the least publicised areas of separation science is that of the application to clinical biochemistry. Many practitioners are based either in academia, or in hospitals yet the degree of knowledge these scientists require is just as much, if not more so, than many in the Big Pharma workplace. The molecular structure of the compounds encountered frequently in this area is often complex and can include several functionalities, which means the development new methods can be very demanding. A major difference is that often the budgets available dictate that equipment is not always state-of-the-art yet improvisation often allows dramatic innovative separations to be developed. One of the best-known and highly respected scientists operating in that field is Dr Chang Kee Lim, who this year celebrated his official retirement.



Chang Kee Lim

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To mark the event a meeting was organised to pay tribute to his work in the field of separation science and biochemistry. Jointly organised by the Royal Society of Chemistry, Separation Science Group and the Association for Clinical Biochemistry, Southern Region, the meeting was held in the Robens Suite at Guys Hospital, London on 13 March this year. A capacity audience heard about Dr Lim's career starting as an undergraduate through his work for the Medical Research Council (MRC) at the Clinical Research Centre (CRC, Northwick Park Hospital), then at the MRC Toxicology Unit at Carshalton and at Leicester, and latterly at Birkbeck College, London, where he held a senior position in the BioAnalytical Science Unit. During his career he instigated, and still edits, the Journal of Biomedical Chromatography, and edited the book 'HPLC of Small Molecules' a work that inspired an interest in that field for many students, some of whom were present at the meeting. Topics covered at the meeting included analysis of porphyrins (Dr Lim's particular area of interest and expertise), vitamin D, purines, catecholamines, and drugs of abuse. Several speakers including Professors David Perrett (Queen Mary, University of London) and Paul Thomas (University of Loughborough) discussed aspects of metabonomics.

Coincidentally this year also marks the twenty-first anniversary of the launch (but not retirement) of Hypercarb™, a porous graphitic carbon (PGC) HPLC packing with some unique properties. Dr Lim was the first to discover the importance of a dominant property of the material (band of delocalised electrons above the planar surface) and harness this to produce some unique separations, which to this day have not been bettered. The mechanism became known as Electronic Interaction Chromatography (EIC).

Bernie Monaghan took the opportunity to question Dr Lim about this particular piece of work and his plans for the future.

Bernie Monaghan (BM): ***Which particular analytes were giving you a problem when you looked at using Hypercarb for the first time and what was their importance in the clinical field? Had other types of HPLC Columns been tried previously (e.g. reversed phase) and what had been the outcome with these?***

Dr. Chang Kee Lim (CKL): The first analyte I looked at was oxalic acid. There was an interest in metabolic diseases and especially organic acidurias at Northwick Park. The determination of oxalic acid in urine is important for the diagnosis of type 1 and type 2 oxalosis. Oxalic acid was difficult to retain sufficiently on silica-based RP columns. I thought the strong hydrophobicity of PGC was ideal for retaining and separating oxalic acid. To my surprise oxalic acid was totally retained and could not be eluted even using a gradient from 100% water (0% acetonitrile) to 100% acetonitrile. Since ion-exchange and ion-pair chromatography could be ruled out, I came to the conclusion that the retention mechanism must be electronically mediated, bearing in mind the layer structure of PGC (*Figure 1*). The lone pairs of electrons on the carboxyl groups of oxalic acid interacted electronically with the circulating electrons on the PGC, resulting in retention.

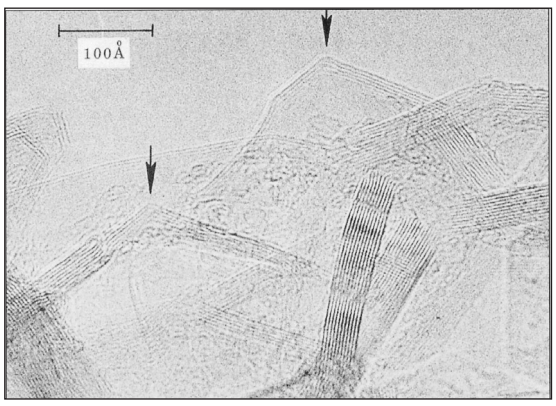

Figure 1. Crystalline structure of PGC with electron cloud (not shown) above surface.

I called it Electronic Interaction Chromatography (EIC) and suggested that to elute oxalic acid from PGC we require an electronic modifier, a compound with lone pair electrons to compete with oxalic acid for interaction. Trifluoroacetic acid (TFA) is such a compound. Other weaker electronic modifiers include sodium acetate, acetic acid and formic acid. With this understanding we developed a method for the determination of oxalic acid in urine using 0.08% TFA as the mobile phase. The method has the added advantage that creatinine could be measured at the same time.

I have proved conclusively that EIC based totally on electronic interaction is possible by separating the oxo anions of Tc and Re on PGC, also with a simple TFA-modified eluent.

At that time the Division of Radioisotopes at the CRC was using $^{99m}TcO_4^-$ to synthesise cationic

^{99m}Tc-amine complexes as the starting material in the synthesis for many potential radiopharmaceuticals used in nuclear medicine. To monitor the reaction and to check the purity of products it is essential to simultaneously separate the oxo anions and the cationic amine complexes. Again EIC was the only technique capable of achieving this. (*Figure 2*)

I believe EIC is still the best technique for the separation of the above compounds.

BM: ***Once you had managed to obtain a separation of cations and anions on the same column did you follow up on this system using the EIC mechanism? Did you publish any of the work?***

CKL: Yes, there were several papers published which featured this work and made reference to the EIC mechanisms and their interaction with other modes of separation [1-3].

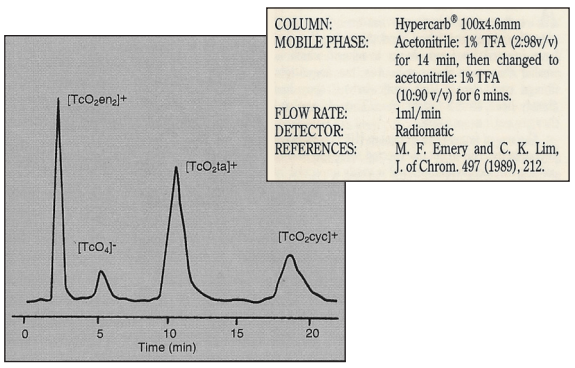

Figure 2. Separation of cations and anions of ^{99m}Tc on Hypercarb in a single run.

BM: ***Once you had proved the validity of EIC, did you use Hypercarb to separate other analytes?***

CKL: Yes, we worked up a very nice separation on Hypercarb using a 3% acetonitrile, 0.1% v/v TFA eluent to separate creatine and creatinine in body fluids (4). Previously it had been very difficult to separate the two components on a reversed phase C18 column, but the greater hydrophobicity of the carbon surface separated the two. The separation was predominantly that of a reversed phase ion pair with the TFA acting as the ion pair reagent, yet some electronic interaction was postulated in controlling the retention of the analytes. The same reference also describes the separation of remoxipride and FLA 981, two potential neuroleptic agents, which utilised the TFA/acetonitrile system on Hypercarb.

BM: ***Hypercarb is a unique stationary phase which theoretically could be viewed as 'the ideal stationary phase' yet it never took off in the field of clinical biochemistry. Is there a reason for this? Are the scientists of today 'missing a trick'? Are there other forms of carbon that could be useful to the clinical scientists to effect, or improve, separations?***

CKL: Hypercarb never took off in clinical biochemistry laboratories possible for the following reasons.

It provides a mixture of interactions, both strongly hydrophobic and electronic, making prediction of retention much more difficult. Clinical samples are complex and many compounds can be immobilised completely by electronic interaction, rapidly modifying the column characteristic. These may be difficult to remove by column washing and reconditioning. Having said that, I am sure there are some applications which can benefit from Hypercarb, like the separation of oxalic acid and the work with the radio-labelled ionic analytes. A good review of the benefits and pitfalls of Hypercarb for clinical research was published around the time of this work [3].

As regards other forms of carbon that have applicability to the clinical field an excellent summary was recently published [5], which indicates applications not just with HPLC systems but also GC, electrically driven separations and SPE (Solid Phase Extraction).

BM: ***Have you had the need to use the Hypercarb columns since that work?***

CKL: My main reseach interest is in porphyrins and related tetrapyrroles. The circulating electrons on the porphyrin macrocycles interact very strongly with PGC. Porphyrins are much better and easier to separate by conventional RP chromatography. I have no need to use Hypercarb for my work.

BM: ***What challenges do the clinical scientists of today still face with regards to choosing stationary phases for their work? Have the advances of high purity silica bases, base deactivated phases and UHPLC Columns brought any advantages for that field of work?***

CKL: The advances of high purity silica phases, base-deactivated phases and UHPLC columns have certainly brought advantages to porphyrin separation in terms of resolution and speed of separation. Column stability and reproducibility have also improved. The clinical laboratories now have a much better variety of good columns to choose from for their particular applications

BM: ***Finally, you were recently honoured with a meeting organised by your peers to celebrate your work and contribution over the years. Do you plan to keep involved with separation science in the clinical field?***

CKL: I shall certainly continue with my interest in separation science in the clinical field. I might even start working with Hypercarb again. Remember, I am only 'officially retired'.

BM: ***Thank you.***

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Polyolefin Analysis Dedicated GPC-IR

Polymer Char has launched a high temperature GPC, dedicated to polyolefin analysis with multiple detectors: Viscometer, Light Scattering and Infrared detector IR4, with concentration and composition sensors for Short Chain Branching measurement, and optionally IR5-MCT for high sensitivity applications. GPC-IR provides substantial advantages in the user daily operation since it is designed in a robust, compact and fully automated way. Sample preparation is fully integrated into the system and doesn't require vial transfer; filling, sample dissolution and injection are all performed automatically. In addition, an in-line filter has been incorporated, with backflush auto cleaning, so no previous filtration step is required.

GPC-IR autosampler is a separate unit with 70 vials (10ml) and two different temperature zones, therefore samples stay at high temperature only the necessary time to prevent degradation. An independent columns oven better protects the columns set since all other hardware parts of the system can be serviced without cooling it down. GPC-IR Virtual Instrumentation Software provides instrument control, process monitoring and complete GPC calculations, integrating all detector signals in a single package.

Mass Spectrometry Systems Accelerate Biomarker Research

Applied Biosystems® part of life technologies™

Applied Biosystems has announced that scientists at bioMérieux, a world leader in the field of in vitro diagnostics, and the Institute of Analytical Sciences at the University of Lyon in France are collaborating on research to advance the discovery of biomarkers that can be used to diagnose or monitor disease. They are using next-generation, mass spectrometry technology with integrated triple quadrupoles and a linear accelerator trap to validate newly identified candidate biomarkers.

To accomplish these goals, they are using AB SCIEX QTRAP 5500 Systems that rapidly quantify dozens of protein and peptide biomarkers in a single analysis. The AB SCIEX QTRAP 5500 System, which was developed by the Applied Biosystems/MDS Analytical Technologies joint venture, offers greater reproducibility and precision than other mass spectrometry platforms and provides faster and more specific results than the current clinical standard of antibody-based testing, making this new technology ideal for protein biomarker validation. These AB SCIEX QTRAP 5500 Systems are being used to develop simple, robust assays for detecting biomarkers that could then be transferred into standard testing procedures in diagnostic laboratories.

Biomarkers are indicators of the presence or progression of a disease that offer a quick and easy method for diagnosing or monitoring patients for a range of conditions, including cancers as well as infectious and degenerative diseases. Extensive research is required to clarify the exact role and significance of each newly discovered candidate biomarker, which is a process that can take several years to complete. The scientists from bioMérieux and the University of Lyon are combining their respective expertise in biomarker discovery and protein mass spectrometry to accelerate this biomarker validation process.

Expanding Optical Sensing Software Options

Ocean Optics has increased the usability and flexibility of its Jaz modular optical sensing platform with two new software options, the Jaz-IRRAD for irradiance measurements and the Jaz Scripting Language for building custom applications. The modular design of the Jaz family allows it to be customized for a range of applications in field, lab or process environments. With the new software options, users will be able to further tailor measurements to their requirements, without any special programming knowledge.

With Jaz-IRRAD absolute irradiance measurement software, the handheld Jaz transforms into a dedicated light meter, allowing users to measure calibrated absolute irradiance without any need for an external computer. Characteristics such as colour temperature, spectral intensity and colour space values of LEDs, radiant sources and the sun are captured with just three pushes of a button. Data captured can be post processed to the intensity parameter of choice-Watts/cm^2, lumen, luz, PAR, or any other light intensity parameter.

The Jaz Scripting Language offers nearly endless possibilities for creating custom applications. This powerful tool is simple enough for non-programmers to build measurement sequences into a self-contained application. Operations such as measuring the sugar content of a liquid, or expressing reflected colour in colour space values such as L*a*b*, can be designed and tested on a PC and loaded onto the Jaz for execution.