The 8th International Conference on Countercurrent Chromatography held at Brunel University, London, UK, July 23-25, 2014

S. N. Ignatova and I. A. Sutherland

Advanced Bioprocessing Centre, Institute of Environment, Health and Societies,
Brunel University London, UB8 3PH, United Kingdom
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ABSTRACT

The 8th International Conference on Counter-current Chromatography (CCC2014) was held at Brunel University London from July 23rd-25th, 2014. It is 14 years since Brunel hosted the first International Conference on CCC (CCC2000) at the beginning of the millennium and therefore, it was a good opportunity to review the progress of this emerging technology and particularly the impact it is having with industry today.

INTRODUCTION

When we hosted the first international conference on counter-current chromatography (CCC2000) here at Brunel University in the year 2000, the technology, from the commercial point of view, was still in its infancy. There were sessions on basics, methodology and theory as the technology was still not well known or recognized by the chromatographic/separation community. There was a lot of emphasis on applications to demonstrate what technology is capable of – natural products, antibiotics, peptides, proteins as well as more general applications. There were also sessions on scale-up and pH zone refining with examples of purification of gram quantities at the laboratory scale. Overall there were 16 invited keynote speakers, 25 oral presentations and only 21 posters – 62 presentations in all with about 90 delegates. Since then this conference series has toured the world: Beijing, China in 2002, Tokyo, Japan in 2004, Bethesda, MD, USA in 2006, Rio de Janiero, Brazil 2008, Lyon, France 2010 and Hangzhou, China 2012 before once more returning "home" to the UK to be hosted by Brunel University London.

The technology has matured considerably in the last 14 years. It has gone from the academic prototyping stage through commercial development to now becoming a robust technology as a valuable scale-up process in industry's toolkit. Aiming to reflect this trend, the 8th international

conference on counter-current chromatography (CCC2014) programme was structured following the steps of developing a CCC/CPC separation. The first day had three sessions: method development, new solvent systems and process development, reflecting the industry's interest in rapid method development. The second day was entirely devoted to industrial applications with the majority of speakers talking about real industrial case studies processing as high as a kilogram per day. The third day was dedicated to novel approaches, emerging technology and new instrumentation as well as numerous applications.

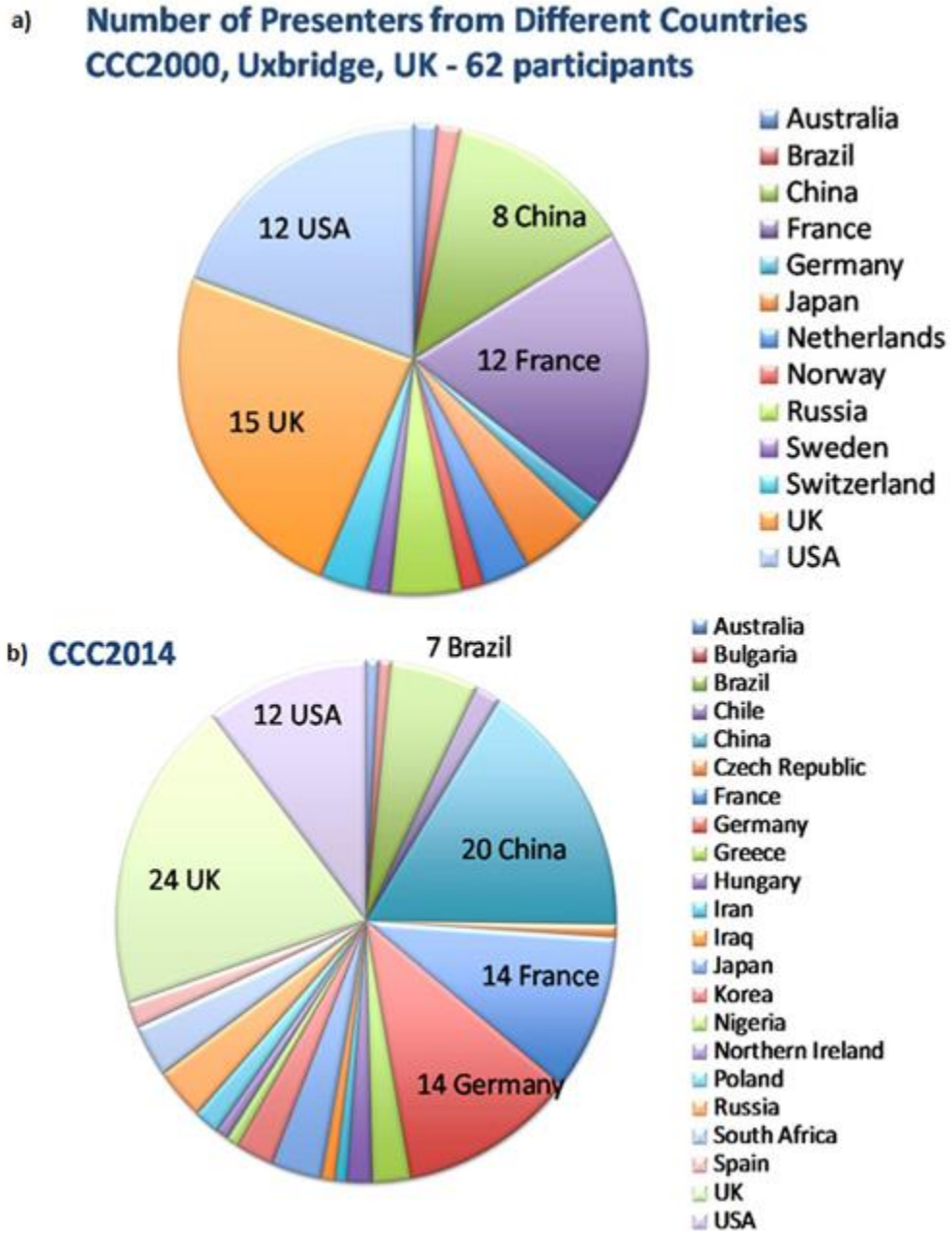

Figure 1 : *The number of presenters from different counties in a) CCC2000 – 66 abstracts from 13 countries and b) CCC2014 – 130 abstracts from 22 countries*

Figure 2: *CCC2014 Chair, Dr Svetlana Ignatova opening the conference*

There were twice as many presentations (126) compared to CCC2000 with only 4 keynotes, 20 full orals, 17 short orals and 85 posters of which 44 were asked to give flash presentations with a maximum of 3 slides each. Figure 1 gives a comparison between CCC2000 and CCC2014 of the number of presenters from different countries. It not only shows the number of abstracts doubling and the number of countries involved increasing from 13 to 22, but that countries like China, Germany and Brazil have significantly enlarged their contribution. The UK share of abstracts is remaining about the same, while the USA and France have proportionally fallen behind.
The aim of this conference was to highlight some key advances and breakthroughs that have occurred in the last 14 years in both counter-current chromatography (CCC) and centrifugal partition chromatography (CPC). After Professor Geoff Rodgers, Deputy Vice Chancellor (Research) opened the conference, CCC2014 Chair, Svetlana Ignatova (Figure 2) reviewed 14 years of progress. This introduction to the CCC2014 special issue gives some of the highlights of the conference, drawing on the papers in this special issue as illustration.

Method Development

Rapid method development was identified by industry as a priority in CCC/CPC. Guido Pauli opened the session with a talk highlighting how the scientific community had embraced their "Generally Useful Estimation of Solvent Systems (GUESS)" with more than 100 citations. He then presented a successful application of a rapid TLC method of "GUESSing". Brent Friesen presented a qualitative and quantitative evaluation of solvent systems using the GUESS approach paving the way to the development of new solvent systems that are amenable to successive orthogonal counter-current separation protocols employed in metabolomics studies [1].
As an alternative Shihua Wu gave a targeted strategy for natural product isolation by CCC using a novel 9x9 solvent map [2] and a new mixture of 14 mimic natural product compounds. The proposed strategy was applied to Chinese herbal extracts for a full validation.

New Solvent Systems

As the technology evolves, it is applied to more specific applications, which often require particular solvents due to safety restrictions, target compounds stability or lack of selectivity of the solvent systems currently available in the literature. One such example was given by Aneta Sporna-Kucab from Cracow University of Technology, Poland. She successfully developed a food grade solvent system for the gradient separation of highly polar Betalains by adding sodium chloride to a phase system containing butanol, different volumes of ethanol and phosphoric acid [3]. The solvent systems had high stationary phase retention, which did not vary too much as the mobile phase gradient changed. This novel approach of using food-grade solvents may lead to future CCC/CPC applications for isolation of compounds with potential health benefits. Mirjana Minceva and her team meanwhile were looking at how the addition of ionic liquids (ILs) to aqueous two-phase systems would influence the partitioning of model protein compounds. Although the viscosities and densities were suitable for CCC/CPC, the addition of ILs increased the partition coefficient outside its chromatographic separation range. However, it opens up the opportunity to use CCC/CPC as a concentration tool for proteins [4].

There were also two papers on the use of 3-phase systems in CCC, which generally allow separation of closely related compounds. The first by Wu et al. [5] of the Institute of Chinese Materia Medica, China used n-hexane, methyl acetate, acetonitrile and water (4:3:4:4) to form 3 phases (upper (UP), medium (MP), lower (LP)), which produced three different 2-phase systems (UP-LP, UP-MP, MP-LP). The authors separated 17 different compounds running systems in reversed phase mode, of which 5 were newly identified. Lee et al [6] of Seoul National University, Korea developed a novel strategy for separating diterpenoid isomers from the root of *Aralia continentalis* for the first time using a three phase system (hexane, dichloromethane, acetonitrile, water). However, in their case the middle phase was redundant, so the authors used "mixing-on-demand" preparation for phases to minimise solvent waste. The paper also proposes a mathematical prediction of CCC separation which should save considerable time in the future and open the door for the separation of isomers from both natural and synthetic sources.

Englert [7] of University of Hohenheim, Germany added Benzotrifluoride, as a solvent system modifier, to a n-hexane/acetonitrile phase system achieving the isocratic separation of lipophilic compounds such as carotenoids from a crude carrot extract. This early research offers the possibility of expanding the use of lipophilic (non-aqueous) two-phase solvent systems to applications involving very non-polar compounds.

Scale-up and Continuous Processing

Scale-up and continuous processing are the most sought after areas for any technology development. This topic was covered by presentations describing scaling up between instruments of different makes as well as scales highlighting the important difference between running separations in normal and reversed phase modes. Some of those case studies are included in this special issue. Mariana Neves Viera et al [8] from Technical University of Braunschweig, Germany demonstrated a 120x scale-up for the fractionation of *Schinus terebinthifolius* Raddi berries dichloromethane extract minimising solvent usage as all the process optimisation was performed at the analytical stage. Simon Hammann et al [9] from University of Hohenheim, Germany showed how they could continuously link online hyphenation of CCC with NMR.

Figure 3: *Sir Richard Sykes, Chancellor of Brunel University London, opening Industry day at the CCC2014 conference.*

Industry Day

Sir Richard Sykes, previously CEO of GSK and Rector of Imperial College, opened Industry day at CCC2014 in his new role as Chancellor of Brunel University London (Figures 3 & 4). He emphasised that Brunel's transformational change programme encourages more interdisciplinary research and working more closely with industry and was delighted that the conference was having an industry day for the first time. "Looking at the programme it is very clear to me that researchers are starting to address issues important to Industry such as: rapid method development, high throughput, ease of use, automation and the need for de-skilling the processes, the ease of scale up and technical transfer from one instrument to another and from one Country to another. It is also interesting to see that natural product research could be having a revival, particularly in the search for novel antimicrobial drugs due to the very serious problem of drug resistance". He concluded by encouraging Industrial delegates to develop the technology to be as versatile and robust as possible so that pharma companies use it as part of their usual separation toolkit.

Starting the scientific part of the day, Ian Sutherland reviewed both hydrodynamic and hydrostatic forms of the technology with emphasis on the professional engineering development and scale-up of the processes to fulfil the needs of industry and to provide a robust technology for future growth. Gary Yanik of PDR-Separations, USA highlighted how method development in CCC had been too labour-intensive. He had worked with CCC manufacturers, researchers, and short-run preparative purification groups to develop a universal hardware/software upgrade (AutoCCC) and a recommended procedure for automated screening methods and purifying compounds. David Thornton of GSK, USA described how his group originally applied these methods with HPLC, to reduce the time to develop a scalable purification process. His group has recently extended this screening concept to counter-current chromatography (CCC) and described the subsequent scale-up to a one litre instrument for processing.

A series of industrial case studies then followed: the first by Adrian Weisz from the FDA's Center for Food Safety, who described their ongoing effort to determine the composition of colour additives used in foods, drugs, cosmetics, and medical devices. He presented for the first time a method for separating three of these hydrophobic impurities from each other using a spiral countercurrent chromatography column [10]. Paul Hellier from Pierre Fabre, France described their recent work investigating the use of a Fast Centrifugal Partition Extractor whilst performing separations in the pH zone refining mode. In particular the separation of the alkaloids vindoline and catharanthine, molecules that occur naturally in the plant *Catharanthus roseus* and are key materials in the semi-synthesis of a number of anti-cancer drug candidates. Continuing the industrial case studies, Svetlana Ignatova, on behalf of Carl DeAmicis of Dow Agrochemicals, USA and Guy Harris of Dynamic Extractions Inc, USA, described the productivity and performance comparison of Countercurrent Chromatography and Reverse Phase HPLC at Pilot Scale using a Spinosyns Purification as a case study. The high-performance counter-current chromatography (HPCCC) process produced a 2-fold higher throughput and consumed approximately 70% less solvent than preparative scale RP-HPLC, the volume of product containing fractions from HPCCC amounted to 7% of that produced by HPLC and so required much less post-run processing. Lijuan Chen of Sichuan University, China described how 1.5 kilogram of Honokiol of 99.9% purity was separated from its isomer, Magnolol, using two Midi HPCCC instruments within four months, which satisfied the further formulation study, pharmacological research as well as preclinical safety evaluation study. Both Honokiol and its formulation honokiol liposome injection have now been submitted for consideration as a new drug for further clinical trials to the Drug Evaluation Centre of the China Food and Drug Administration (CFDA).

A series of talks demonstrating the versatility of CCC/CPC technologies started with Nathalie Douillet of GSK, UK presenting a breakthrough in oligonucleotide purification using Kromaton CPC columns in ion-exchange mode. Peter Hewitson of Brunel University London, UK described the successful size fractionation of bio-polymer particles using a DE-Spectrum in a field-flow fractionation (FFF) mode with a single phase solution as the mobile phase. Jonas Krause [11] of TU Dortmund University, Germany used Kromaton CPC technology for continuous reactive extraction using enzymes as catalysts with direct product removal. There were more presentations about method development from the industry point of view when the solvent system search was based on molecular structure. Firstly Sian Marsden-Jones [12] explained how Quantitative Structure Activity Relationship (QSAR) computational models were being developed to predict the partitioning of compounds in two phase systems in an effort to speed up method development in CCC/CPC. It was followed by a presentation from Philip Wood of Dynamic Extractions Ltd, UK about their approach to method development using either computer prediction based on a known molecule structure or solvent system screening when structures are not known, all built in one workstation. He also talked about using CCC as a template purification process for removal of a common impurity of triphenylphosphine oxide from a synthetic reaction mixture. Another example of using CCC for purification of reaction mixture was described by Stefanie Kuhnert, TU Braunschweig, Germany. Firstly she used CCC for a primary fractionation of the crude extract from *Afzelia bipendensis* for preparing proanthocyanidins for semi-synthesis, followed by second CCC purification of the reaction mixture.

The work presented by David Ward of UCL, UK [13] described the first steps in using Kromaton CPC technology in bio-refinery context for purification of monoccharides from hydrolysed sugar beet pulp. This is one of many attempts in waste valorisation research using various technologies and aiming to get more value out of renewable resources and reduce waste across the UK and EU.

The industry day was finalised by two examples of biological purifications using aqueous two-phase systems (ATPS). In first instance, Jonathan Huddleston of Brunel University London, UK explained how CCC can be used for integration of clarification and purification steps for purifying monoclonal antibody-based material from *E.coli* fermentation broth. The second instance by Christopher Ladd Effio et al of Karlsruhe Institute of Technology, Germany [14] was for purifying virus like particles (VLPs). Normally ATPS have low solubilising capacity, but the authors demonstrated how ATPS (PEG-salt systems) can be optimised on a high throughput screening platform to achieve high solubility of VLPs for their case study on isolation of human B19 parvoVLPs from crude Sf9 insect cell lysate.

Figure 4: *Deputy Chair, Ian Sutherland (founder of CCC conference series), Chair Svetlana Ignatova and Chancellor Sir Richard Sykes by the CCC2014 display welcoming delegates message in 22 different languages.*

Emerging Technology

The theme of CCC/CPC versatility was further demonstrated in other papers addressing particle separations using subtly different strategies.

Fedotov et al. [15] gave a detailed review of using CCC instruments in centrifugal field flow fractionation (FFF) mode to achieve high throughput purification of nano- and micro-particles of a different nature. Normally FFF in unit gravity can only manage samples of 1mg for analytical purposes, but Fedotov could process up to 1000x more using standard multilayer CCC columns due to the high fluctuating g-field of any CCC instrument.

Liu et al at Beijing University of Chemical Technology, China [16] used ionic liquids to modify magnetic multifunctional nanospheres with chiral ionic liquids to be utilised as either a stationary phase on its own or as an additive to alcohol-water system for a separation of chiral amino acids, D- and L-tryptophan, in spiral column. The authors also used a density gradient to enhance the separation. Using the same model mix of amino acids, Tong et al [17] employed classical ATPS with bovine albumin as a biological chiral selector. Comparing both studies, one should bear in mind the

difference in loading and racemates ratios. The elution volume for the second peak is almost the same for both studies. However, the lower flow rate for the Tong paper might have caused tailing of the L-typtophan peak.

This session also included examples of single wall carbon nano-tube purification with ATPS as well as red blood cell fractionation with single phase saline solution using various configurations of CCC columns.

Process Modelling, Theory and Instrumentation

Modelling, theory and instrument development were always part of every CCC conference. It is vital for any technology to be predictable and innovative to provide solutions for various scientific and industrial problems. The CCC2014 conference carried on this tradition and dedicated most of the last day to these topics.

Modelling of CPC and studying the hydrodynamics of mixing zones in the chambers has revealed some interesting insights that will lead to better CPC separation efficiency and scale-up. Schwienheer et al [18] have compared different chamber geometries showing that some had dead zones that reduced efficiency despite having superior retention characteristics. They also emphasise the importance of having a high aspect ratio favouring the flow direction in order to effectively flush out chambers between runs. Goll et al [19] compare two CPC columns with identical 250mL column volume: one with 1800 small chambers and one with just 220 larger ones. While the former had lower retention and demonstrated significantly higher resolution/efficiency with aqueous/organic phase systems the 220 chamber one could retain ATPS while the 1800 chamber one could not. Collet et al. [20] propose a methodology for CPC column sizing based on the characterisation of the efficiency of advanced cell or chamber shapes, taking into account the hydrodynamics. They illustrate 5 different geometries from 25-5000mL for two applications with excellent predictive modelling using mass transfer coefficient for their scale up. They claim they can now predict the optimum CPC column length leading to the highest productivity for a given application. Further CPC modelling is presented by Kotland et al [21] again with excellent correlation between practice and theory, but this time for pH zone refining separation of catharanthine and vindoline.

Berthod & Faure [22] revisit resolution in hydrodynamic CCC highlighting the importance of tubing bore size. They propose an optimum range between 1-3mm but also mention that larger bores with their higher retention values can lead to shorter separation times and equivalent efficiency as the smaller bore instruments.

Finally Shinomiya et al [23] have designed a completely new centrifuge with three axes of rotation, which they call a coil satellite centrifuge (CSC). They validate it with the separation of various sugar derivatives in ethyl acetate/butanol/water phase systems and compare it with a standard HSCCC J Type centrifuges. While there appeared to be some incremental improvements, there was no step change or wow factor in the results, but it will be interesting to see if they find any breakthrough applications in the future.

Applications

Following tradition, there were a very large number of applications of CCC/CPC technologies at the conference – 50 in total with a keynote by Gerold Jerz from Technical University Braunschweig,

Germany to set the scene followed by 1 full oral, 3 short oral, and 18 flash presentations. In addition, there were also 27 posters. This special issue contains only 4 selected papers representing groups from each continent across the world. Dalene DeBeer et al [24] from Agricultural Research Council, South Africa describe the sample loading and compound stability considerations taken when isolating aspalathin and nothofagin from Aspalathus linearis (rooibos). Fernanda Costa et al [25] from Federal University of Rio de Janeiro, Brazil address the selectivity of solvent systems in CCC using *Salicornia gaudichaudiana* separation as a practical example. Sun et al [26] Shandong Academy of Sciences, China describe the preparative separation of quaternary ammonium alkaloids from *Coptis chinensis* Franch by pH-zone-refining CCC and finally Hammann et al [27] from University of Hohenheim, Germany describe the isolation of Δ5 polymethylen interrupted fatty acids from *Podocarpus falcatus*.

Figure 5: *Conference Dinner in the Newton Room of the Hamilton Conference Centre at Brunel University London.*

Conference Dinner

130 people attended the conference dinner (Figure 5) – an occasion where speeches were made, awards presented and there were announcements about the next CCC meeting in Chicago, USA (CCC2016)

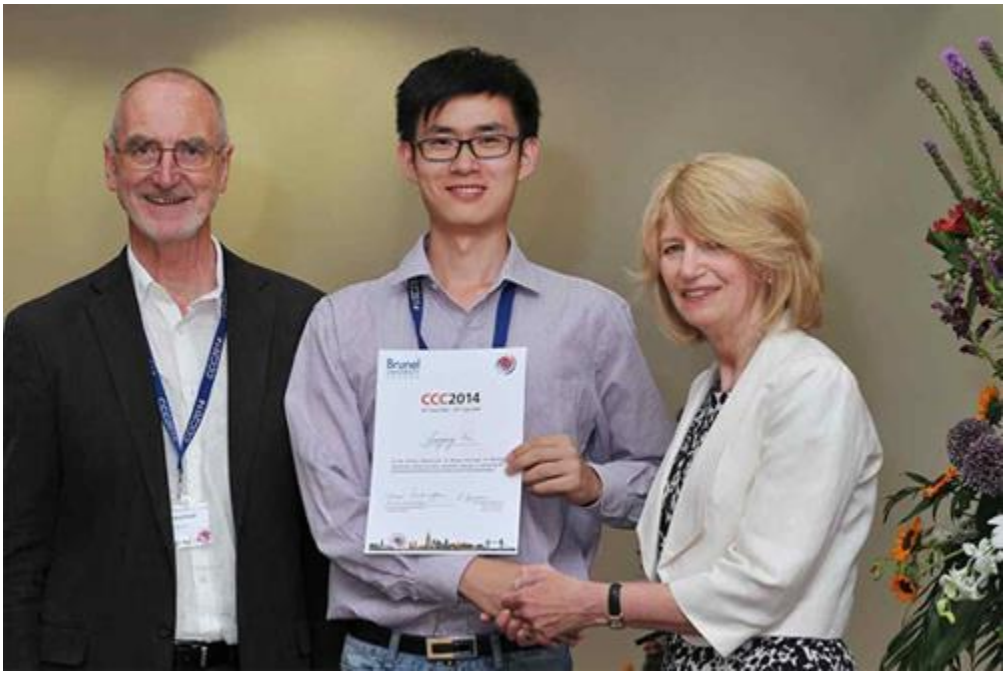

Figure 6: *The deputy chair, Professor Ian Sutherland and the Vice Chancellor, Professor Julia Buckingham presenting Jiangning Xu with his student bursary*

Awards

Four student received awards at the conference dinner. They were Jiangning Xu (Figure 5) of the State Key Laboratory of Chemical Resource Engineering, Beijing University of Chemical Technology, Beijing, China for his poster "Separation of seven phenolic acids from the bran of oats by pH-zone-refining counter-current chromatography"; Michael Englert, Institute of Food Chemistry, University of Hohenheim, Stuttgart, Germany for his paper "Potential of counter-current chromatography for the isolation of high-priced Carotenoides" [7]; Stephanie Kuhnert of the Institute of Food Chemistry, Technische Universitat Braunschweig, Germany for her poster on "Semisynthetic preparation of proanthocyanidins from *Afzelia bipindensis* and isolation using HSCCC" and Tuba Esatbeyaglu from the Institute of Food Chemistry, Technische Universitat, Braunschweig, Germany for her poster on "Isolation of dimeric, trimeric, tetrameric and pentameric procyanidins from cocoa beans (*Theobroma cacao* L.) using counter-current chromatography". Finally at the end of the conference the Young Scientist Crafty Chromatographer Award was presented to Svetlana Ignatova of Brunel University, UK and the Edward Chou Award to a Senior Investigator for life time achievement was presented to Peter Winterhalter, TU Braunschweig.

International Committee Meeting

Traditionally members of the International committee have a separate meeting at the biannual conference to discuss possible locations for the following conferences and any other urgent issues. The next conference, the 9th International Conference on Counter-Current Chromatography (CCC2016) will take place at Dominican University, River Forest, near Chicago, Illinois, USA on August 1st -3rd , 2016 (www.CCC2016.com). There had been talk at the conference about changing the name of counter-current chromatography as users argued that it was not really countercurrent as there was a stationary phase. Yoichiro Ito [28], the founder of the technology, had submitted a paper defending the term counter-current chromatography as the combination of counter-current

distribution and liquid chromatography, which can under certain circumstances be set up with counter-current flow. Alain Berthod replied [29] by giving a summary of what was concluded at CCC2014: “the counter-current or countercurrent terms and the CCC acronym are accepted to represent all chromatographic techniques working with two liquid phases without any solid support. However, there is no obligation to use them. Other terms such as centrifugal partition chromatography with the CPC acronym or countercurrent separation with the CS acronym or others can be used. It was strongly recommended to put (in all cases) the “counter-current chromatography” words and CCC acronym in the Keyword section of all articles dealing with chromatographic separations using two immiscible liquid phases and centrifugal fields”. He then went on to acknowledge there could be countercurrent flow in a closed coiled tube, but claimed this was not chromatography as there was no second pump. At risk of continuing the discussion through to CCC2016, the Brunel team have shown that the coil planet centrifuge itself is the second pump (Wood et al [30]).

Following the closing down address by the chair, Svetlana Ignatova, the remaining delegates lined up for the conference group photo (Figure 7). The next day (a Saturday) was set aside for group visits to Oxford and Windsor.

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Figure 7: *CCC2014 Group Photograph*