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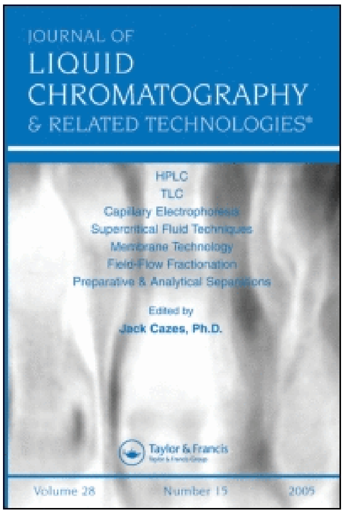

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Countercurrent Chromatography and the Journal of Liquid Chromatography: A Love Story

Alain Berthod[a]
[a] Université de Lyon, Laboratoire des Sciences Analytiques, Villeurbanne, France

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Countercurrent Chromatography and the Journal of Liquid Chromatography: A Love Story

Alain Berthod
Université de Lyon, Laboratoire des Sciences Analytiques, Villeurbanne, France

Abstract: Countercurrent chromatography (CCC) is a separation technique that works with a support-free liquid stationary phase. Since the mobile phase is also a liquid, biphasic solvent systems are used. The problem with CCC is due to its nature: it is difficult to maintain a stable liquid stationary phase when a liquid mobile phase is pushed through it. All modern CCC columns use centrifugal fields. The hydrodynamic CCC columns use open tubes coiled on spools that rotate in a planetary manner. The hydrostatic CCC columns use channels interconnected by ducts, mounted in a centrifuge rotor. It is difficult to work with a support-free liquid stationary phase, but the advantages of this chromatographic configuration are so important that it is worth finding better ways and to invent better CCC columns. The main advantage is that all injected compounds can access the whole volume of the liquid stationary phase, compared to the necessary solid-liquid interphase contacts with classical solid stationary phases. Saturation of the stationary phase, or column overload, is much more difficult in CCC than in classical preparative LC with silica gel based stationary phases. CCC is definitely a preparative technique. The other advantages of a liquid stationary phase include the wide range of polarities that can be obtained using the numerous available solvents and mixing them. No irreversible adsorption is possible in CCC, since the liquid stationary can always be recovered. These advantages and the modern commercially available CCC columns are presented in this article celebrating the 30th birthday of the Journal of Liquid Chromatography that always, with its

Address correspondence to Alain Berthod, Université de Lyon, Laboratoire des Sciences Analytiques, CNRS 5180, Bat. CPE, 69622, Villeurbanne, France. E-mail: berthod@univ-lyon1.fr

editor, strongly supported the technique by publishing, at certain occasions, half of the whole scientific CCC production of the world.

Keywords: Countercurrent chromatography, Hydrostatic columns, Hydrodynamic columns, Large scale purification, Preparative chromatography, Special issues, Symposium issues

INTRODUCTION

Countercurrent chromatography (CCC) is a separation technique that uses the chromatographic principles with two liquid phases. This means that the mobile phase and the stationary phase are the two liquid phases of a biphasic liquid system in equilibrium.[1–3] There is not necessarily a countercurrent circulation of one liquid phase versus the other. CCC was invented in 1966 by Yoichiro Ito[4] who named it after countercurrent distribution liquid-liquid apparatuses (“Craig machines”) and dedicated his professional life to develop the technique.[5] He has published hundreds of articles establishing the triple C acronym so well that it is now accepted worldwide for all separation techniques using a support-free liquid stationary phase.

The very name of the technique has led people who do not know CCC to ask: “I just see one pump on this CCC set-up; where is the second pump needed to establish the countercurrent flow?” No, CCC is a classical liquid chromatography (LC) technique working with only one pump. CCC is just an LC technique with a support-free liquid stationary phase.[3]

As can be guessed, the problem is to obtain a stable support-free liquid stationary phase. Here is the real difficulty of the CCC technique. The CCC column cannot be a simple tube with two frits at each end. In all modern CCC “columns”, centrifugal fields are used to hold the liquid stationary phase when the liquid mobile phase is pushed through it. Centrifugal fields imply rotating parts, rotors, gears, spools, rotating seals, motors, speed regulators, noise, and wear problems, all things that had hindered the development of CCC. It should be clearly understood that the whole CCC device is simply a chromatographic column.

This review article will rapidly present why it is so interesting to work with a liquid stationary phase. The modern CCC columns, their uses, and some applications will be briefly presented. The focus will be on the historical bond, the Love Story, that exists between *Journal of Liquid Chromatography & Related Technologies*® (JLC&RT) and CCC.

A LIQUID STATIONARY PHASE

It was already mentioned that it is difficult to work with a liquid stationary phase. What are, then, the advantages of a liquid stationary phase?

A Preparative Technique

The content of a working HPLC column is: mobile phase, silica skeleton of the stationary phase, bonded moieties on the silica stationary phase (in reversed phase LC), and stagnant mobile phase in stationary phase pore volume. The solid stationary phases differ by surface area, pore size, pore volume, bonding nature and density, residual silanols, purity of the silica skeleton, and special chemical treatment, all parameters making it difficult to find two similar RPLC columns even though they both are, e.g., octadecylsilane bonded silica stationary phases.[6] The solute exchanges between the mobile and the solid stationary phase occur at the interface on the surface of the bonded silica. This surface can be rapidly overloaded, producing peak distortion and overlapping, and loss of resolution.

The content of a working CCC column of volume, V_C, is just a mobile phase volume, V_M, and a stationary phase volume, V_S related by:

$$V_M + V_S = V_C \tag{1}$$

Liquid-liquid partitioning is the only exchange responsible for solute retention according to the retention equation:

$$V_R = V_M + K_D V_S \tag{2}$$

Scaling-up is almost straightforward. If the instrument volume, the liquid system phase retention, and solute partition coefficients are known, Eq. (2) allows us to predict peak positions very reliably.

A recent example of such scaling-up and of the prep capabilities of CCC is presented here. Glycosinates are valuable molecules found in plants with highly desirable properties. They could be added to functional food. Glycosinates are highly polar molecules which are very difficult to separate. 500 mg of glucoraphanin was separated from 200 mg of glucoiberin by CCC in two hours using a 325 mL hydrodynamic CCC column and a 21/11/68% v/v propanol/acetonitrile/2.1 M ammonium sulfate (275 g/L $(NH_4)_2SO_4$ solution) liquid system.[7] This separation was transposed onto an 850 mL hydrodynamic column with the same liquid system. In 3 days and 34 runs, injecting 17 g of methanolic broccoli extract each time, it was possible to produce 53 g of 98% pure glucoraphanin.[8]

A Versatile Technique with No Irreversible Solute Adsorption

Another essential advantage of a liquid stationary phase is its versatility. There is no objection to working with the upper mobile phase, or with the lower mobile phase. In both cases, the other phase is the stationary phase. It is even possible to change the phases' roles during the run; this is the dual-mode method (Fig. 1). The elution-extrusion method was developed recently. It uses the fact that the

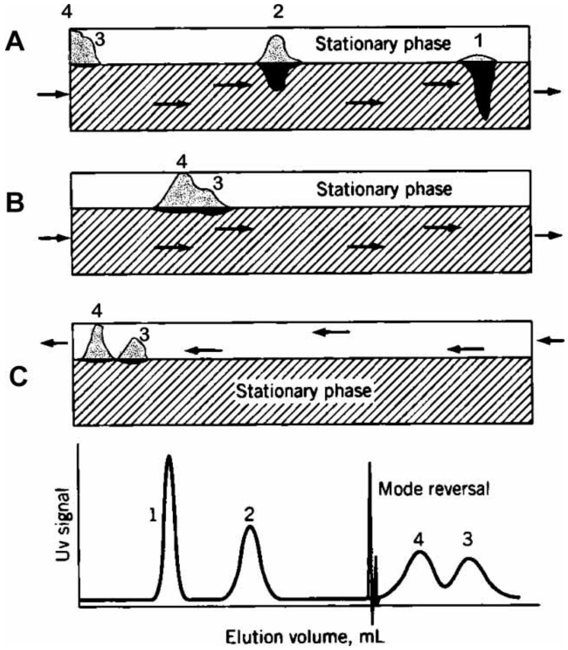

Figure 1. The CCC dual-mode method. A: Initial separation of the less retained Solutes 1 and 2 that elute rapidly. B: A large volume of mobile phase would be needed to elute Solutes 3 and 4. C: The phase roles are switched and what was previously the stationary phase (open liquid phase) is flowed in the opposite direction. Solutes 3 and 4 elute in the order 4 first then 3 after the mode reversal.

stationary phase is a liquid, so it can be used as a piston to extrude the column content after normal elution.[9,10] It was demonstrated that, inside the column, the peak band widths were very narrow; these narrow bands could be successfully extruded in CCC, allowing for unexpectedly high resolution factors.[11] True moving bed CCC or co-current CCC was also possible thanks to the liquid nature of the stationary phase. In this situation, two pumps are used. The "stationary" phase, that should better be called the slower phase, slowly moves in the same direction as the mobile phase, thus speeding up the elution of otherwise highly retained solutes.[11] The extremely valuable property of these different ways to use the liquid nature of the stationary phase is that there is no possible irreversible adsorption inside the CCC column. Whatever mixture is injected, it is guaranteed that everything will be recovered.

A Separation Technique Differing from HPLC

CCC is a separation technique complementary to HPLC with a solid stationary phase. Figure 2 illustrates an essential point of CCC. The four chromatograms

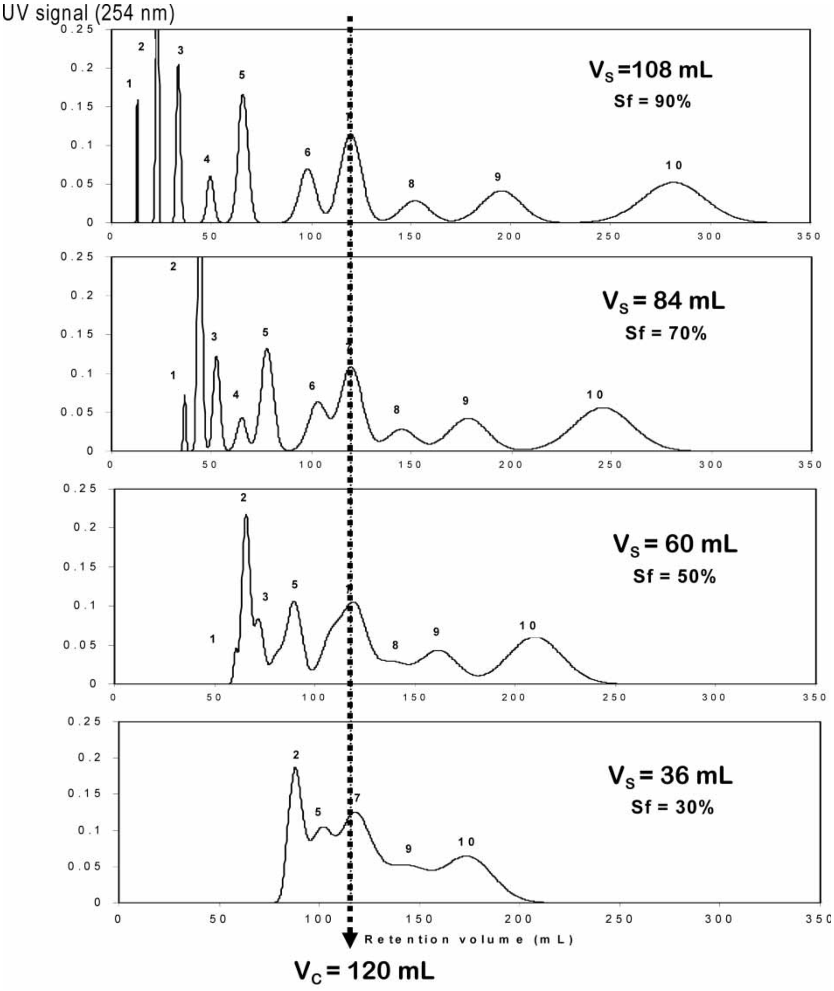

Figure 2. Four chromatograms obtained with the same CCC column, the same liquid system and the same sample. The dramatic influence of the stationary phase retention volume on chromatographic resolution is evidenced. Column volume: 120 mL (vertical dotted arrow). Adapted from Ref. [3].

presented by Fig. 2 were all obtained with the same CCC column, using the same solvent system, and injecting the same sample. In HPLC and all other separation techniques, these working conditions would produce the same chromatograms. This is actually the way to test HPLC equipment. In CCC, mobile and stationary phases are linked. Figure 2 shows a dramatic loss of resolution due to the decrease of the volume of the liquid stationary phase retained inside the CCC column. This factor is so important that a special parameter, Sf, was introduced to CCC to quantify it:

$$\mathrm{Sf} = \mathrm{V_S}/\mathrm{V_C} \tag{3}$$

In the Fig. 2 chromatograms, the Sf factor was intentionally decreased from 90% (or 0.9, top chromatogram) down to 30% (or 0.3, bottom chromatogram), clearly showing the resolution loss: only five peaks are barely seen on the chromatogram obtained with Sf = 30% and the ten compound injected sample (bottom chromatogram). Obviously, if the CCC column does not retain any liquid stationary phase (Sf = 0), all injected solutes will elute together at $\mathrm{V_C} = 120$ mL, the column volume. Equation (2) shows that all compounds having a $\mathrm{K_D}$ coefficient higher than one have a decreasing retention volume as Sf decreases. Compounds with $\mathrm{K_D}$ coefficients lower than unity have an increasing retention volume in the same situation (Fig. 2). The vertical dotted arrow corresponds to the CCC column volume and also to the retention volume of solute 7 with a $\mathrm{K_D}$ coefficient exactly equal to one ($\mathrm{V_R} = \mathrm{V_C}$, Eqs. (1) and (2)).

Mobile and stationary phases are also linked chemically. In HPLC, the column is selected: C_{18}, C_4, phenyl, etc... bonded column and next the mobile phase is prepared mixing, e.g., water, methanol, and/or acetonitrile in the desired proportions. It is not possible to work that way in CCC. The biphasic liquid system should be selected, meaning that the stationary phase and the mobile phase should be selected together. Any change in the composition of the liquid mobile phase will change the composition of the liquid stationary phase. These changes can be predicted working with phase diagrams. But this fact should be known when envisaging CCC.[3]

TWO KINDS OF CCC COLUMNS

Figure 3 is a sketch illustrating the hydrodynamic and hydrostatic modes used to maintain a liquid phase stationary phase while an immiscible liquid phase is flown through it.

Hydrodynamic CCC Columns

Open tubes coiled on one or several spools are the core of hydrodynamic CCC columns. These columns were exclusively developed by Ito.[1,4,5] All modern hydrodynamic CCC columns are based on what Ito called its Scheme IV. In this configuration, the spools are enclosed in a rotor and a gear system which produces a planetary motion of the spools around two axes: they rotate around their own axes and also around the central rotor axis that bears a stationary gear. This combined planetary rotation produces a

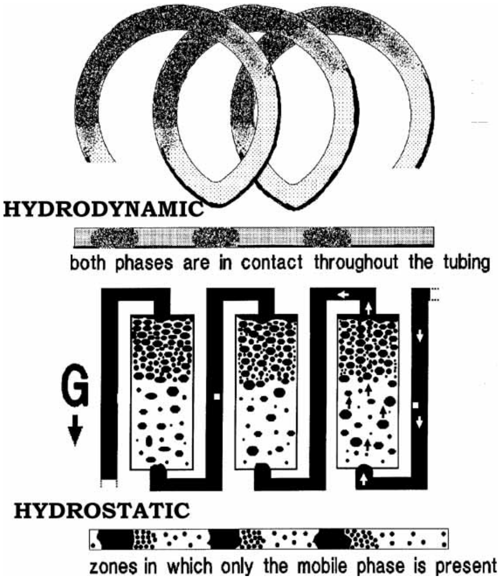

Figure 3. Top: hydrodynamic CCC columns contain coiled tubes rotating in a planetary manner. The periodic centrifugal field induces decantation and mixing zones. Bottom: hydrostatic columns have geometrical interconnected containers, locules or channels, retaining the stationary phase (open liquid). The dark liquid is the lighter upper phase entering tail-to-head (hydrodynamic CCC) or in the ascending way (hydrostatic CCC) in the columns. The open liquid is the denser or lower stationary phase.

centrifugal field that changes in intensity and direction in a periodic way. The high centrifugal field is obtained when there is addition of rotor and spool fields, i.e., the considered tube point passes at the rotor exterior. Then, this high field produces a phase decantation. A half turn after this situation, the same tube point passes near the central axis where there is subtraction of the spool and rotor fields with a resulting centrifugal field in the opposite direction. This produces the mixing of the two phases. When equilibrium is reached, a succession of mixing and decantation zones exist inside the length of the tube, as illustrated by the unfolded tube shown in Fig. 3. An Archimedean force is created by the thread of the coiled tubing. This force tends to push the lighter liquid phase towards one end of the coil called "head" by similarity with gravity pushing the lighter phase on top of the denser one in a graduated cylinder. The described hydrodynamic equilibrium is created only if the mobile phase enters through the right side of the coil. If the mobile phase is the lower denser liquid, it should enter in the coil through the head side, against the Archimedean force, in the head-to-tail direction. If the mobile phase is the upper lighter liquid, it should enter the CCC column through its "tail" in the tail-to-head direction. Otherwise, the CCC column will not retain any liquid stationary phase, thus precluding separation.

Figure 4 shows a modern hydrodynamic CCC column of 18 mL, the smallest CCC column on the market at the moment. The inset shows the biggest hydrodynamic CCC column.

Hydrostatic Columns

In hydrostatic columns, there are geometrical volumes, in the form of tubes, channels, or locules, in which the two liquid phases are contacted. The volumes are interconnected, forming a pattern. They are contained in a rotor whose rotation generates a constant gravitational field, G (Fig. 3, bottom). For almost twenty years, commercial hydrostatic machines were made by the Japanese company, Sanki Engineering Ltd, that marketed them under the trade name "Centrifugal Partition Chromatographs" (CPC). Hydrostatic CCC columns are still often called CPCs. The constant gravitational field pushes the denser phase at the bottom of the channel (open phase in Fig. 3). As for hydrodynamic columns, there is a right way to introduce the mobile phase into the column. If the mobile phase is the light upper phase, it should enter at the bottom of the channels, in an ascending way (dark

Figure 4. The smallest and the biggest hydrodynamic CCC columns available on the market (2007). Both columns are built by Dynamic Extraction, London, UK. The "Milli" is an 18 mL hydrodynamic column with one spool and a counterweight in a temperature regulated box. Lower left: The "Maxi" is a 5 liter double coil column. Ian Sutherland (Brunel University of West London) is shown inside the rotor to show the scale.

liquid in Fig. 3, bottom) similar to the tail-to-head mode of the hydrodynamic CCC columns. The advantage of hydrostatic CCC columns is their quiet operation and very good retention of the liquid stationary phase in the channels. A drawback is that the connecting ducts only contain the mobile phase. Since there is only one phase, the chromatographic mobile-stationary phase exchanges are not possible: the connecting ducts can be considered as dead volumes.

Figure 5 shows a modern hydrostatic CCC column and the discontinued Sanki LLN-CPC 200 mL column. Figures 4 and 5 clearly show that the CCC columns are apparatuses of significant size and complexity. This is due to the centrifugal field needed in all modern columns to tightly hold the support-free liquid stationary phase. It is not possible to recommend one type of CCC columns or another. It depends on the separation or purification problem. If the liquid system is difficult to retain (e.g., aqueous two phase liquid systems), a hydrostatic CCC column would be recommended. If a high efficiency is needed, a hydrodynamic CCC column may be preferred. Table 1 compares the features of the two types of CCC columns. Table 2 lists most of the world manufacturers of the two types of CCC columns.

Figure 5. Hydrodynamic CCC column. Top right, the Sanki CPC-LLN, a 200 mL hydrostatic column sold for years in the US by Jack Cazes. The JLC&RT issue gives the scale. Bottom left: The Armen Elite one liter CPC (Armen Instruments, Vannes, France). Top left: close view of the patented dual chamber channel design of the Armen Elite CPC hydrostatic column.

Table 1. Compared properties of the two types of CCC columns

CCC Column	Hydrodynamic	Hydrostatic
The liquid stationary phase is retain in or by	Coiled PTFE tubing	Channels or locules
Axis number	2, planetary motion obtained with a gear system	1, simple rotation
Gravitational field	Highly variable, planetary motion	Constant, simple rotation
Efficiency	Good, 4 plates per tubing turn or ~10 plates per mL	Average, 0.5 plate per channel or ~5 plates per mL or less
Pressure	Low Pressure (1–10 atm or less than 140 psi)	Average Pressure (10–95 atm or 140–1000 psi)
Maintenance	Flying connecting leads	Rotating seals
Noise	Very Noisy	Quiet

APPLICATIONS

CCC does not challenge HPLC, since it is not a technique dedicated to analysis. The main use of CCC is separation and purification of molecules. CCC may challenge preparative LC in terms of purity of the final product, solvent consumption, productivity, and general cost of the production line. CCC can go from milligram size, e.g., search and purification of plant extracts; gram size, e.g., preparation of standards and reference materials; up to kilogram size.[13]

Figure 6 shows a study done of 242 articles published in 2004 and 2005 dealing with the CCC technique. By far, the isolation of compounds coming from natural products from vegetal origin, e.g., roots, leaves, plants, trees, and from animal origin, e.g., proteins, lipids, enzymes, is the main CCC field, with 157 articles. Liquid-liquid exchanges involved in the CCC separation processes are gentler for delicate and complex natural molecules than the silica-mobile phase exchanges of classical LC. The glucoraphanin purification presented earlier is a typical example.[7,8] More examples are seen with CCC used with kg/day production range.[14]

Figure 6 also shows that the other applications of CCC presented in 2004 and 2005 include purification of pharmaceutical compounds (19 articles) coming from a synthetic pathway, methodology exploring the properties of a liquid stationary phase and new ways to use it (17 articles), the description of new CCC columns or even new methods (15 articles), purification of inorganic compounds, e.g., metal ions (15 articles), original solvent systems and hydrophobicity studies (19 articles). Extensive lists of applications and examples of separation and purification can be found in books and reviews dedicated to CCC.[1–3,12,15]

Table 2. CCC column manufacturers (2007)

Company name	Model	Spool number	Volume (L)	Comments and Web sites http://*
Hydrodynamic Columns				
Dynamic Extractions Brunel, London, UK	Milli	1	0.015	GIP complient *dynamicextractions.com*
	Midi	2	0.6 or 1.2	
	Maxi	1	5 or 15	
New Technology Application, Beijing, China	GS20	2	0.03	Available in Asia only *binta.com.cn*
	GS10A2	1	0.24	
Pharma Tech Research, Baltimore, USA	TCC-1000	1	0.05	*pharma-tech.com*
	CCC 3000	3	0.3	
	CCC 1000	3	0.12–0.85	
AECS, Bridgend, UK	Quattro	2	0.1–0.5	Taylor made CCC columns *ccc4labprep.com*
	Labprep	2	0.5–3	
Conway Centrichrom Inc., Buffalo, NY, USA	DP-1000	2	0.03–1	variable volume *centrichrom.com*
Tauto Biotech Ltd, Shenzhen, China	TBE-300	2	0.3	*tautobiotech.com*
	TBE-1000	2	1	

(*continued*)

Table 2. Continued

Company name	Model	Spool number	Volume (L)	Comments and Web sites http://*
Channel Number				
Hydrostatic Columns				
EverSeiko, Tokyo, Japan	HPCPC1000	2000	0.22	Sanki Machines *everseiko.co.jp*
	LLL-1.5	1300	1.4	
	LLI-1.7	1040	5.4	
Partus Technology, Reims, France	Partitron	1000	5	Rotor in one titanium block *rodolphe.margraff@wanadoo.fr*
SEAB, Kromaton, Angers, France	FCPC	1300	0.2	Different volumes available on demand *Kromaton.com*
Armen Instrument, Vannes, France	Elite CPC	1000	1	Integrated pumping system *armen-instrument.com*
	Prep CPC	1000	5	

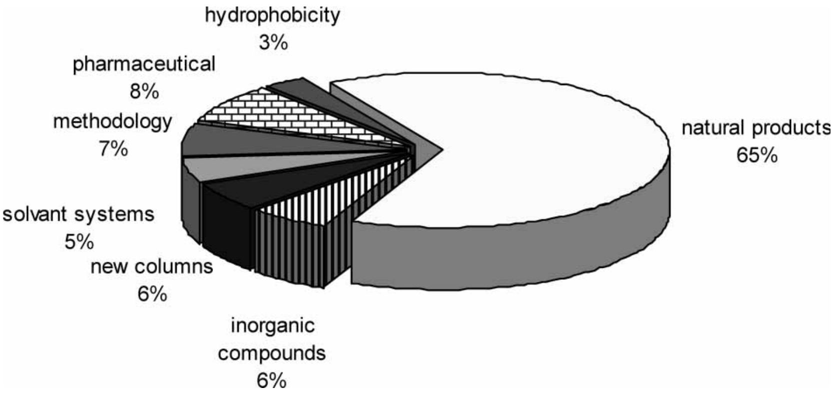

Figure 6. Topics treated by 242 articles published during the two years 2004 and 2005 in countercurrent chromatography. Data obtained from SciFinder®, CAPlus® and Scopus®.

CCC AND THE JOURNAL OF LIQUID CHROMATOGRAPHY

This special issue celebrates the birth date of *Journal of Liquid Chromatography* (JLC) that first appeared thirty years ago, in 1978, to be a tribune for emerging new techniques using a liquid mobile phase. At the time, high pressure liquid chromatography was gaining an increased importance over gas chromatography, being able to efficiently separate non-volatile and/or thermo labile and biological compounds. Its editor, Jack Cazes, was always watching for new potential separation methods.

The Pittsburg Conference Era

Jack noticed, early, the young CCC technique and, in 1983, he invited Yoichiro Ito, its inventor to edit a special issue which was fully dedicated to the young, new CCC technique. Dr. Ito worked and still works at the National Institutes of Health in Bethesda, Maryland. He was setting up a special CCC session at the Pittsburg Conference. He proposed to his neighbor, Dr. Bhushan Mandava, working at the Environmental Protection Agency, in Washington, DC, to edit the special issue. It was published as number 2 of Volume 7 of JLC.[16] It contained 13 articles coming from seven different laboratories. Five of its authors are still active in the CCC field, twenty five years after this first special issue. They are Yoichiro Ito (authoring seven articles out of thirteen!), Kurt Hostettmann, Walter Conway, Martha Knight, and Ian Sutherland.[16] This first special issue was very well received and, besides maintaining his JLC editor position, Jack Cazes accepted the charge of CEO of Sanki USA with the exclusive sales

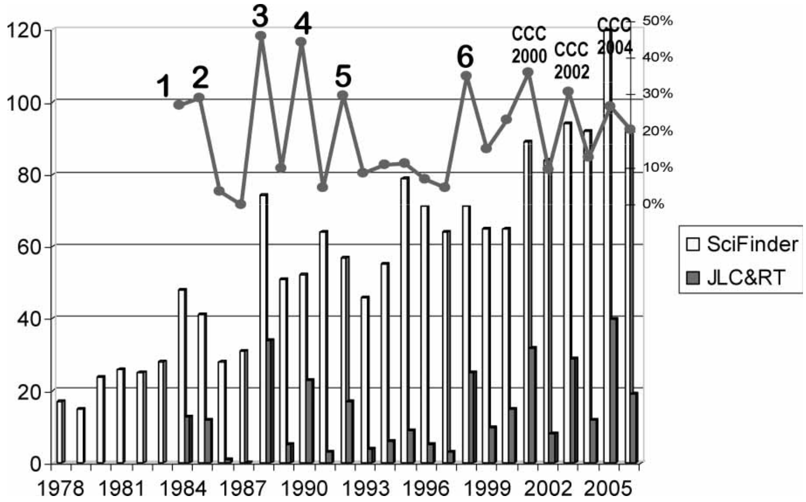

Figure 7. Journal of Liquid Chromatography & Related Technologies® and Countercurrent Chromatography. White bars: Total number of articles dealing with CCC published yearly (counted using SciFinder®); Dark bars: Articles in JLC and JLC&RT; Line and right scale: part of JLC&RT expressed in percentage of the whole number of published articles. N° 1 to 6 correspond to 6 special issues. CCC 2000 to 2004 correspond to Symposium issues.

in North America of the hydrostatic CCC columns made by Sanki Engineering, Kyoto, Japan. He managed to combine his two jobs, organizing CCC special sessions at the Pittsburg Conference and having Bhushan Mandava editing five more JLC special issues entirely dedicated to CCC.[17–21] The second 1990 special issue[20] was dedicated to CPC columns, the products sold by Sanki, which was the sole hydrostatic CCC column provider in the world at the time. All other special issues, including the last one, in 1998, but in the red journal JLC&RT,[22] presented novelties and applications involving both types of CCC columns.

Establishment of a Bi-Annual International Symposium

The late Edward Chou (1944–2004) and Ian Sutherland of the Brunel University of West London, respectively, funded and organized CCC 2000 in Brunel in September 2000. CCC 2000-London was so successful that an International CCC committee was established and it was decided that this meeting was the first of a bi-annual international symposium dedicated to the CCC technique. The next meeting was scheduled for April 2002 in Beijing (China). JLC&RT and Jack Cazes were the tribune where the best talks presented in London were published in a Symposium special issue.[23] The Beijing Symposium was followed by CCC 2004 in August in Tokyo (Japan) with, again, Symposium volumes in JLC&RT.[24,25] So many excellent articles were proposed to the CCC 2004 symposium issue that Jack and the symposium volume editors had to ask authors to publish their work in other journals: the CCC success transcended the available double issue page number of the journal. A discussion of the international CCC committee during CCC 2006 symposium in the home town of Yoichiro Ito, Bethesda, took the decision to propose the Symposium articles to a competitor journal able to host a larger number of articles. This article is the proof that the CCC authors will never forget what Jack and JLC&RT did and keep going to do to the technique.

CONCLUSION

Countercurrent Chromatography is a separation technique working with a liquid stationary phase and mainly oriented towards purification of compounds. Comparing CCC with silica based preparative chromatographic techniques, at similar column volumes, CCC has a significantly higher loading capacity to process mixtures in a similar time scale using less solvent volumes. Another essential advantage is the absence of irreversible column adsorption: everything that is injected into a CCC column can be recovered. Biphasic liquid systems are used. They provide a very wide range of polarities. The separation mechanism is exclusively related to liquid-liquid partitioning. No silanol interaction, no size exclusion effect due to solid porosity, no strong interaction on silica surface. The two-liquid-phase interaction process is much gentler for delicate molecules than liquid-solid interactions involved with a solid stationary phase. The major problem of the technique: how to maintain, tightly, a liquid stationary phase, is increasingly well handled by modern CCC columns, either following the hydrodynamic way or the hydrostatic way.

Figure 7 shows the steadily increasing number of articles dealing with CCC (open bars). The dark bars are the JLC and, after 1995, JLC&RT contribution. The line at the top of the figure expresses this contribution in yearly percentage, clearly showing the significant surges due to the CCC special issues (numbered 1 to 6) and symposium volumes. In a year with a special issue or a symposium issue, JLC&RT publishes more than one article out of three (30%) appearing in the whole international literature referenced in international databases. In 1988 and 1990, this proportion approached 50% of the work in CCC done in the world published in JLC&RT. Figure 7 clearly proves that Journal of Liquid Chromatography and its unique editor, Jack Cazes, were always actively concerned with the technique going to the point where half of the scientific world production of CCC articles was found in the journal. Happy 30th birthday JLC&RT and thank you so much for the continued support to the emerging CCC technique.

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REFERENCES

1. Mandava, N.B.; Ito, Y. Chromatographic science series. In *Countercurrent Chromatography, Theory and Practice*; Marcel Dekker: New York, 1985; Vol. 44.
2. Conway, W.D. *Countercurrent Chromatography, Apparatus, Theory and Applications*; VCH Publishers: Weinheim, 1990.
3. Berthod, A. Comprehensive analytical chemistry. In *Countercurrent Chromatography, The Support Free Liquid Stationary Phase*; Elsevier: Amsterdam, 2002; Vol. 38.
4. Ito, Y.; Weinstein, M.A.; Aoki, I.; Harada, R.; Kimura, E.; Nunogaki, K. The coil planet centrifuge. Nature **1966**, *212*, 985–987.
5. Ito, Y. Origin and evolution of the coil planete centrifuge: a personal reflection of my 40 Years of CCC research and development. Sepn. Purif. Rev. **2005**, *34*, 131–154.
6. Claessens, H.A.; Vermeer, E.A.; Cramers, C.A. A comparison of reversed-phase performance for some commercially available C_{18} HPLC columns. LC-GC **1994**, *12* (2), 114–121.
7. Fahey, J.W.; Wade, K.L.; Stephenson, K.K.; Chou, F.E. Separation and purification of glucosinates from crude plant homogenates by HSCCC. J. Chromatogr. A **2003**, *996*, 85–93.
8. Fisher, D.; Garrard, I.J.; Van der Heuvel, R.; Sutherland, I.A.; Chou, F.E.; Fahey, J.W. Technology transfer and scale-up of extraction of glucopharanin, a potential anti-cancer, from plant extract. J. Liq. Chromatogr. Rel. Technol. **2005**, *28*, 1913–1922.
9. Berthod, A.; Hassoun, B.; Harris, G. Using the liquid nature of the stationary phase: the elution-extrusion method. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 1851–1866.
10. Berthod, A.; Ruiz-Angel, M.J.; Carda-Broch, S. Elution-extrusion CCC; use of the liquid nature of the stationary phase to extend the hydrophobicity windows. Anal. Chem. **2003**, *75*, 5886–5894.
11. Berthod, A. Band broadening inside the chromatographic column: the interest of a liquid stationary phase. J. Chromatogr. A **2006**, *1126*, 347–356.
12. Foucault, A.P. Chromatographic Science Series. In *Centrifugal Partition Chromatography*; Marcel Dekker: New York, 1995; Vol. 68.
13. Sutherland, I.A.; Hawes, D.; Ignatova, S.; Janaway, L.; Wood, P. Review of progress toward the industrial scale-up of CCC. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 1877–1891.
14. Garrard, I.G.; Janaway, L.; Fisher, D. Minimizing solvent usage in high speed, high loading, and high resolution isocratic dynamic extraction. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30*, 151–163.
15. Ito, Y.; Conway, W.D. Chemical analysis. In *High Speed Countercurrent Chromatography*; Wiley: New York, 1996; Vol. 132.

16. Mandava, N.B. Special issue on countercurrent chromatography. J. Liq. Chromatogr. **1984**, *7*, 227–440.
17. Mandava, N.B. Special issue on countercurrent chromatography. J. Liq. Chromatogr. **1985**, *8*, 2127–2342.
18. Mandava, N.B. Special issue on countercurrent chromatography. J. Liq. Chromatogr. **1988**, *11*, 1–307.
19. Mandava, N.B. Special issue on countercurrent chromatography. J. Liq. Chromatogr. **1990**, *13*, 2307–2511.
20. Cazes, J. Special issue on centrifugal partition chromatography. J. Liq. Chromatogr. **1990**, *13*, 3559–3687.
21. Mandava, N.B. Special issue on countercurrent chromatography. J. Liq. Chromatogr. **1992**, *15*, 2639–2939.
22. Mandava, N.B. Special issue on countercurrent chromatography. J. Liq. Chromatogr. Rel. Technol. **1998**, *21*, 1–278.
23. Berthod, A.; Sutherland, I.A. J. Liq. Chromatogr. & Rel. Technol. CCC 2000 London: The First International Conference on CCC 2001, 24; 1523–1869.
24. Berthod, A.; Sutherland, I.A.; Zhang, Y. J. Liq. Chromatogr. & Rel. Technol. CCC 2002 Beijing: The 2nd International Conference on CCC 2003, 26; 1335–1676.
25. Berthod, A.; Sutherland, I.A.; Oka, H. J. Liq. Chromatogr. & Rel. Technol. CCC 2004 Tokyo: The 3rd International Conference on CCC 2005, 28; 1789–2110.

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