Review

Cross-axis coil planet centrifuge for the separation and purification of polar compounds

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ABSTRACT

A new counter-current chromatograph, named the cross-axis coil planet centrifuge, has been optimized through various versions since 1987. Features of this recently commercialized apparatus are reviewed. It allows the use of biphasic polar solvent systems, of aqueous solutions of polymers and systems containing an organic phase of small or moderate hydrophobicity. Separations and purifications achieved on the cross-axis device demonstrate that it provides a good efficiency and resolution with very high retention of the stationary phase at convenient flow-rates, whatever the solvent system used.

CONTENTS

1. INTRODUCTION

Counter-current chromatography (CCC) is a liquid–liquid partition chromatographic method.

The stationary phase is liquid and is retained in the column by a chosen centrifugal force field. The mobile phase, immiscible to the stationary phase, percolates through the latter. At present, CCC has limitations related to the design of the devices. The type J coil planet centrifuge (CPC)

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allows the use of high flow-rates of the mobile phase but cannot be used reliably with biphasic polar solvent systems [1]. In contrast, the centrifugal droplet counter-current chromatograph (CDCCC) is able to retain these solvent systems but the flow-rates are limited because of the internal pressure [2].

The cross-axis CPC has emerged as a universal counter-current chromatograph. It combines the respective advantages of the two previous devices, *i.e.*, the use of high flow-rates and of biphasic polar solvent systems. Moreover, the column efficiency is at least comparable to or even higher than that of the type J CPC, which in turn is higher than that of the CDCCC.

This review describes the genesis and evolution of cross-axis devices, along with their principles and operation. Some applications are detailed, ranging from the purification of complex media, natural products and antibiotics to separations of biological interest. They allow comparison with the two other CCC devices and demonstrate the capability of the cross-axis device to provide faster separations without any loss of efficiency and resolution.

2. INSTRUMENTATION

2.1. Genesis

In 1981, Ito [3] built a new apparatus based on planetary motion, named the high-speed counter-current chromatograph (HSCCC) because of its ability to achieve fast separations. The central axis of the apparatus and the axis of the column are parallel. For all the devices described in this section, the chromatographic column is made of a PTFE tube wound around a cylindrical holder. The design is referred as a type J CPC [4] (coil planet centrifuge). Its principle is shown in Fig. 1A.

In 1986, he designed a new prototype, called the type J-L angle rotor CPC [5]. The motion is also planetary. However, the column axis and the central axis are no longer parallel; in this prototype they have a 25° angle to each other, as shown in Fig. 1B.
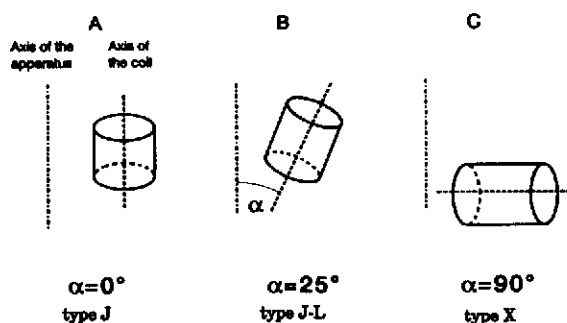
The obvious end for these designs was to

Fig. 1. Genesis of the cross-axis CPC.

incline the column axis· so that it would be perpendicular to the central axis. Such a prototype was realized in 1987 by Ito, who named it the type X CPC [6], shown in Fig. 1C.

2.2. General principle

The vertical axis of the apparatus and the horizontal axis of the coil are always kept perpendicular to each other at a fixed distance. The cylindrical column revolves around the central axis at the same rotational speed as it rotates on its own axis. As the PTFE inlet and outlet flow tubes can rotate on themselves without any twisting, the device is rotary seal free. Three parameters displayed in Fig. 2A explain the various versions of the cross-axis prototypes: r is the radius of the column holder, R is the distance between the two axes and L is the measure of the lateral shift of the column holder along its axis. The name of a cross-axis device is based on the ratio L/R, when defined. Types X and L represent the limits for the column positions; the first type involves no shifting of the column holder while the second corresponds to an infinite shift. Some examples of Ito's prototypes are shown in Fig. 2B. Table I gives the characteristics of the six cross-axis prototypes built by Ito and co-workers [6–11]. They have different L/R ratios, hence their various names.

Studies of the ratio β ($=r/R$) led to various columns by varying the radius of the column holder. However, another idea was to change the holder configuration. Fig. 3 shows the two types of column holders used on type J and

A

B

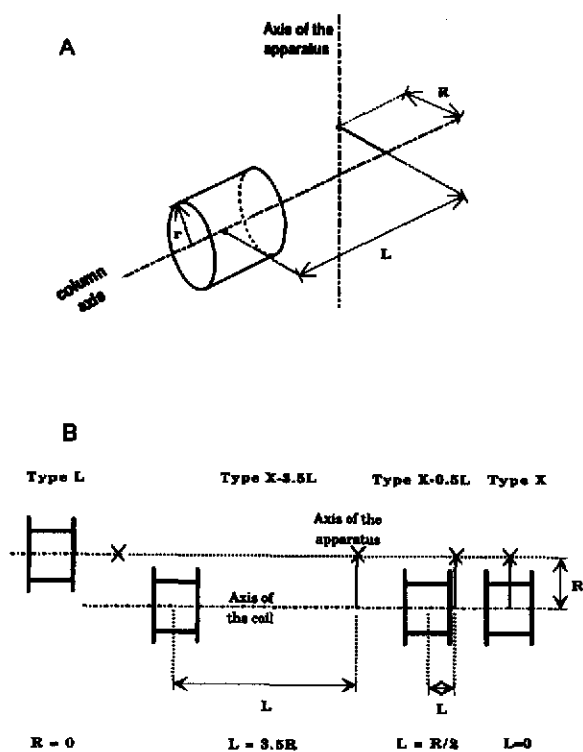| Type L	Type X-3.5L	Type X-0.5L	Type X

Fig. 2. General principle of various cross-axis CPCs. (A) All the fundamental parameters for the design of a cross-axis apparatus are shown. (B) Some examples of Ito's prototypes are displayed. Type X was built in 1987, type X-0.5L in 1988, type X-3.5L in 1991 and type L in 1992.

type I

type II

Fig. 3. Two types of columns used on type J and cross-axis CPCs (modified from ref. 11). The description is given in the text.

cross-axis prototypes. Type I is the best known and the simplest as the PTFE tube is directly wound on a cylinder. This standard type (Ia) consists of alternate layers of right- and left-handed coils. A second subtype (Ib), mainly used for cross-axis CPCs, consists of entirely left- or right-handed coils with interconnecting tubes between each layer. The type II column is an eccentric coil assembly [12]. It consists in a given number of column units, each prepared by winding a PTFE tube on a cylinder. The set of these units is arranged symmetrically around the holder; they are parallel to the holder axis, at the same distance. All of them are left- or right-handed coils. For this type of column, β is defined as the ratio of the radius of a cylinder forming a column unit on R. The purpose is to decrease β, which can be as low as 0.01. The internal volume of such columns is small (a few
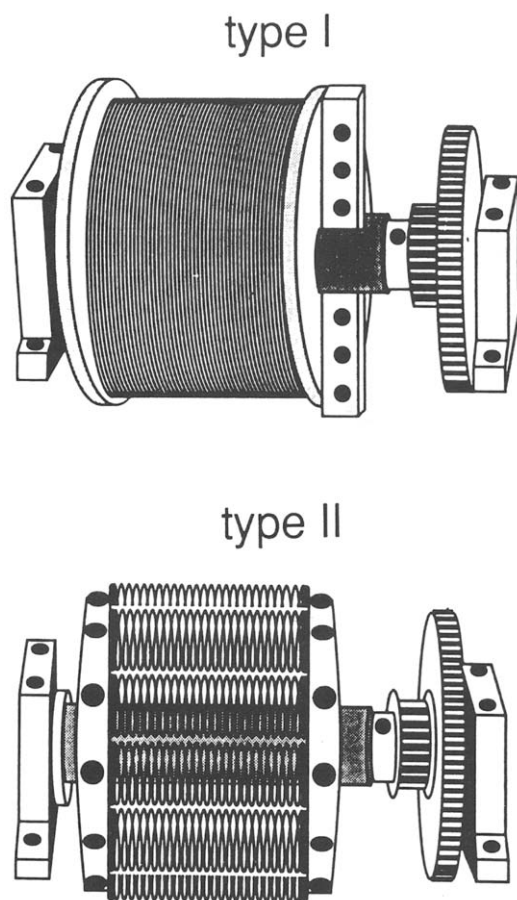
tens of millilitres) so that they have an analytical purpose. Moreover, using small β values increases the efficiency (number of theoretical plates) of the column [11].

Except for the first prototype, it was possible to assemble two columns in series. The advantage is to equilibrate continuously the whole apparatus, from a mechanical point of view.

The latest prototype [11] (X-1.5L type), commercialized by Countercurrent Technologies (CTI [13]), is shown in Fig. 4. All the parts are made of stainless steel, except for the gears and the cylindrical holders, which are of Delrin. Its external dimensions are 60 cm × 60 cm × 35 cm. A horizontal section, perpendicular to the central axis, is shown in Fig. 5. The two columns, made of several layers of PTFE tubing wound on

TABLE I

CHARACTERISTICS OF CROSS-AXIS CPCs

Year	Column position	L (cm)	R (cm)	β^a	Column volume (ml)
1987 [6]	X	0	10	0.25	15
				0.50	15
				0.75	15
				0.5–0.8	400
				0.19–0.9	?
1988 [7]	X or X-0.5L	0 or 10	20	0.125	15 or 28
				0.375	15 or 28
				0.625	15 or 28
				0.375–0.625	800
1989 [8]	X-1.25L	12.5	10	0.5	28
				0.75–1.75	750
1991 [9]	X-LL	15.2	7.6	0.5	20–30
				1	20–30
				1.6	20–30
				0.25–0.60	280
				0.50–1.00	250
				1.00–1.20	450
1991 [10]	X-3.5L	13.5	3.8	0.50–1.30	150
				0.44–1.50	220
1992 [11]	X-1.5L or L	16.85	10.4 or 0	0.26 or 0.16	20
				0.48 or 0.30	41
				0.26–0.48	
				or	287
				0.16–0.30	
				0.10 or 0.06	18
				0.02 or 0.01	17

^{a} β Calculated as defined in the text ($\beta = r/R$), except for the L position: $\beta = r/L$.

the cylindrical holder, are mounted in series. The inside diameter of the tube used for the columns is 2.6 mm. The rotational speed is regulated up to 1000 rpm, the usual operating speed being 750–800 rpm. The configurations of the stationary and planetary mitre gears and the pulleys and belts force the column to rotate around its own axis at the same speed as its revolution speed around the central axis. Two counter-axes, rotating due to the plastic gears, are required to prevent the twisting of the inlet and outlet PTFE tubes. The prototype was built to allow the counter-axis to be exchanged with the column holder. In that case, the type would be L.

3. ADVANTAGES

3.1. Retention of stationary phase

Ito [14] defined three solvent system groups, according to the hydrophobicity of the non-aqueous phase. The first, called "hydrophobic", includes solvent systems containing a hydrophobic organic phase, such as heptane–water or chloroform–water. Such systems are easily retained by the type J [15] and cross-axis CPCs [16] and by the CDCCC [17], as shown in Table II, with a particularly high value for the X-axis.

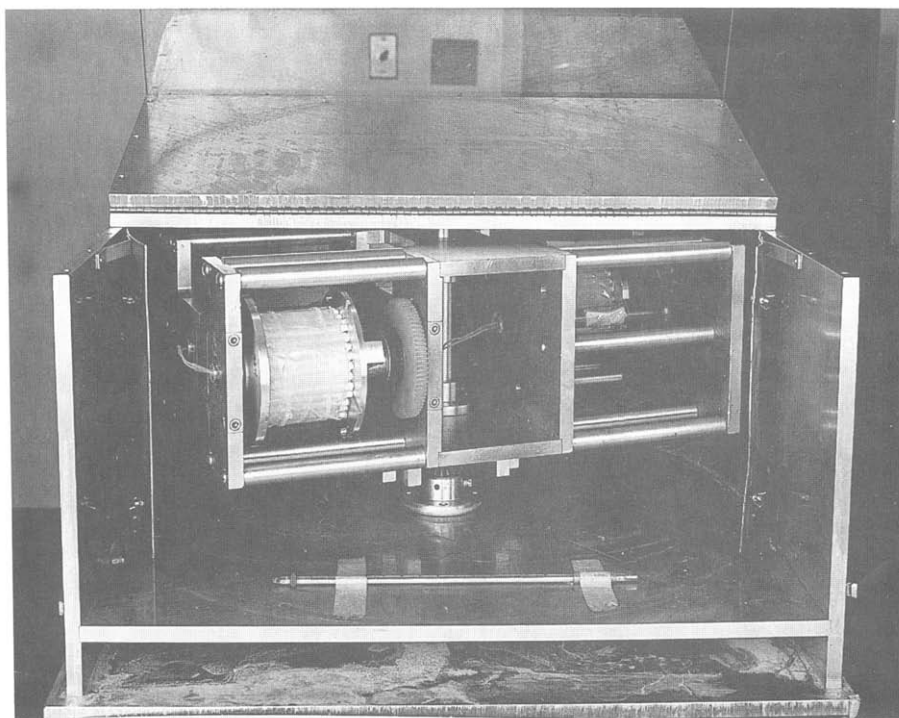"Intermediate" solvent systems involve a more hydrophilic organic phase; examples are chloro-

Fig. 4. Photograph of the type L and X-1.5L prototype (courtesy of Dr. Y. Ito). All the rotating parts are enclosed in a box. The pulley driven by a belt from the electric engine is at the bottom of the vertical central axis. Two cylindrical holders (white parts) are mounted in the X-1.5L position. The inlet and outlet PTFE tubes go through the upper part of the central axis. One counter-axis for each column is installed to prevent the twisting of the PTFE tubes. A small circular plate around the holder of the columns and the counter-axis decreases the required engine torque by 30%. Owing to the sufficient distance between one column axis and its counter-axis, the connecting tubes are reliable and do not need to be replaced often.

form–acetic acid–water and *n*-butanol–water. Their tendency to evolve after mixing to a more stable emulsion than the "hydrophobic" system decreases the retention of stationary phase. Type J CPC undergoes the highest decrease [15], whereas the other two devices show only a small decrease [16,17].

The "hydrophilic" group encompasses biphasic systems containing a polar phase, such as *n*-butanol–acetic acid–water and *sec.*-butanol–water. Their emulsions are nearly stable. Consequently, their stationary phase is even less retained in the column; the type J device leads to such low values that it can hardly be used with such systems [15]. The CDCCC allows good retention of the stationary phase [17] at a low flow-rate limited by the internal pressure drop due to the viscosity of the organic phase [2]. The cross-axis apparatus does not encounter

such a limitation [11,16]; its internal pressure drop is similar to that observed inside the type J CPC, which is much smaller than that obtained on a CDCCC.

This advantage is magnified when systems containing at least one polymer phase are used. The retention of the stationary phase ranges from 30% to 70% at an average flow-rate of 3 ml/min [11] for the cross-axis CPC, whereas the CDCCC permits a smaller range but at a flow-rate lower than 1 ml/min [17]. The type J CPC using type I columns does not retain these solvent systems [18], whereas the use of type II columns allows a small retention of stationary phase (typically 20%) [19]. Therefore, all the values shown in Table II emphasize one of the advantages of the cross-axis CPC, *i.e.*, that it allows a universal choice of solvent systems without diminished flow-rates.
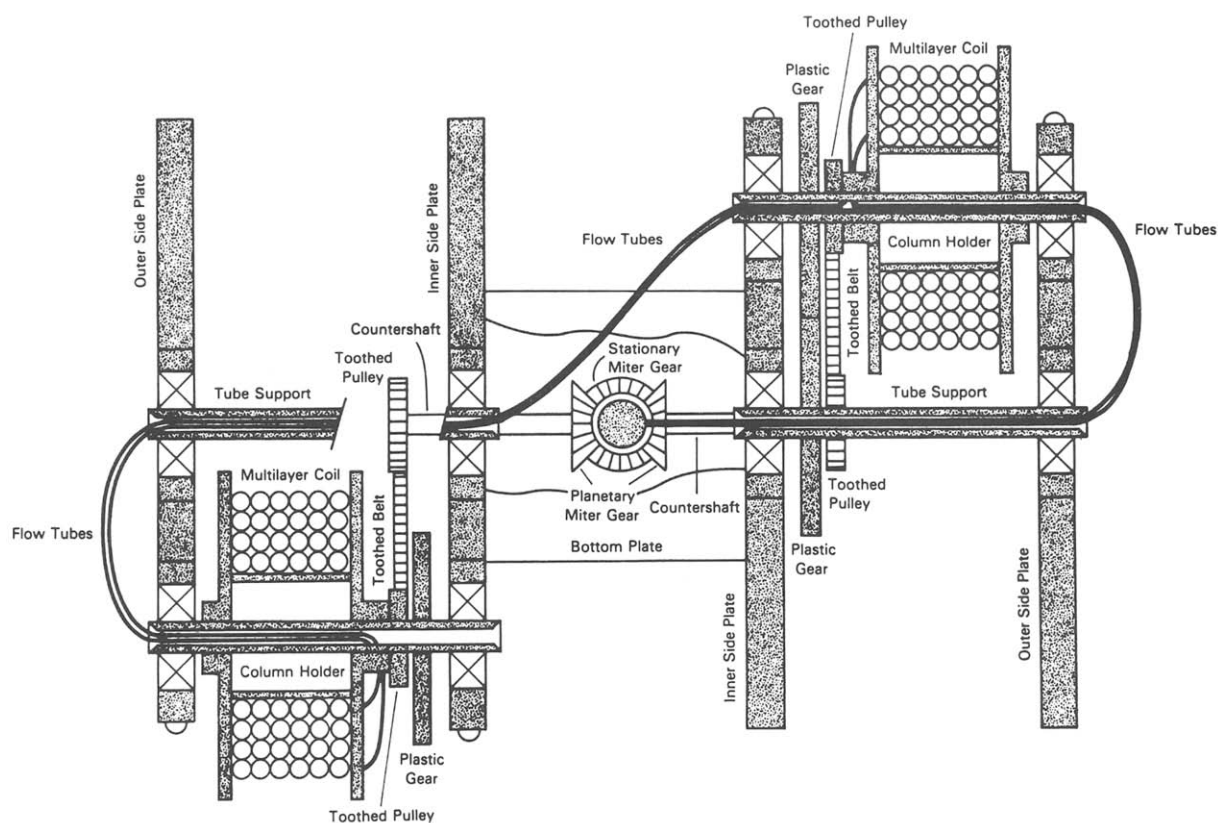
Fig. 5. Section of the latest cross-axis device [11]. The plane of the section is perpendicular to the central axis of the apparatus. The two columns and their counter axes are shown in the X-1.5L position. The paths for the PTFE tubes are shown. The inlet tube comes from the central axis, goes through one counter-axis and undergoes a small loop before entering the axis of the column. Then the outlet of the column goes to the counter-axis, undergoes the small loop and enters the axis of the second column. The outlet tube must take exactly the same reverse path otherwise twisting occurs.

TABLE II

MAXIMUM RETENTIONS OF STATIONARY PHASE FOR TYPE J AND CROSS-AXIS CPCs AND CDCCC

The retention of the stationary phase is given as a percentage of the total volume of the column. For hydrophilic systems and those containing a polymer phase, the values are obtained on the cross-axis CPC at an average flow-rate of 3 ml/min, whereas they are typically obtained at 1 ml/min on the CDCCC.

Solvent system group	Type J CPC	Cross-axis CPC	CDCCC
"Hydrophobic"	90 [15]	>95 [16]	75 [17]
"Intermediate"	50 [15]	>80 [16]	75 [17]
"Hydrophilic"	20–30 [15]	50–90 [11,16]	50–70 [17]
Containing a polymer phase	0 [18]	30–70 [11]	<50 [17]

3.2. Resolution and efficiency

According to the equation

$$R_s = 2V_s(K_2 - K_1)/(w_2 + w_1) \qquad (1)$$

where R_s is the resolution between two adjacent peaks, K_2 and K_1 the partition coefficients of the separated solutes and w_2 and w_1 are the peak base widths in volume units, the resolution between two peaks is proportional to the volume of stationary phase (V_s) inside the column. As a result, the higher the retention of stationary phase, the higher is the resolution. The high retention of the stationary phase obtained with the cross-axis CPC is consequently particularly helpful in achieving good separations.

Using the same solvent system, a separation of three proteins (e.g., cytochrome c, myoglobin and ovalbumin) allows a comparison of type J and cross-axis CPCs [19]. To obtain a sufficient retention of the polyethylene glycol (PEG)-rich stationary phase, type II columns were used with a type J CPC. However, the limited flow-rate of 0.65 ml/min leads to 19% retention of the stationary phase, which is low compared with the 49% obtained with the cross-axis at a flow-rate of 2 ml/min. The low flow-rate for the type J CPC increases the separation time to 10 h, as shown in Fig. 6A, two times longer than the experiments carried on the cross-axis CPC (Fig. 6B). Moreover, the latter shows a higher resolution, partly due to the increase in stationary phase retention. The efficiencies, measured as the number of theoretical plates using the myoglobin and ovalbumin peaks, are similar for the two devices.

3.3. Column choice

The cross-axis apparatus is very versatile because the volume of type I columns is easily chosen and adapted to the purpose of the separation. A small cylinder radius and inside diameter and one layer of PTFE tube lead to an "analytical" version. A multi-layer coil of a larger inside diameter allows a "semi-preparative" use. Type II columns are adapted to "analytical" separations, as they are made of several single-layer coils of low volume.
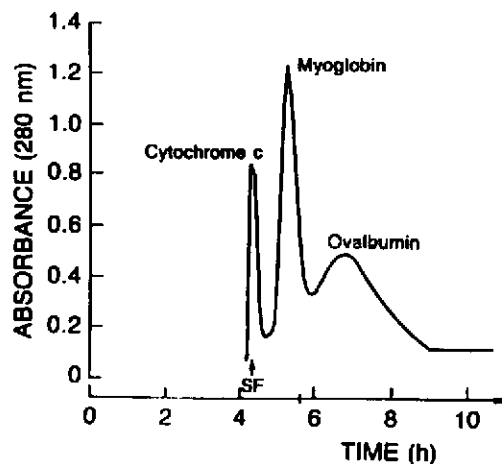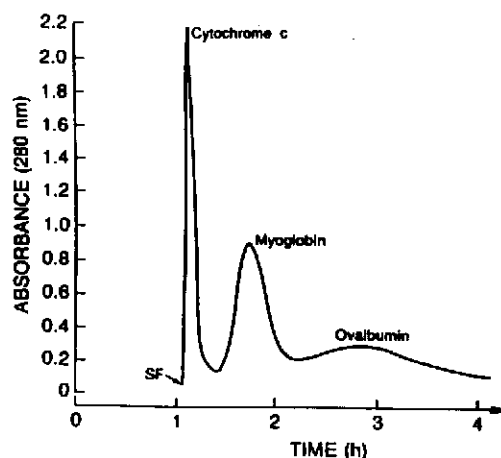

Fig. 6. Comparison of separations of a three-protein mixture achieved on type J and cross-axis CPCs (modified from ref. 19). (A) Type J CPC equipped with Type II columns with a 220-ml total capacity; sample, 10–100 mg of each protein; solvent system, 12.5% (w/w) PEG-1000–12.5% (w/w) K$_2$HPO$_4$ with a heavier mobile phase; flow-rate, 0.65 ml/min; rotational speed, 800 rpm. (B) Type X-LL cross-axis CPC with a 250-ml total capacity; two type Ib columns in series. Same experimental conditions as above, except flow-rate 2 ml/min and rotational speed 750 rpm.

4. OPERATION

4.1. Operating parameters

A cross-axis device is operated the same way as a type J CPC. However, more parameters intervene: they are displayed in Table III for

TABLE III

OPERATING PARAMETERS FOR TYPE J AND CROSS-AXIS CPCs

Available parameter	Type J CPC	Cross-axis CPC
Column type	×	×
Direction of winding	×	×
Rotation direction	×	×
Elution mode	×	×
Elution direction	$-^a$	×
Column position	$-^a$	×
Rotational speed	×	×
Flow-rate	×	×
Choice of a lighter or a heavier mobile phase	×	×

^{a} Not available.

both CPCs. Related to the column fabrication are the *column types* already discussed and the *directions of winding*. The latter can be left- or right-handed. Both devices have two *rotation directions*, *i.e.*, clockwise or counter-clockwise, around the central axis, consequently setting the rotation direction of the column. Choosing the directions of winding and of column rotation allows the definition of the head and the tail of the column. When the column is rotated, a small solid ball introduced into the tube migrates towards one end, which is called the head. The tail is the other end. For type J and cross-axis CPCs, the choice of winding and of rotation direction around the central axis determines the head and tail of the column. The direction of the mobile phase pumped in the column is described by the head-to-tail or tail-to-head *elution mode*. Another parameter is consequently required: the *elution direction*. This indicates the direction of the mobile phase relative to the central axis, *i.e.*, inward or outward. The *position of the column* (X, L, X-L, . . .) is an additional parameter for the cross-axis.

Three other usual parameters are common to both devices: the *rotational speed*, ranging from 50 to 1000 rpm, the *flow-rate*, ranging from 0.2 to 10 ml/min, and the choice of a *lighter* or a *heavier mobile phase* are also important parameters influencing the efficiency of the column, the resolution and the retention of the stationary phase.

4.2. Separation procedure

The liquid phase chosen as the stationary is pumped in the column while the apparatus is stopped. When the columns are completely filled, the CPC is rotated at the desired speed and direction. The mobile phase is then pumped and the hydrodynamic equilibrium of the phases is achieved when the mobile phase emerges from the outlet tube. The sample can be introduced using an injection valve. The solutes are dissolved in the stationary phase, the mobile phase or the mixture of the latter, depending on the experiments.

Optimization for the best retention of the stationary phase is often required concerning the elution mode and direction, the choice of the mobile phase and the rotation direction [20]. Whatever the cross-axis CPC and the solvent system, the heavier mobile phase needs to be pumped in the outward elution direction, while a lighter mobile phase requires an inward elution direction. Type Ib columns consequently enhance the major influence of the elution direction as it is the same in each layer. In contrast, type Ia columns reduce this influence by alternately changing the elution direction for neighbouring layers. Other parameters are fixed before the experiment, such as the column position, its direction of winding and its type. An increase in rotational speed usually increases the retention of stationary phase, whereas a higher flow-rate always means a lower retention of stationary phase [11]. A study based on the experimental designs is now being carried out to evaluate the independent influence of each parameter, along with their multiple interactions [21].

For on-line solute monitoring, the outlet tube is connected to detectors which are widely used in liquid chromatography, such as a UV detector [22], a fluorimeter [23] or an evaporating light-scattering detector [24].

5. APPLICATIONS AND EXAMPLES

Table IV shows separations or purifications achieved with the various cross-axis prototypes. They have analytical or preparative purposes and can be classified into three groups. One en-

TABLE IV

EXAMPLES OF SEPARATIONS AND PURIFICATIONS

Separated compounds	Amount	Solvent system
DNP amino acids [25–28]	100 mg–10 g	Chloroform–acetic acid–0.1 M HCl
Dipeptides (containing a tyrosine moiety) [25,26]	100 mg–2.5 g	n-Butanol–dichloroacetic acid–0.1 M ammonium formate (100:1:100 to 100:0:100, v/v/v) or n-butanol–acetic acid–water
Indole auxins [27,28]	3 g	n-Hexane–ethyl acetate–methanol–water
Proteins (containing a haeme group, lipoproteins, globulins, histones, recombinant enzyme) [10,19,29]	10 mg–1 g	PEG-1000–potassium phosphate buffer or PEG-8000/Dextran T500 + potassium phosphate buffer
Polysaccharides [30]	–[a]	n-Butanol–0.13 M NaCl + HPC[b] (15 g/l)
Steroids (crude synthetic mixture) [27]	2.4 g	n-Hexane–ethyl acetate–methanol–water
Flavonoids (from a crude extract of sea buckthorn) [27]	100 mg	Chloroform–methanol–water
Antibiotics (bacitracin) [28]	5 g	Chloroform–95% ethanol–water

[a] Data not available.
[b] Hexadecylpyridinium chloride.

compasses the experiments carried out with biphasic polar solvent systems. Solutes separated include dipeptides and polysaccharides. Fig. 7
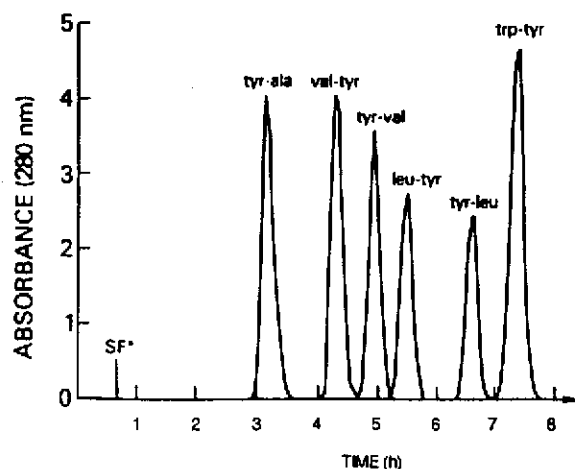

Fig. 7. Chromatogram of a dipeptide separation on a type X cross-axis CPC [25]. Amounts of 100 mg each of tyrosylalanine, valyltyrosine, tyrosylvaline, leucyltyrosine, tyrosylleucine and tryptophyltyrosine. Solvent system, n-butanol–dicloroacetic acid–0.1 M ammonium formate, with a gradient (100:1:100 to 100:0:100) with a heavier aqueous mobile phase; flow-rate, 120 ml/h; rotational speed, 800 rpm. One type Ia column, total volume 400 ml. Retention of stationary phase, 55%.

shows a chromatogram of the analytical separation of six dipeptides containing a tyrosine moiety [25], which was obtained on the first prototype (X type). The solvent system consisted of two polar phases, i.e., a butanol-rich phase and an aqueous phase. A gradient of dichloroacetic acid from 0.01 to 0% (v/v) was used. The retention of the stationary phase was 55%, which is high for such a solvent system.

The second group of experiments concerned aqueous solutions of polymers. These systems have been extensively studied [31] because they are particularly suitable for the separation or extraction of cellular organelles and biological molecules. PEG–potassium phosphate buffer allows the pH for the two liquid phases to be fixed between 4 and 9. Moreover, varying the molecular mass of the polymer modifies the partition coefficient of the solute. Such a system was used on the cross-axis prototypes for various applications to proteins, all shown in Table IV. Another well known system is based on a PEG–dextran biphasic mixture. The partition coefficients of the solutes can also be adjusted by changing the molecular masses of the polymers; adding a phosphate buffer sets the pH. The very low interfacial tensions of these solvent systems (as low as 0.0001 dyn/cm) are suitable for fragile
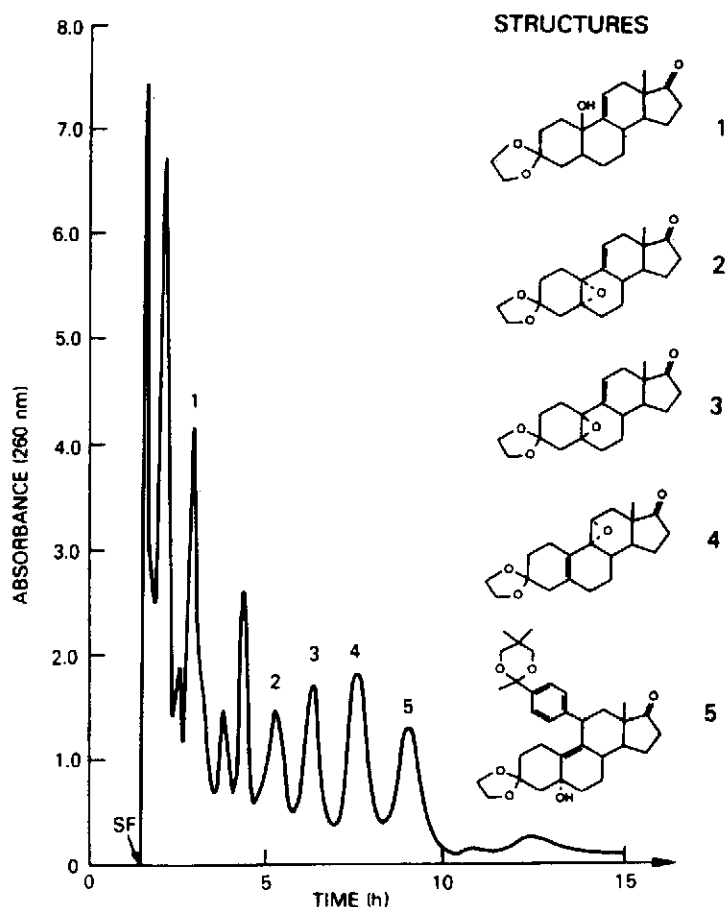
Fig. 8. Chromatogram of a steroid reaction mixture on a type X-0.5L cross-axis CPC [27]. A 2.4-g amount of a mixture of synthetic steroids was used. Solvent system, n-hexane–ethyl acetate–methanol–water (6:5:4:2, v/v) with a heavier aqueous mobile phase; flow-rate, 240 ml/h; rotational speed, 450 rpm. Two type Ia columns in series, total volume 1600 ml. Retention of stationary phase, 71%.

molecules, such as proteins in which the quaternary structure may be broken. A PEG-8000–dextran T500 system containing potassium phosphate buffer allowed the separation of various histones and globulins [10].

The third group of experiments included more "classical" solvent systems, based on a low-polarity organic phase and an aqueous phase. Fig. 8 shows a chromatogram of 2.4 g of a crude reaction mixture of synthetic steroids [27]. The semi-preparative separation was carried out at a high flow-rate of 4 ml/min. However, the retention of the stationary phase remained high (71%). Five products were identified by NMR spectroscopy and their formulae are given in Fig.

8. The same solvent system also separated a 3-g mixture of indole auxins [27,28]. Solvent systems based on a chloroform-rich organic phase were used to separate DNP-amino acids and flavonoids from a crude extract of sea buckthorn (Hippophae rhamnoides) [27]. An antibiotic, i.e., bacitracin, was purified with the same system [28].

6. CONCLUSIONS

The cross-axis coil planet centrifuge is a useful counter-current chromatograph. Its design enhances the retention of the liquid stationary phase inside the chromatographic column for

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biphasic polar solvent systems in comparison with previous CCC devices. As a result, the resolution is increased. The efficiency, which is at least similar to that of the type J CPC, is satisfactory, partly owing to three-dimensional mixing. Moreover, the choice of columns of small or large internal volume determines the analytical or preparative purpose of the device.

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