Inamuddin · Ali Mohammad *Editors*

Green Chromatographic Techniques

Separation and Purification of Organic and Inorganic Analytes

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Editors
Inamuddin
Department of Applied Chemistry,
Faculty of Engineering & Technology,
Aligarh Muslim University,
Aligarh
India

Ali Mohammad
Department of Applied Chemistry,
Faculty of Engineering & Technology,
Aligarh Muslim University,
Aligarh
India

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Preface

Green technologies demand the use of safer instrumental techniques for the separation, identification and purification of organic and inorganic analyte. Nowadays, special emphasis has been given to the separation and purification of bioactive components like vitamins, amino acids, toxins, carbohydrate etc. However, the concern about the use of toxic solvents has been completely ignored. Therefore, to make the system convenient, it is advisable to use non-toxic solvents or techniques for the separation and purification. So that life can sustain a long without exposure of harmful toxic solvents.

This book would be a wide spectrum scientific resource on the use of green chromatographic techniques for the separation, identification and purification of bioactive as well as inorganic analyte. The aim of this book is to provide in depth knowledge of the green techniques which utilize green solvents. In some chapters the technique itself is green or using non toxic environment. However, the book is basically aimed to merge the terms green techniques and green solvents as **green chromatography** which may prove the useful resource to the scientist working in the field of analytical, organic and pharmaceutical chemistry.

Green Chromatographic Techniques: Separation and Purification of Organic and Inorganic Analytes' edition with *most up-to-date reference* work will prove a necessary resource for scientists, R&D industrial specialists, researchers, upper-level undergraduate and graduate students, Ph.D. students, college and university professors working in chemistry, chemical and biochemical fields. Based on thematic topics, the book edition contains the following 9 chapters:

Chapter 1 addresses some practical and theoretical aspects of counter current chromatography (CCC), highlighting the specific advantages of this support-free liquid stationary phase purification greener technique.

Chapter 2 is dealing with new preparative method of separating concentrated solutions of mineral electrolytes into individual components by size exclusion chromatography on neutral nanoporous hyper-crosslinked polystyrene sorbents. Basic principles of the method as well as factors determining the selectivity of separations are discussed.

Chapter 3 is dealing with the supercritical fluid chromatography which is known as a green approach for the separation and purification of organic and inorganic analyte.

Chapter 4 provides historical development of thin-layer chromatography towards becoming modern, automated, high resolution technique in the form of high-performance thin-layer chromatography, and their further advances in miniaturization of chromatographic beds in the form of ultra-performance thin-layer chromatography (UPTLC).

Chapter 5 highlights the different aspects of gas chromatography in the light of green techniques starting from sample preparation to the selection of mobile phase and chromatographic columns. Coupling other analytical tools with GC to focus the versatility and high accuracy of analysis with dual system of separation and detection is also discussed.

Chapter 6 is dealing with the green reversed-phase high-performance liquid chromatography (RP-HPLC), and green thin layer chromatography (TLC) methods used for preparing and purifying allicin, a garlic-derived organosulfur compound.

Chapter 7 is dealing with some important green sample preparation techniques used for the separation of organic analyte in complex matrices.

Chapter 8 is focusing the determination of organochlorine pesticides was made through the gas chromatography method using capillary columns and detector with electrons capture.

Chapter 9 describes the retention mechanisms for size exclusion chromatography (SEC) and applications of SEC in the biomedical and pharmaceutical sciences. Finally, the use of SEC as a technique for speciation analysis of polydimethylsiloxanes (PDMS) is presented.

Inamuddin
Ali Mohammad

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Inamuddin
Ali Mohammad

Contents

Contributors

Filip Andrić Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia

Runa Ghosh Auddy Department of Chemical Technology, University of Calcutta, 92 A. P. C Road, Kolkata 700009 West Bengal, India
e-mail: auddyruna@gmail.com

Alain Berthod Institut des Sciences Analytiques, CNRS ISA UMR 5280, Université de Lyon, 5 rue de la Doua, 69100 Villeurbanne, France

Dipa Biswas Department of Chemical Technology, 92, A P C Road, 700009, Kolkata, India
e-mail: dipa_bis@yahoo.com

Zinaida Blinnikova Nesmeyanov-Institute of Organo-Element Compounds, Russian Academy of Sciences, 119991 Moscow, Russia

Adriana Chiş Department of Food control, Environment Protection Faculty, University of Oradea, Oradea, Romania
e-mail: andichis@yahoo.com

Purcărea Cornelia Department of Food control, Environment Protection Faculty, University of Oradea, Oradea, Romania

Sriparna Datta Department of Chemical Technology, University of Calcutta, 92 A. P. C Road, Kolkata 700009 West Bengal, India
e-mail: sriparna_d@yahoo.com

Vadim Davankov Nesmeyanov-Institute of Organo-Element Compounds, Russian Academy of Sciences, 119991 Moscow, Russia

Amit De Department of Chemical Technology, University of Calcutta, 92 A. P. C Road, Kolkata 700009 West Bengal, India

Karine Faure Institut des Sciences Analytiques, CNRS ISA UMR 5280, Université de Lyon, 5 rue de la Doua, 69100 Villeurbanne, France
e-mail: karine.faure@univ-lyon1.fr

Shalini Gupta Protein Research Laboratory, Research Resources Center, University of Illinois at Chicago, 835 S. Wolcott Avenue, Chicago, IL 60612, USA

Cristina Horga Institute of Public Health, Cluj-Napoca, Romania

Jin-Sheng Huang Protein Research Laboratory, Research Resources Center, University of Illinois at Chicago, 835 S. Wolcott Avenue, Chicago, IL 60612, USA

Lasanthi P. Jayathilaka Protein Research Laboratory, Research Resources Center, University of Illinois at Chicago, 835 S. Wolcott Avenue, Chicago, IL 60612, USA

Bao-Shiang Lee Protein Research Laboratory, Research Resources Center, University of Illinois at Chicago, 835 S. Wolcott Avenue, Chicago, IL 60612, USA
e-mail: boblee@uic.edu

Jenny Lee Illinois Mathematics and Science Academy, 1500 Sullivan Rd, Aurora, IL 60506-1000, USA

Nazim Mekaoui Institut des Sciences Analytiques, CNRS ISA UMR 5280, Université de Lyon, 5 rue de la Doua, 69100 Villeurbanne, France
e-mail: davank@ineos.ac.ru

Dušanka Milojković-Opsenica Faculty of Chemistry, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia
e-mail: dusankam@chem.bg.ac.rs

Debarati Mitra Department of Chemical Technology, 92, A P C Road, 700009 Kolkata, India
e-mail: debarati_che@yahoo.com

Krystyna Mojsiewicz-Pieńkowska Faculty of Pharmacy, Department of Physical Chemistry, Medical University of Gdańsk, Al. Gen. Hallera 107 Gdańsk 80-416, Poland
e-mail: kpienk@gumed.edu.pl

Cíntia Alessandra Matiucci Pereira Centro Universitário Central Paulista (UNICEP), R. Miguel Petroni 5111, São Carlos, SP 13563-470 Brazil
e-mail: cmatiucci@gmail.com

Maria Tsyurupa Nesmeyanov-Institute of Organo-Element Compounds, Russian Academy of Sciences, 119991 Moscow, Russia

Vânia Gomes Zuin Universidade Federal de São Carlos (UFSCar), Rodovia Washington Luiz, Km 235, São Carlos, SP 13565-905 Brazil
e-mail: vaniaz@ufscar.br

Chapter 1
Saving Solvents in Chromatographic Purifications: The Counter-Current Chromatography Technique

Karine Faure, Nazim Mekaoui and Alain Berthod

Abstract This chapter addresses some practical and theoretical aspects of counter current chromatography (CCC), highlighting the specific advantages of this support-free liquid stationary phase purification technique. The focus is on the latest instrumental developments which demonstrates that the poorly known CCC technique may exhibit a high, unrealised potential for greener purification processes.

1.1 Introduction

Counter-current chromatography (CCC) is a purification technique that is based on the partitioning of the solutes between two immiscible liquid phases, one being immobile (i.e. stationary phase), while the other liquid (i.e. mobile phase) passes through. This definition includes both the advantages and drawbacks of the technique. First to be noticed, there is absolutely no mention of any counter current flow. Indeed, only one liquid phase flows through the CCC instrument. The unfortunate choice of the technique name originates from historical reasons (Conway 2011) and will prevail as the cute abbreviation "CCC" became so popular. Secondly, the separation of solutes is based only on their distribution between two liquid phases. This is the simplest mechanism that can be found in chromatographic separations since one liquid phase is the mobile phase and the other liquid phase is the stationary phase. The characteristic feature of total absence of solid matrix in the CCC instrument promises a huge versatility for purification of complex samples.

If CCC does not ring any bell to the reader, the main reason is that solid/liquid chromatography has long established itself as the main purification technique, with a large instrument development over decades, while CCC has long suffered from poor engineering and unreliable instruments.

K. Faure (✉) · N. Mekaoui · A. Berthod
Institut des Sciences Analytiques, CNRS ISA UMR 5280,
Université de Lyon, 5 rue de la Doua, 69100 Villeurbanne, France
e-mail: karine.faure@univ-lyon1.fr

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This review will address the theoretical aspects of CCC, highlighting the specific advantages of the technique. It will focus on the latest instrumental developments and demonstrate that the poorly known CCC technique may exhibit a high, unrealised potential for greener purification processes.

1.2 CCC Theory

The CCC retention mechanism is based on solute partitioning between the two non miscible liquid phases. The solute retention relies only on its distribution ratio, K_D, between the two phases. No adsorption occurs, as no solid material is present. The theory behind the separation mechanism is expressed by the simple equation:

$$V_R = V_M + K_D \cdot V_s \tag{1.1}$$

where

V_R is the retention volume,

V_M and V_S are, respectively, the mobile and stationary phase volumes inside the CCC instrument of total volume, V_C ($=V_M+V_S$),

K_D is the solute distribution ratio between the two phases that is the concentration of all forms of the solute in the stationary phase divided by the concentration of all forms of the solute in the mobile phase (Berthod 2002). K_D is also called solute liquid-liquid partition coefficient.

1.2.1 High Loadability

Because the separation process occurs solely in liquids allowing for a volume access, the sample mass that can be injected and treated in one run is generally more important in CCC than in conventional preparative liquid chromatography (prep-LC). In prep-LC, the stationary phase is a porous solid, most often silica particles. The solutes interact with the limited surface of the solid stationary phase that is rapidly overloaded. In CCC, the solutes interact with the much larger volume of liquid stationary phase so the amount of sample that can be introduced in the CCC column is much larger than in prep-LC, only limited by solubility. The samples can be solubilised either in the mobile phase or in the stationary phase, or even in a mixture of both phases.

The useable volume of liquid stationary phase can make up to 70–80 % of the overall CCC column volume, so overloading happens at significantly higher loadings compared to prep-LC where the useable solid stationary phase volume makes rarely more than 1 % of the column volume. The capacity of purification of the CCC technique is therefore significantly enhanced compared to classical prep-LC working with similar volume columns. Consequently, the number of injections/runs to produce a certain amount of pure compound is reduced. High loadability is the ma-

jor incentive that CCC purification solution has. This appealing potential throughput can provide a fair solution to purify otherwise unprofitable products.

1.2.2 Scale up Capability

A CCC separation is governed solely by the distribution of the sample between the two immiscible liquid phases; hence its scale up is simple and predictable. Indeed, Eq. 1.1 is valid for any CCC column not involving the CCC column volume. It remains true whatever the total instrument volume, $V_M + V_S = V_C$, is. However the injection capacity, hence the productivity, both depend directly on V_C. The solvents used with small CCC columns are exactly the same as those used with a larger volume CCC column; hence Eq. 1.1 allows predicting accurately the sample peak positions. In contrast, scale up in solid/liquid chromatography is not straightforward since it is usually difficult to get the very same chromatographic particles that were used in the analytical columns to develop the large scale preparative column.

Figure 1.1 shows an example of school separation of benzyl-alcohol and p-cresol optimized on a small 5 mL CCC hydrodynamic column. The separation was scaled-up one thousand times using a 4.6 l column. In this work the authors have increased the flow rate, the injection volume and mass by three orders of magnitude as to keep the run time identical working with higher processed amount (g in the 4.6 L column) instead of milligram quantities injected in the 5 mL column (Sutherland et al. 2005).

1.3 Instrumentation

CCC provides a very simple yet productive way to purify complex mixtures working with solvent mixtures. Despite these assets, this technique is not widely used in industry because of the very simple reason: Eq. 1.1 shows that solutes are separated if and only if a large volume, V_S, of stationary phase can be retained in the CCC instrument. After several CCC design working with gravity such as the Craig machine or the so-called droplet CCC columns (Conway 2011; Berthod 2002), centrifugal forces were found to be the best way to retain a liquid stationary phase while the mobile phase is pushed through. Necessarily, the implementation of centrifugal forces comes with rotating parts, motors, belts or gears subject to failure, together with vibration, noise and security issues. These unattractive features had major consequences in the slow development of CCC in the industry. Early CCC instruments containing rotors were often poorly engineered and the large equipments available made method development both time- and sample-consuming.

In the past years, the CCC column design and technology made significant improvements. Reliable CCC columns allow for serious instrumental control and fast method development, with smaller columns and automated process control. The

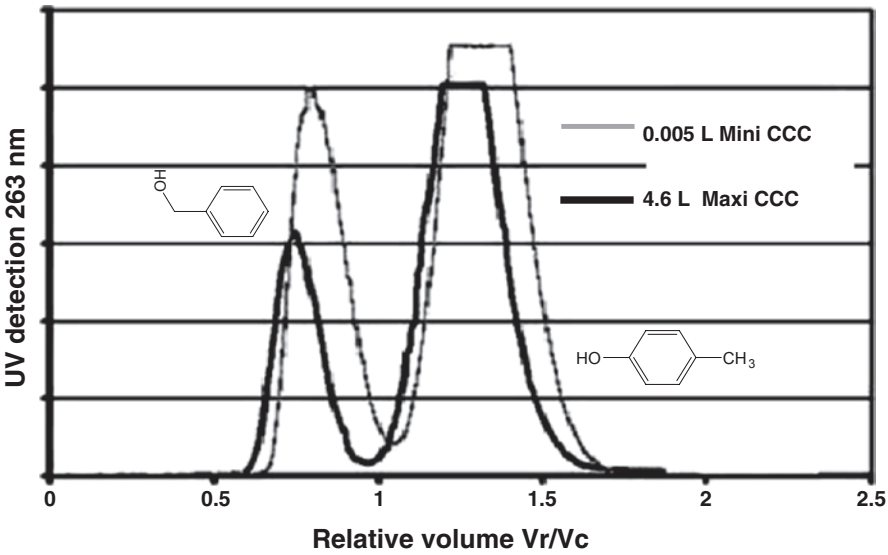

Fig. 1.1 Chromatograms resulting from the separation of a mixture of benzyl alcohol (*BA*) and p-cresol (*PC*) on a 5 mL-column (26 mg BA and 12 mg PC injected in 0.5 mL aqueous phase, F = 1 mL/min lower aqueous mobile phase, 2100 rpm, Sf = 64%) and a 4.6 L-column (24 g BA and 11.5 g PC injected in 460 mL of lower phase, F = 6 L/min, 600 rpm, Sf = 47%), both loadings are 10% of column volumes. Liquid system: heptane 4.7, ethyl acetate 0.3, methanol 1.7, water 3.3 (v/v) in the reversed phase mode with upper organic stationary phase and aqueous mobile phase in the descending or head-to-tail flowing direction. UV detection 263 nm. Adapted from (Sutherland et al. 2005) with permission of Taylor & Francis

latest commercially available instrumentations are presented in Table 1.1 along web sites and contact addresses. No doubt CCC can now integrate its full potential in industrial strategy for purification.

1.3.1 Hydrostatic and Hydrodynamic Instruments

From the numerous CCC prototypes developed during thirty years by Ito, two kinds of instruments were found economically viable and are described hereby (Conway 2011; Berthod 2002).

Sometimes called centrifugal partition chromatographs (CPC), the *hydrostatic* CCC instruments are build from stacked disks in which chambers are engraved in a radial direction, while being linked to each other by small ducts (Fig. 1.2 top). The disks are brought in rotation in a centrifuge. The disk rotation induces a constant radial centrifugal field (hydrostatic force). The liquid stationary phase (colorized in red in Fig. 1.2) is held in the chamber, while the mobile phase percolates through the channels. Chromatographic separation occurs in the chambers while the ducts remain filled with mobile phase. There are two possible working ways. In the *as-*

Table 1.1 Technical characteristics of commercially available CCC equipments

Model name	Column volume (mL)	Max rotation speed (rpm)	Max back-pressure (kg/cm^2)	Max flow rate (mL/min)	Typical handling size (g)
HYDROSTATIC CCC columns (Centrifugal Partition Chromatographs)					
Armen Instrument -Gilson—Z.I. de Kermelin, 16 rue Ampère, 56890 Saint Ave, France www.armen-instrument.com					
SCPC250	250	3000	100	50	5
TCPC250	250	3000	100	100	Continuous
SCPC1000	1000	3000	100	100	20
TCPC1000	1000	3000	100	100	Continuous
Kromaton-Rousselet-Robatel—BP 129 — 07104 Annonay, France www.kromaton.com					
FCPC analyt	50	2000	100	30	1
FCPC semi-prep	200	2000	100	100	5
FCPC prep	1000	2000	100	100	20
EverSeiko Corp. 4-39-5 Senzoku, Taitoku, Tokyo, Japan http://www.everseiko.co.jp					
CPC80	80	1500	60	10	1
CPC240	240	1500	60	20	5
CPC1400	1400	1100	60	80	20
HYDRODYNAMIC CCC columns					
Dynamic Extractions, 890 Plymouth road, Slough, Berkshire, SL14LP, UK http://www.dynamicextractions.com					
Mini	18	2000	~15	2	0.2
Spectrum[a]	140 (20)	1600	~15	10	2
Midi	940	1400	~15	100	20
Maxi	18000	800	~15	1500	1000
AECS-QuikPrep, 55 Gower street, London, WC1 6HQ, UK http://www.quattroprep.com					
IcMSPrep[a]	110 (30)	1200	~10	5	2
QuikPrep[a]	750	1200	~10	20	15
LabPrep[a]	2000	1200	~10	50	20
Tauto Biotech Ltd., 326 Aidisheng road, Zhangjiang Park, Shanghai, China http://www.tautobiotech.com					
TBE-20A	16	2000	~10	1	0.1
TBE-300B	260	1000	~10	4	2
TBE-1000A	1000	600	~10	10	10
CC-Biotech LLC, 9700 Great Seneca Highway, Rockville, MD 20850, USA http://www.ccbiotech.us					
STS-80	80	([b])	~15	([b])	2
STS-135	135	([b])	~15	([b])	4

[a] Device with several possible volumes (smaller volume in parentheses)
[b] STS stands for Spiral Tubing Support; the assembly must be placed in an existing hydrodynamic CCC rotor

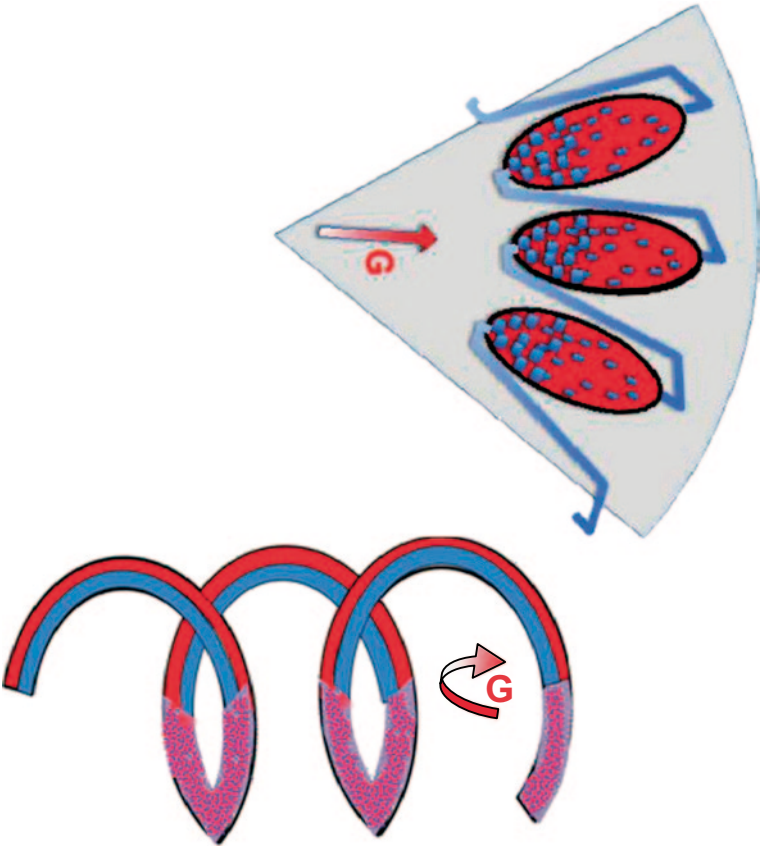

Fig. 1.2 Scheme of the two major CCC instruments. In red color: stationary phase, in blue color: mobile phase. Top—portion of a disk contained in a hydrostatic instrument showing the pattern of channel and ducts retaining the liquid stationary phase with a constant centrifugal field G; bottom—sketch of three turns of a coil contained in a hydrodynamic instrument. The variable centrifugal field G is high at the top inducing liquid phase decantation. It changes direction at the bottom inducing strong phase mingling

cending mode, the chambers are filled with the denser liquid stationary phase and the lighter mobile phase is pushed against the centrifugal field hence the ascending term by analogy with motion in the gravitational field is justified. In the *descending* mode, the stationary phase is the lightest and it fills the chambers, while the denser mobile phase is percolated through it in the direction of centrifugal forces. In both configurations, each geometrical channel is responsible for a significant back hydrostatic pressure proportional to the height of the liquid phase to cross and to the square of the rotating speed. In general, this design easily holds solvent systems, even with small density differences, but it suffers by the limited chromatographic efficiency due to the unused "dead" volumes introduced by the ducts containing only the mobile phase without any chromatographic exchanges.

Hydrodynamic CCC instruments are based on at least one coil mounted in a rotating frame. A gear arrangement generates a planetary rotation motion of the coil(s) with unsymmetrical and rotating centrifugal forces. Many complex designs have been attempted, mainly by the founder of the technique Yoichiro Ito and his group (Conway 1989). The most common instrument is called J-type hydrodynamic CCC. It is based on a planetary motion, with the helical coil rotating around its own axis, while the gear assembly rotates around a central axis, both axes being parallel and revolving at the same speed. During rotation, an oscillating or rotating force field is generated. When put in contact, the two immiscible liquid phases are decanting far from the central rotor axis with a strong centrifugal field (cumulating rotor and coil rotation), and mingling immediately after when passing close to the central axis where the centrifugal field due to coil rotation is subtracted to the rotor centrifugal field (Foucault and Chevolot 1998). The elevated number of mixing/decantation steps results in very good chromatographic efficiency, with high number of theoretical plates.

Due to the thread of the coil tubing, an Archimedeous pumping effect occurs, resulting in the suction of liquid phase at one coil end (called tail), while the pressure increases at the other end (called head). The denser phase of the biphasic liquid system used is always located at the tail side forcing the lighter upper phase to gather at the high pressure coil head. Consequently, if the mobile phase is the denser phase, it will be introduced in a *head-to-tail* direction in a similar way described as descending for the hydrostatic CCC column since it is against the Archimedeous pumping effect. If the mobile phase is the lighter phase, it will be pushed in opposite direction called *tail-to-head* or ascending. The main weakness of these J-type hydrodynamic CCC instruments may rely in liquid stationary phase retention. Some biphasic liquid systems, especially the polar solvent systems, are poorly retained due to high viscosity, low interfacial tension or small density differences between the two liquid phases. When a liquid system is well retained by hydrodynamic CCC columns, the observed chromatographic efficiency (peak sharpness) is higher than what can be obtained with the same liquid system and hydrostatic CCC columns of similar volume.

1.3.2 Liquid Systems

One has to keep in mind that the commercial hydrodynamic CCC instruments are in fact only empty tubes. As opposed to HPLC columns that are sold packed with chromatographic phases and are actually stationary phases, the mobile phase being selected independently, CCC columns have to be generated by the operator chemist prior to every analysis. The major advantage of this point is that a fresh column can be prepared on request, by simply bringing in contact two immiscible liquid phases. While HPLC deals with a limited number of stationary phases, CCC has to deal with the fine selection of the adequate biphasic liquid system to choose between unlimited numbers of possible systems. A CCC solvent system actually

represents two chromatographic systems as either the lighter or the denser phase can be chosen as stationary phase. Any change in composition of either phase will affect the composition of the other phase. The choice of the solvent system fully depends on the sample and its polarity. Many reviews are dedicated to the selection of solvents (Foucault and Chevolot 1998; Hopmann et al. 2011; Yoichiro 2005; Berthod 1991). The range of polarity is wide, from least polar systems not containing water(e.g. heptane/methanol; heptane/acetonitrile, heptane/DMSO) to the most polar systems containing water in their two phases and called aqueous two-phase systems (ATPS), such as the phosphate/polyethyleneglycol (PEG)/water or dextran/PEG/water systems.

Standardization of solvent selection has been attempted, especially for natural product analyses. The heptane or hexane/ethyl acetate/methanol/water system is made by mixing selected volumes of these four solvents that forms two liquid phases. It was introduced by Oka in 1991 (Oka et al. 1991). These four solvents are appropriate having wide polarity difference, low viscosity, UV transparency, strong interfacial tension between the aqueous and organic phases and fast equilibration upon mixing associated with an acceptable impact on environment. Refining this system, a series of compositions with decreasing polarity was proposed. In the ARIZONA system, the alkane/ethyl acetate volume ratio is exactly the same as the methanol/water volume ratio. For example, the first composition, labelled A, is made with no heptane, 1 volume of ethyl acetate (ratio 0/1) and the same for methanol and water, that is, no methanol and 1 volume of water. The A composition is the binary ethyl acetate/water most polar system. The least polar composition Z is the binary waterless system heptane/methanol. This series of compositions (Table 1.2) was called the ARIZONA system because its compositions were referred to using the A to Z letters (Table 1.2). These compositions were found to cover the maximum range of polarity (Lu et al. 2009).

1.4 Counter Current Chromatography, a Green Process

1.4.1 Saving Solvents

In terms of ecological aspects, chromatographic techniques are characterized by a large consumption of organic solvents. Developing a greener process in chromatographic purification can consist in using smaller volumes of solvents. In this aspect, CCC appears at first sight as dealing with large volumes of solvents, but its high capacity results in an overall saving of solvents. A practical example will illustrate this saving. Spinetoram J is a powerful natural insecticide that needs to be purified from Spinetoram L, its fermentation by-product (DeAmicis et al. 2011). Using classical, solid phase based, liquid chromatography, a typical purification run by prep-LC of 2.5 g lasts 8 min with a flow rate of 800 mL/min, which leads to a consumption of 6.4 L of solvents per 2.5 g run. In a simple comparison, a CCC run lasts 140 min at a flow rate of 360 mL/min, i.e., *50 L per run but for a 111 g of crude load*. Since the

Table 1.2 Compositions forming the so-called Arizona liquid system

letter	Solvents as v/v/v/v				Upper phase composition % v/v				up/low	Density
	heptane	ethyl acetate	Methanol	water	Heptane	ethyl acetate	methanol	water	R	g/mL
A	0	1	0	1	0.0	97.0	0.0	3.0	0.88	0.903
B	1	19	1	19	5.3	94.7	0.7	2.6	0.92	0.920
C	1	9	1	9	10.3	85.8	1.4	2.5	0.965	0.878
D	1	6	1	6	13.7	82.0	1.9	2.4	0.96	0.870
F	1	5	1	5	17.2	78.1	2.4	2.3	0.95	0.862
G	1	4	1	4	19.7	75.6	2.6	2.1	0.95	0.856
H	1	3	1	3	25.8	69.4	2.8	2.0	0.945	0.842
J	2	5	2	5	30.8	64.4	3.1	1.7	0.91	0.831
K	1	2	1	2	36.2	58.8	3.5	1.5	0.88	0.818
L	2	3	2	3	46.1	49.9	3.0	0.9	0.84	0.795
M	5	6	5	6	54.6	41.8	2.8	0.7	0.80	0.777
N	1	1	1	1	62.5	34.4	2.6	0.5	0.70	0.760
P	6	5	6	5	69.4	28.2	2.0	0.4	0.69	0.746
Q	3	2	3	2	75.8	22.6	1.5	0.2	0.68	0.733
R	2	1	2	1	83.0	15.6	1.2	0.13	0.68	0.716
S	5	2	5	2	88.8	10.1	1.0	0.09	0.70	0.704
T	3	1	3	1	89.7	9.3	0.9	0.06	0.735	0.701
U	4	1	4	1	94.0	5.2	0.8	0.05	0.76	0.693
V	5	1	5	1	97.3	1.9	0.7	0.04	0.78	0.685
W	6	1	6	1	97.6	1.7	0.7	0.03	0.775	0.685
X	9	1	9	1	98.0	1.3	0.7	0.03	0.77	0.684
Y	19	1	19	1	98.0	1.2	0.8	0.02	0.71	0.684
Z	1	0	1	0	97.6	0.0	2.4	0.0	0.45	0.683

The upper over lower phase volume ratio, *R*, is related to the phase volume percentages as: lower phase percentage = 100/(R + 1); upper phase percentage = 100R/(R + 1); e.g. the phase ratio of *A* is *R* = 0.88 giving 53.2 % of lower phase and 46.8 % upper phase. Data at room temperature (22 °C ± 1 °C) taken from (Lu et al. 2009)

important point of view is productivity, the volumes of solvent per kg of purified compound must be compared for each technique. In this example, CCC uses *733 L* per 1 kg produced in 9 runs, while prep-LC uses *2560 L per kg produced in 400 runs*. Prep-LC needs three times more solvent volume than CCC.

The total time needed to perform the 9 CCC runs is at least 1260 min or 21 h; more likely 25 h including the setting time between runs. The 400 prep-LC runs make 3200 min or 53 h and more likely 60 h total time. Then the throughput of CCC is 40 g/h of purified Spinetoram compared to 17 g/h for HPLC. In short, for this particular example, CCC is able to produce a kilogram of purified Spinetoram 2.5 times faster than prep LC and using three-fold lesser solvent (DeAmicis et al. 2011).

This comparison cannot be generalized to any purification process, but it clearly demonstrates that in some cases, despite its negative image, CCC, based on liquid-liquid exchanges, can be a much greener process than conventional purification techniques based on liquid-solid phase exchanges such as prep LC.

Another interesting point in solvent management in CCC is the fact that the two liquid phases being non miscible, it is possible to recycle both mobile and stationary phases after each run. Recycling is compulsory when dealing with industrial

purifications, due to economical reasons. In CCC, the majority of separations are achieved in isocratic mode. The collection of mobile phase in between chromatographic peaks affords direct recycling. The stationary phase itself can be extruded and monitored to check its purity before recycling.

Solvent systems are generally prepared by mixing the appropriate solvent proportions (Table 1.2) and allowing equilibration and the appearance of the two non miscible phases. But thanks to precise GC analyses of the composition of each phase, it is now possible to prepare each phase individually. Finally, the possibility to recover solvents in product fractions after evaporation and readjustment to final composition before reuse in CCC has been demonstrated (Garrard et al. 2007).

1.4.2 Improving Process Parameters

Another strategy to improve both economical and ecological aspects is by improving process parameters, in terms of mass factors (intensity, separation, efficiency and greenness) (Zhang et al 2011). The throughput can be improved by implementing multiple injections in the purification process, i.e. injecting while the previous separation is still running. One of the objectives to greatly improve throughput is to obtain online purification, meaning a continuous injection mode. A unique advantage of the CCC technique is that any one of the two liquid phases of the biphasic liquid system can be used as the stationary phase. It is even possible to switch the phase role during a separation. This is called working in "dual-mode" (Berthod 2002; Conway 1989). Using such phase role switch several times was called "multiple dual-mode" (Delannay et al. 2006). Multiple dual mode strategy consists in an elution based on frequent switching between mobile phase and stationary phase. Solutes that partition more in the upper phase will elute at one end while solutes that partition in the lower phase will elute at the other end of the column. Injections can be performed either in the middle of the CCC column (or technically speaking between two CCC instruments), as shown in Fig. 1.3 (Van den Heuvel et al. 2009) or at one end of the column as on Fig. 1.4 (Delannay et al. 2006). The injected solutes are moving back and forth, virtually increasing the chromatographic column length then allowing for complete separation.

1.4.3 Injecting Crude Samples

The liquid nature of the two phases allows the direct injection of crude or heavily loaded samples. Solubility can be achieved in either (mobile or stationary) phase or in a mix of both phases. Because no adsorption can occur and open tube clogging is limited, it is possible to load crude samples, limiting the sample preparation of natural products to a simple liquid extraction. For example, Armbuster et al. (Armbruster et al. 2001) achieved the purification of 64 crude plant extracts. Pushing this advantage to its limit, it was found possible to inject samples containing particulate matter (Sutherland 2007) as shown in Fig. 1.5.