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Review

Hydrodynamic and hydrostatic high-speed countercurrent chromatography and its coupling with various kinds of detectors

Application to biochemical separations

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ABSTRACT

A general introduction is given to modern countercurrent chromatography, high-speed countercurrent chromatography using the coil planet centrifuge and centrifugal droplet countercurrent chromatography (centrifugal partition chromatography). The two techniques offer new capabilities for the retention of the liquid stationary phase in the column via a strong centrifugal field and for increased efficiency. Thus, the flow-rate of the mobile phase permits faster separations in comparison with previous techniques such as droplet countercurrent chromatography or rotation locular countercurrent chromatography. Examples are given to show that this is a powerful technique for the preparative-scale separation of natural compounds and it can be coupled to various detection system.

CONTENTS

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1. INTRODUCTION

Countercurrent chromatography (CCC) refers to support-free liquid–liquid chromatography with two immiscible solvents. Recently developed instruments allow the liquid stationary phase to be kept in the column while the mobile phase is pumped through it at high flow-rate, because a strong centrifugal field is created by the motion of the column. Thus, the separation time is much lower than in previous techniques such as droplet countercurrent chromatography (DCCC).

The main features of interest of modern CCC arise from the use of a liquid stationary phase instead of a solid phase: adsorption or degradation of solutes by the solid phase are avoided, the stationary phase is more stable to acidic or basic conditions than silica-based supports, the volume ratio of the stationary phase to the total column volume is much higher than that in reversed-phase HPLC [1], giving higher column capacity and limiting column overloading, and the volume of the stationary phase can be easily fixed by the operator. Dirty samples or complex matrices can be accepted without clean-up; total recovery of the sample is ensured because both the mobile and the stationary phases are liquids and can be collected after the separation. This makes CCC very attractive for preparative-scale chromatography and competitive with preparative-scale HPLC [2].

From an economical point of view, only the cost of the solvents has to be considered; the consumable operating cost of preparative CCC per gram of product is much lower than that of preparative HPLC [2].

Numerous types of CCC apparatus have been developed over the past 25 years, mostly as prototypes by Ito. In our opinion, too much different apparatus combined with a complicated original technique discouraged newcomers to the field, leading to very poor development of this technique. Here, only the commercially available modern and fast systems that have been used in our laboratory will be considered: a hydrostatic equilibrium system (HSES) known as centrifugal partition chromatography (CPC) or centrifugal droplet CCC (CDCCC) where the "locular column" is arranged in a centrifuge; and hydrodynamic equilibrium systems (HDES) in which the coiled column (or two or three columns in series) has a planetary motion, promoting mixing and settling of the two phases. The latter type of system is generally refered as Ito's machine, coil planet centrifuge (CPC) or high-speed countercurrent chromatography (HSCCC).

As in modern liquid chromatography, on-line detection is of major importance for monitoring the HSCCC column effluent and will be discussed here.

This paper also presents some recent advances in CCC from the points of view of separations and loadability. For convenience, HSES and HDES will be treated separately and briefly presented. More details concerning the fundamentals, instrumentation and applications of CCC can be found in books and other papers [1,3–8]. Special issues of the *Journal of Liquid Chromatography* have also been published on this topic (see ref. 1) and one is actually in preparation. Curiously, only a few papers have dealt with the chromatographic fundamentals of HSCCC or comparison with HPLC and are also of interest [3,4,9,10]. Basics and major references concerning CDCCC can be found in ref. 8.

2. HYDRODYNAMIC EQUILIBRIUM SYSTEM: THE COIL PLANET CENTRIFUGE (HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY)

2.1. Principle and apparatus

2.1.1. Column and rotation. The HDES apparatus exhibits a complex motion of the column that has to be described for a better understanding of the technique.

The column (Fig. 1) consists of a PTFE multilayer coiled column rotating around its own axis. The radius (r) of the coiled column depends on the number of layers. During rotation, an Archimedean effect is experienced by the contents of the column, which migrate toward the head of the column; this effect is assumed to be responsible for the retention of one liquid phase (the stationary phase) in the column. The column is also rotating around the axis of revolution of the system to lead to a planetary motion defined as type IV by Mandava and Ito [3]. The revolution radius is R. The ratio r/R is defined as the parameter β, which generally varies between 0.25 and 0.75 depending on the apparatus and the number of layers of PTFE tubing constituting the column.

Owing to this motion, the movement of a layer of

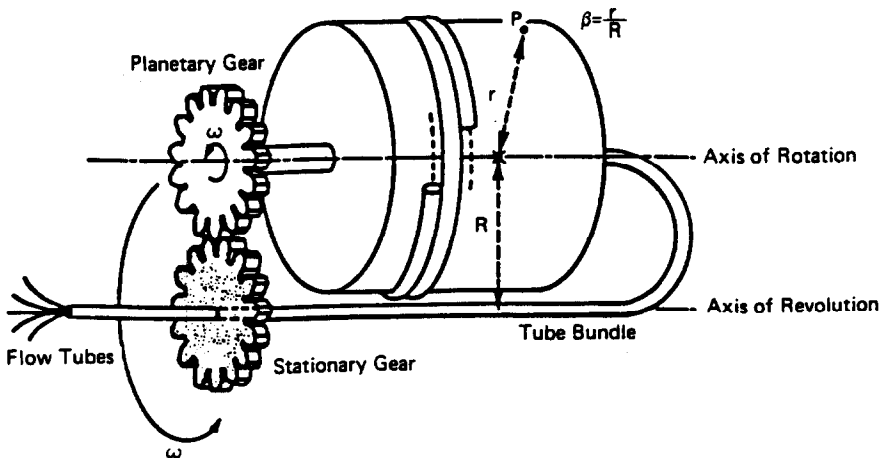

Fig. 1. Schematic diagram of an HSCCC column. The column rotates around its axis at angular velocity ω and around the main axis at ω. Flow tubes are placed in the central axis and the column axis to avoid twisting and breaking; hence no rotational seal is required.

the column can be represented as shown in Fig. 2. When β exceeds 0.5, the trajectory exhibits a loop where the force field is much lower (or is zero for $\beta = 0.25$) than in the opposite case. This difference in force field leads to two different behaviours of a two-phase system in the CCC column: when the force field is strong, the two phases are separated (settling step); when the intensity of the field is low (in the loop), mixing of the two phases occurs [4]; this can lead to the formation of droplets depending on the properties of the solvents [11]. Thus, during revolution, mixing and decantation steps are automatically produced that simulate a series of separating funnels.

2.1.2. Solvent systems. In CCC, there are three main criteria for selecting a solvent system to perform separations. First, solvent systems have to be composed of two immiscible phases; second, their selectivity towards samples of interest has to be sufficient to lead to separations with good resolution; and third, the stationary phase must be retained in the column when applied in a CCC unit.

The most important criterion is the second one. Depending on the polarity of the solutes to be separated, one will select a hydrophobic or non-aqueous, hydrophilic or intermediate solvent system (trends are given in Table 1). Generally, the systems are based on two immiscible solvents and the partitioning of the solutes in the two phases is adjusted by addition of one or two solvents (modi-

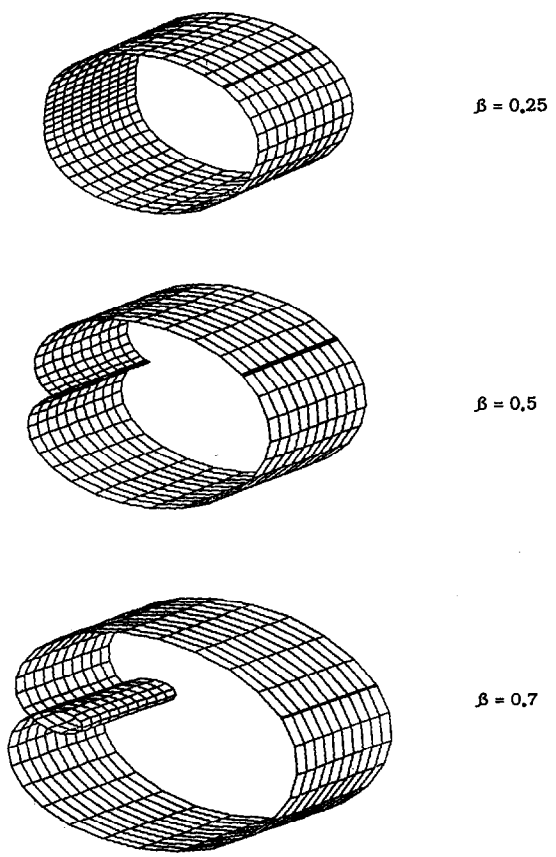

Fig. 2. Motion of a layer of the column in a coil planet centrifuge. In the small loop, at high β values, mixing of the two phases occurs. At the opposite of this small loop, settling is observed.

TABLE 1

GENERAL TRENDS FOR SELECTING SOLVENT SYSTEMS FOR COUNTERCURRENT CHROMATOGRAPHY AS A FUNCTION OF POLARITY OF SOLUTES

Pumping direction of mobile phase *versus* density of the mobile phase and solvent system classification in a coil planet centrifuge.

	Polarity of solutes		
	Non-polar	Medium polarity	Polar
System of solvent (Ito's classification)	Non-aqueous or hydrophobic	Intermediate	Hydrophilic
Pumping direction of the lighter (L) and heavier (H) phases in the coil planet centrifuge	L: tail-to-head H: head-to-tail	$B > 0.25$ as hydrophobic $B < 0.25$ as hydrophilic	L: head-to-tail H: tail-to-head
Basic solvents	Hydrocarbons–acetonitrile, methanol (or water)	Chloroform–methanol–water	Butanol–ethanol–water
Modifiers	Methyl acetate Ethyl acetate Dichloromethane Ethanol	Ethyl acetate Propanol Acetic acid	Methanol Acetone Acetic acid

fiers) that dissolve in the two phases. The pH can also be controlled. Evaluation of the selectivity of different solvent systems can be done by determination of the partition coefficients (K) of the components; K is the ratio of the concentration in the stationary phase to that in mobile phase. TLC, HPLC, UV absorptiometry, etc., can be used to determine K. A high selectivity (α) between adjacent solutes ($\alpha = K_2/K_1$) is needed [1] ($\alpha \approx 1.5$–2 at $K \approx 1$) and is easily obtained [12] to ensure separations require only 1000–2000 theoretical plates in the CCC column.

The two previous points have to be considered for any CCC separation. However, in HSCCC, the third criterion is very important because the behaviour of the stationary phase depends on the nature of the solvents used to form the system: one must check the class to which the system belongs before operation in order to obtain maximum retention of the stationary phase by pumping the mobile phase in the right way depending on the material chosen as the stationary phase (Table 1); calculation of settling velocity and of capillary wavelength [13] can be of major interest for systems for which the behaviour in the HSCCC column is unknown.

Moreover, in CCC the resolution can be expressed as

$$R_s = 2\ V_s \cdot \frac{K_2 - K_1}{w_1 + w_2} \quad (1)$$

where V_s is the volume of stationary phase in the column, K_i the partition coefficient of solute i and w_i the peak base width (in volume units). The higher is V_s in CCC (and in CDCC), the higher is the resolution between adjacent peaks. Hence V_s must also be considered for optimizing resolution in CCC [9].

Finally, to achieve on-line detection, a stable stationary phase retention is also required, which sometimes can be difficult [14,15].

2.1.3. Apparatus. In HDES apparatus, the Ito coil planet centrifuge (PC, Potomac, MD, USA), the use of rotary seals is avoided by placing the flow tubes in the central and column axis to make connections between the injection valve, the column and the detector. In this model, the column is balanced by a counterweight (Fig. 3). More recently, apparatus have been developed by Pharmatech (Baltimore, MD, USA), Models 800, 2000 and 3000, SFCC (Eragny, France), Model CPHV 2000 (not avail-

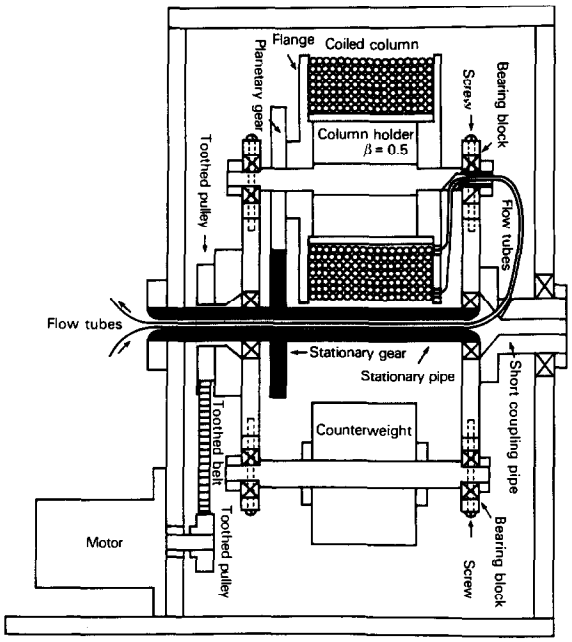

Fig. 3. Schematic diagram of a single-column coil planet centrifuge. A counterweight is used to equilibrate the system.

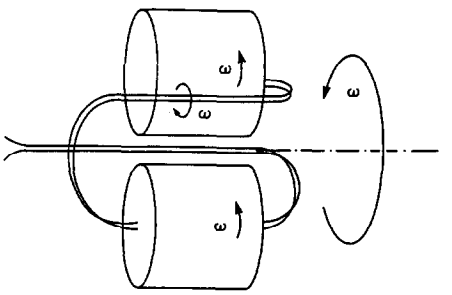

Fig. 4. Schematic diagram of a two-column coil planet centrifuge. The columns balance each other and no counterweight is required.

able), and SEAB (Villejuif, France), Model Kromaton, where two or three columns are connected in series and balance each other (Fig. 4); to avoid breaking of the flow tubes between two adjacent columns, they must be placed in a counter-rotating axis. Generally, HSCCC columns are not thermostated, except the Model CPHV 2000. Pumps and injectors are identical with those in analytical or semi-preparative HPLC.

Generally, UV detection is performed off-line, after laborious collection of fractions; on-line detection is to be preferred for high-speed separations but it can be difficult because bleeding of the stationary phase can occur and a difference in temperature may exist between the column and the detector, leading to demixing of the two phases in the detection cell [14]. Hence other detection methods can be used, such as evaporative light-scattering detection, which removes the solvents before the solutes enter the detection cell [15], or fluorescence detection; mass spectrometry [16–18] and FTIR spectrometry [19] have been applied in several applications coupled to HSCCC. Several devices have also been proposed to enhance on-line UV detection: heating of the transfer line between detection cell and column [14] to reduce the difference in temperature; installation of a pressure restrictor after detection to avoid bubble formation in the detection cell [14]; and prior detection, addition of a solvent (such as 2-propanol) to the column effluent that increases the miscibility between the stationary phase and the mobile phase in case of bleeding of the stationary phase [15,20]; an extra pump (pulse-free) and a mixing chamber are required.

In the next section, several examples are given to demonstrate the use of on-line detectors with HDES–HSCCC.

2.2. *Applications*

Several applications of HSCCC are presented here with special emphasis devoted to the separation of pristinamycin macrolide antibiotics. Table 2 gives general fields of applications of HSCCC (also valid for CDCCC).

Pristinamycins are macrolide antibiotics active on Gram-positive microorganisms (Fig. 5). An extract of fermentation medium can be injected directly into the HSCCC column, leading to a raw separation, the polar stationary phase retaining the ballast (which is yellow). The solvent system chloroform–ethyl acetate–methanol–water (2.5:1.5:3:2, v/v) can produce fractions containing up to 60% of PIIB compared with 9% in the sample. Fig. 6 shows the chromatograms obtained by HSCCC by injecting 500 mg of raw extract (dissolved in the aqueous stationary phase to obtain 50 ml). Chromatogram (a) is a first fractionation: peak compression occurs owing to selection of the stationary phase as the solvent for

TABLE 2

GENERAL APPLICATIONS OF COUNTERCURRENT CHROMATOGRAPHY

H = Heavier phase; L = lighter phase; MS = mass spectrometry; TLC = thin-layer chromatography; UV and Visible = absorptiometry in ultraviolet or visible region; on-/off-line = effluent is continuously monitored on-line but off-line detection is also performed on collected fractions; n.a. = data not available.

Application	Solvent system	Mobile phase and detection	Ref.
Insecticides	Pentane–methanol–water (6:5:1)	H	21
Demeton		TLC of fractions	
Herbicides	Hexane–ethyl acetate–methanol–water (8:2:5:5)	L	22
Triazines		UV 254 nm	
		MS off-line	
Terpenes	Hexane–acetonitrile–methanol–water (5:5:4:2)	H	7
Triterpenes		n.a.	
Antibiotics	Hexane–ethyl acetate–methanol–water (1:1:1:1, 2:3:3:2 or	L	3
Arizonins	2:5:3:2)	n.a.	
Various antibiotics	Chloroform–methanol–water	H	3, 4, 7
Norerythromycins	*n*-Heptane–benzene–2-propanol–acetone–buffer (5:10:3:2:5) or	H	23
	carbon tetrachloride (or chloroform)–methanol–buffer (1:1:1)	TLC	
Steroids	Hexane–dichloromethane–methanol–water (1:4:1.7:0.8)	H	3
Hydrocortisone		n.a.	
DNP-amino acids	Chloroform–0.1 *M* hydrochloric acid–acetic acid (2:1:2)	L/H	24
		UV of fractions	
Hormones	Hexane–ethyl acetate–methanol–water (3:7:5:5)	H	25
Indole auxins		UV 260 nm	
		on-/off-line	
Alkaloids	Chloroform–methanol–acetic acid–water (5:3:1:3)	H	3, 7
Digitonin		n.a.	
Tannins	*n*-Butanol–0.1 *M* sodium chloride (1:1)	L	26
		UV 280 nm	
Dipeptides	*n*-Butanol–acetic acid–water (4:1:5)	H/L	27
		UV 280 nm	
		on-/off-line	
Purine and	*n*-Butanol–phosphate buffer (pH 6.5) (1:1)	L	24
pyrimidines		UV off-line	
Proteins	*n*-Butanol–dichloroacetic acid–water (100:1:100)	H	28
Bombesin		UV 280 nm	
		on-/off-line	
Cholecystokinin	*n*-Butanol–0.2 *M* ammonium acetate (pH 9)	L	29
analogue		UV 280 nm	
		HPLC of fractions	
Pigments	*n*-Butanol–ethanol–water (10:1:5)	L	7
Dyes		n.a.	
Methyl violet	Chloroform–water–acetic acid (2:1:2)	H	30
		Visible/MS off-line	
Catecholamines	*n*-Butanol–barium chloride (saturated solution, pH 6.5) (1:1)	L	31
Noradrenaline		UV 278 nm	
Dopamine			

the samples [12]; the second chromatogram is obtained by re-injecting the collected fraction into the HSCCC system and leads to 85% purity of PIIB. Pristinamycins were detected on-line ELSD. On-line spectrofluorimetry was also used for the detection of PI in analytical-scale HSCCC (Fig. 7). The selectivity of the solvent system was obtained by adding formic acid to the previous one. Stable detection was

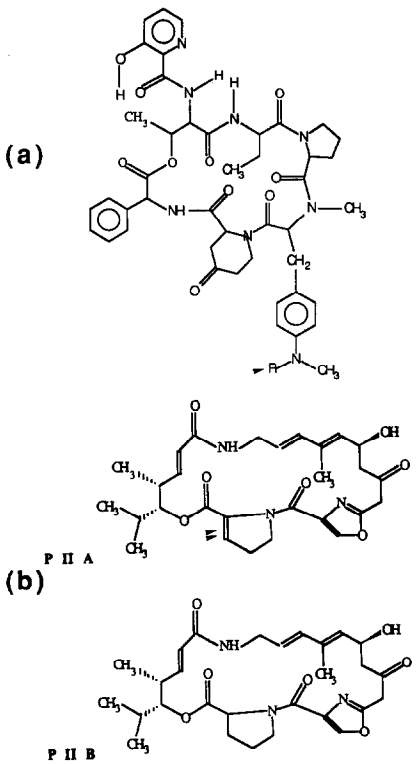

Fig. 5. Structures of (a) pristinamycins IA (R = CH_3) and B (R = H) and (b) pristinamycins IIA and B.

ensured by using specific detection. UV detection with similar conditions of solvents and flow-rate required addition of 2-propanol to the column effluent prior to detection [15].

Using a 1-l column and the aqueous phase as the mobile phase to increase capacity factors and resolution, injections of 11 g of sample treated as for preparative HPLC were made. Comparisons with preparative HPLC demonstrated that HPLC is twice as fast but much more expensive: the solvent consumption is five times higher in HPLC (without considering the cost of the stationary phase). The final purities of PIIB were 92.3% and 91.6% for preparative HPLC and preparative CCC, respectively [2,32].

As Figs. 6 and 7 show, analytical separations performed by HSCCC approach HPLC separations from the point of view of efficiency, resolution and separation time; thus, mass spectrometry on-line with HSCCC can be a powerful tool for the identifi-

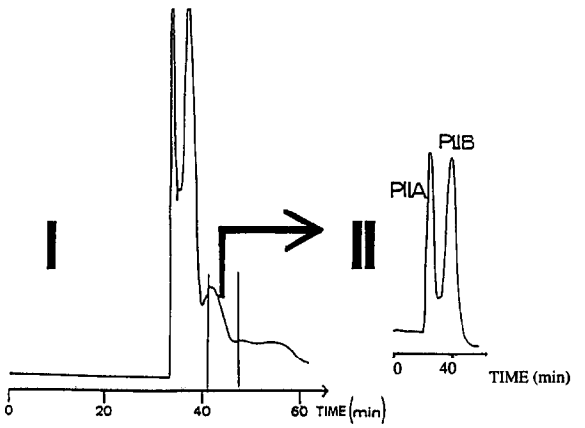

Fig. 6. Direct two-step purification of raw extract of pristinamycins from fermentation medium. After the first separation, the fraction containing PIIA and B is collected and re-injected into the countercurrent chromatograph. PIIB recovered is of 85% purity. Conditions: column, volume 300 ml, 700 rpm (PC high-speed countercurrent chromatograph); solvent system, chloroform–methanol–ethyl acetate–water (2.5:3:1.5:2, v/v); mobile phase, organic phase; flow-rate, 4 ml/min; detection, evaporative light scattering detector at 40°C; sample, 500 mg of raw extract dissolved in the stationary phase to obtain peak compression; volume injected, 50 ml.

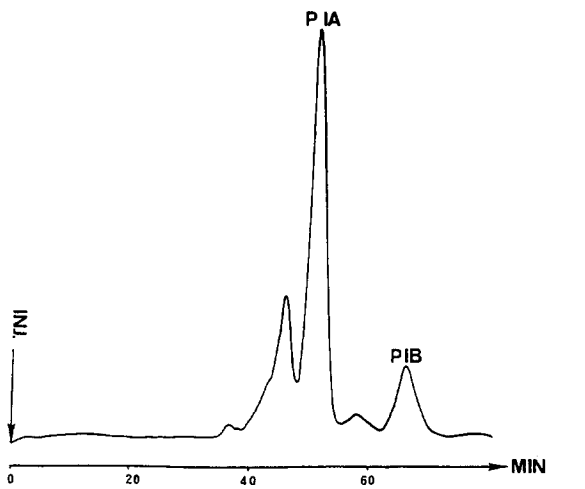

Fig. 7. Separation of pristinamycins IA and IB by countercurrent chromatography with fluorescence detection. Conditions as in Fig. 6 except 0.4% formic adid added to the solvent system and mobile phase flow-rate 2 ml/min; sample injected, mixture containing 79% of PIA and 11% of PIB dissolved in 450 μl of mobile phase. Fluorescence detection, excitation at 365 nm, emission at 415 nm, 2 mV full-scale.

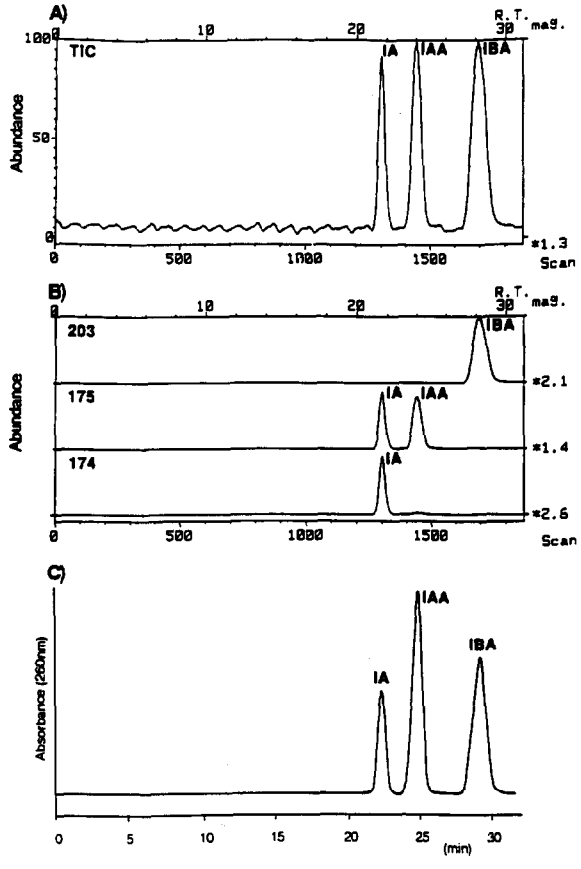

Fig. 8. High-speed countercurrent chromatograms of indole auxins: (A) total ion current and (B) mass chromatograms obtained by electron impact mass spectrometry using splitting of column effluent before frit interfacing to the mass spectrometer; (C) UV trace. Solutes, IA = indole-3-acetamide; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid. From ref. 18.

cation of compounds. This is demonstrated in Fig. 8; separation of indole auxins has been achieved using HSCCC on-line with electron impact mass spectrometry using a frit interface for introducing the column effluent after splitting [18].

3. HYDROSTATIC EQUILIBRIUM SYSTEM: CENTRIFUGAL DROPLET COUNTERCURRENT CHROMATOGRAPHY (CENTRIFUGAL PARTITION CHROMATOGRAPHY)

Centrifugal droplet countercurrent chromatographic or centrifugal partition chromatographic

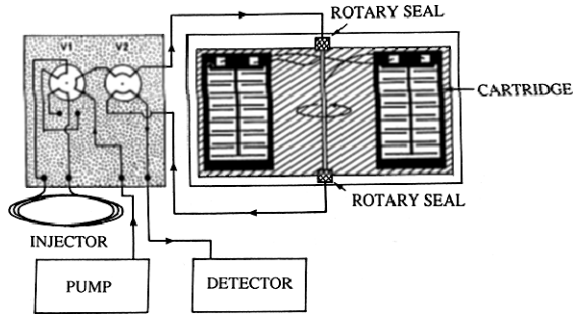

Fig. 9. Schematic diagram of centrifugal droplet countercurrent chromatograph. Two rotary seals are used to connect the injection valve and detector to the column placed in a centrifuge. Ascending or descending modes are selected using a valve located in the injection module. Two to twelve cartridges can be placed in the centrifuge to vary the column volume from 40 to 240 ml.

instruments are manufactured by Sanki Engineering (Kyoto, Japan). The Model LLN is shown in Fig. 9. Rectangular PTFE cartridges engraved with channels and ducts (Fig. 10) are connected in series with capillary tubing and arranged in a circle in a centrifuge to constitute the "column". Two to twelve cartridges can be mounted in the rotor to give 800 to 4800 partition channels and a total volume ranging from 20 to 240 ml. Preparative cartridges can also be placed in the centrifuge to give 480 partition channels and a total volume of 900 ml. The

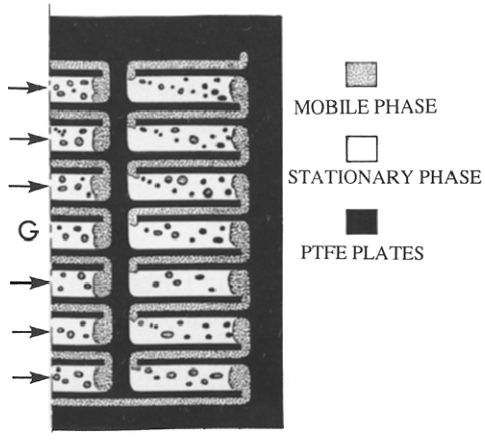

Fig. 10. Schematic diagram of channels in a cartridge of a centrifugal droplet countercurrent chromatograph. In the descending mode, the heavy mobile phase flow in the same direction as the centrifugal field indicated by an arrow; in the ascending mode, the direction of the flow is reversed.

centrifuge can be thermostated. This model is actually being replaced with a new system with a fixed volume of 240 ml equiped with a single disc engraved with 2136 partition channels.

The complete apparatus, including pumps, injection valve and detector, is very similar to an HSCCC system, except for the column. The column is connected to the pump and to the detector via two rotary seals and can be rotated at *ca.* 700–1500 rpm.

Using a selection valve, two elution modes can be performed: in the descending mode the heavier (lower) phase is used as the mobile phase and flows through the lighter (upper) phase used as the stationary phase; the mobile phase has the same direction as the centrifugal field (the channels are parallel to the direction of the centrifugal field); in the ascending mode the upper phase is the mobile phase and the lower phase is the stationary phase. In the ascending mode, the motion of the droplets of the mobile phase is the contrary of the direction of the centrifugal field G (Fig. 10).

Owing to excellent stationary phase retention in the column, original two-phase systems can be used such as aqueous systems or systems having a low interfacial tension in which it is difficult to retain the stationary phase in HDES apparatus. Thus, on-line detection is generally easy to perform after CPC separation. When selecting a solvent system, one does not need to worry about retention of the stationary phase; the only requirement is to obtain a high selectivity in order to achieve resolution of the mixture to be separated with a plate number close to 1000.

The main drawback of CPC is related to the HSES, where partitioning occurs between droplets of the mobile phase dispersed in the stationary phase without promotion of mixing by the motion of the column. Hence the efficiency of the HSES is limited and lower than that of HDES apparatus where the column motion creates mixing/settling sequences.

In CPC, the pressure drop in the system also has to be considered [33]; mainly, it depends on the square of the angular velocity, the flow-rate and the viscosity of the mobile phase. When viscous solvents are used at elevated rotational speed, the pressure drop can exceed the mechanical resistance of the PTFE cartridges. Hence the maximum pressure allowed in CPC has been fixed at 60 bar by the manufacturer; users have to maintain the pressure within this limit by reducing the flow-rate of the mobile phase or the rotational speed of the column. The former can lengthen the duration of separation and reduce the efficiency because the variation of efficiency *versus* the mobile phase flow-rate exhibits

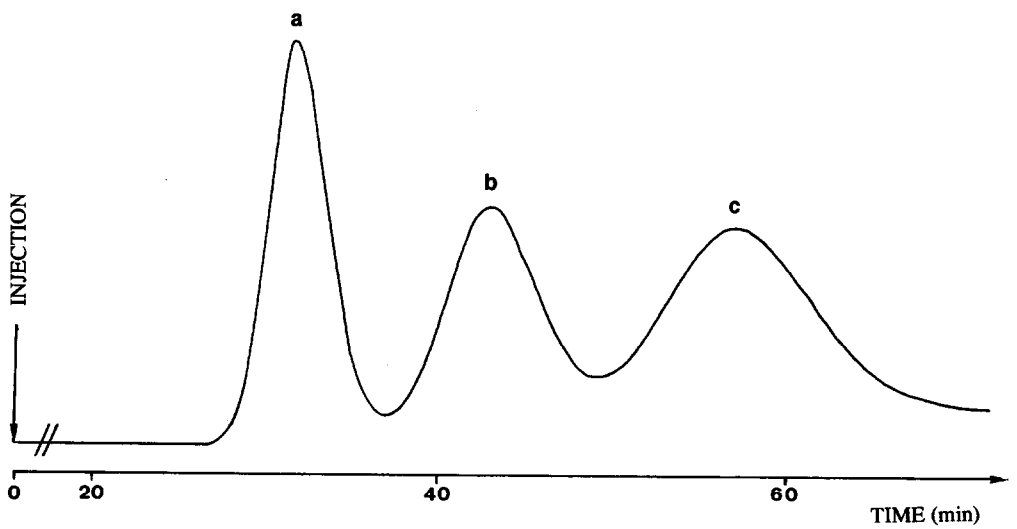

Fig. 11. Separation of phenol derivatives by centrifugal droplet countercurrent chromatography. Conditions: column, six cartridges, 120 ml, 900 rpm; solvent system and mobile phase as in Fig. 6, flow-rate, 2 ml/min; pressure, 43 bar; sample injected, 1 ml of 1 g/l solution dissolved in the mobile phase; detection, UV at 270 nm. a = 2,3-Di-*tert.*-butylphenol, b = *o*-cresol, c = 4-ethylresorcinol.

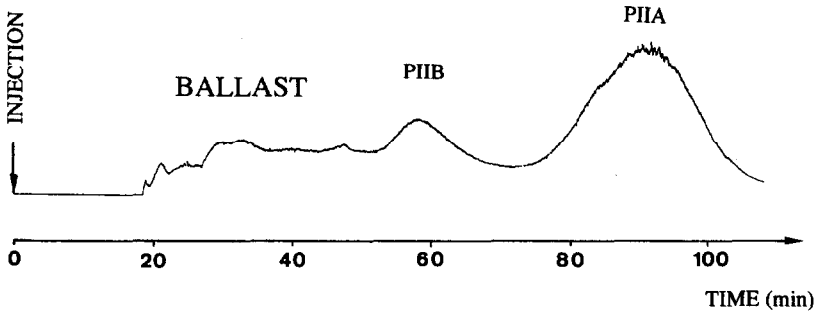

Fig. 12. Centrifugal droplet countercurrent chromatogram of pristinamycins II. In comparison with the separations in Figs. 6 and 7, the mobile phase is the aqueous phase and the composition has been changed to improve the selectivity to compensate for the lower efficiency. Conditions: column, 120 ml (six cartridges), 800 rpm; solvent system, chloroform–methanol–ethyl acetate–water (2:3:2:2, v/v); mobile phase flow-rate, 5 ml/min; pressure drop, 30 bar; detection, evaporative light scattering detector at 40°C; sample, 400 mg of extract dissolved in the stationary phase; volume injected, 8 ml.

a minimum at 1–2 ml/min [34] instead of a maximum in HPLC; thus, flow-rates of 3–5 ml/min are preferred; the latter can be a prejudice for the volume of the stationary phase retained in the column and, consequently, for the resolution, R_s (eqn. 1).

Three examples of applications of CPC are presented in Figs. 11–13. The separation of phenol derivatives summarizes the points developed here concerning efficiency (theoretical plates), pressure drop (43 bar), easy on-line detection (2-propanol is not required after the separation to obtain sensitive and stable detection; it was required for a similar separation by HSCCC [15]). The semi-preparative and preparative capabilities of CPC are demonstrated in Figs. 12 and 13. In Fig. 12, using six analytical cartridges, 400 mg of pristinamycin extract can be injected to lead to more than 92% purity PIIB (more than 95% purity of PIIA). In comparison with Figs. 6 and 7, the solvent system has been changed to enhance the selectivity in order to compensate for the lower efficiency of CDCCC compared with HSCCC. An increase in ethyl acetate content allows the selectivity to be high enough to lead to baseline separation with only a few hundred theoretical plates and one does not have to worry about the stationary phase retention in the column. This was not the case in HSCCC, where a solvent system containing more chloroform was preferred to ensure stable retention of the mobile phase [12]. Fig. 13 shows an example of preparative-scale purification of 130 g of a mixture containing dihomo-γ-linolenic acid performed with a 7-l centrifugal partition chromatograph. Other examples of applications can be found in ref. 8.

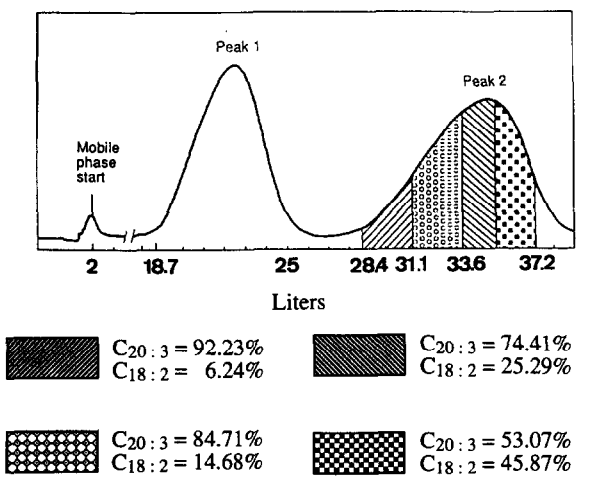

Fig. 13. Preparative centrifugal droplet countercurrent chromatogram of ethyl dihomo-γ-linolenate. Conditions: column, 7 l, 700 rpm; solvent system, hexane–acetonitrile; mobile phase, acetonitrile; flow-rate, 150 ml/min; detection, UV at 210 nm; sample, 130 g of a mixture containing 17.08% of ethyl dihomo-γ-linolenate dissolved in 400 ml of 75% stationary phase–25% mobile phase. Peak 1 is a mixture of ethyl linolenate and ethyl arachidonate. Peak 2 was analysed by gas chromatography: percentages of ethyl dihomo-γ-linolenate ($C_{20:3}$) and ethyl linoleate ($C_{18:2}$) are indicated for the four fractions collected. From ref. 1.

A promising application of CPC for the determination of partition coefficients should also be pointed out because an excellent correlation between shake-flask and CPC measurement of K has

been reported [35,36]. HSCCC has also been used for this purpose [37].

4. CONCLUSION

CCC has already received numerous applications, mostly for the separation or fractionation of natural compounds because raw samples are easily accepted. Generally, the amount injected is a few hundred milligrams for a 300-ml column volume. Results on pristinamycins show that a higher input can be obtained after studying the sample medium. Thus, CCC can compete with preparative HPLC because similar resolution and purity are obtained. It has also been demonstrates that this is a powerful technique for fractionation prior to separation by CCC or preparative HPLC [38] and it can be used for extraction purposes [39,40] (centrifugal partition chromatography with a "modular column volume" is very convenient for extraction). Recognition of CCC by the US Food and Drug Administration is also expected for the determination of octanol–water partition coefficients.

The commercial systems described here offer reliability. Very high selectivity of solvent systems associated with a higher efficiency per unit time than previous CCC techniques such as DCCC permit separations to be completed in less than 1 h instead of days. Nevertheless, more studies of the fundamentals of this technique are required for a better understanding of the mechanism of retention of the stationary phase (HSCCC) and for optimization of separations; the effects of the volume and repartition of the stationary phase in the CCC column, linear velocity of the mobile phase, rotational speed, partition coefficient, etc., on efficiency and resolution (and loadability) have to be clearer than at present. Work is in progress in many laboratories. Difficulties arise from the complex motion of the column used for HSCCC.

When the mechanisms involved in CCC are better understood, the technique should be improved to approach the efficiency per unit time of HPLC.

5. LIST OF ABBREVIATIONS

CCC	Countercurrent chromatography
CPC	Centrifugal partition chromatography
DCCC	Droplet countercurrent chromatography
ELSD	Evaporative light-scattering detection
FTIR	Fourier transform infrared
G	Centrifugal field
HDES	Hydrodynamic equilibrium system
HPLC	High-performance liquid chromatography
HSCCC	High-speed countercurrent chromatography
HSES	Hydrostatic equilibrium system
K	Partition coefficient
MS	Mass spectrometry
MP	Mobile phase
PI, PII	Pristinamycins I and II, respectively
r	Rotation radius
R	Revolution radius
R_s	Resolution
TLC	Thin-layer chromatography
UV	Ultraviolet
V_s	Volume of stationary phase
w_1	Peak base width of solute i
α	Selectivity
β	Ratio of rotation radius to revolution radius

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