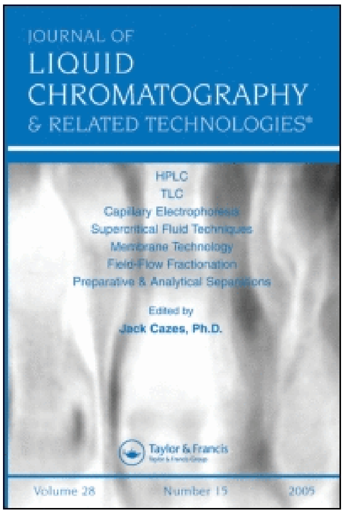


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Original Uses of the Liquid Nature of the Stationary Phase in CCC. I. Extraction and Separation of Transition Metal Ions

K. Talabardon[a]; M. Gagean[a]; J. M. Mermet[a]; A. Berthod[a]
[a] Laboratoire des Sciences Analytiques UMR CNRS 5619 Université de Lyon, Villeurbanne Cedex, France

To cite this Article Talabardon, K. , Gagean, M. , Mermet, J. M. and Berthod, A.(1998) 'Original Uses of the Liquid Nature of the Stationary Phase in CCC. I. Extraction and Separation of Transition Metal Ions', Journal of Liquid Chromatography & Related Technologies, 21: 1, 231 — 250

To link to this Article: DOI: 10.1080/10826079808001952

URL: http://dx.doi.org/10.1080/10826079808001952

J. LIQ. CHROM. & REL. TECHNOL., 21(1&2), 231-250 (1998)

ORIGINAL USES OF THE LIQUID NATURE OF THE STATIONARY PHASE IN CCC. I. EXTRACTION AND SEPARATION OF TRANSITION METAL IONS

Karine Talabardon, Mathias Gagean, Jean Michel Mermet, Alain Berthod

Laboratoire des Sciences Analytiques
UMR CNRS 5619
Université de Lyon 1
CPE Lyon
69622 Villeurbanne Cedex, France

ABSTRACT

Displacement chromatography is a way of using the chromatographic technique, in which the solutes are separated in bands in the stationary phase. Because the stationary phase is a liquid in CCC, it possible to stop the displacement process when the separated bands are still in the stationary phase. The collection of the stationary phase bands is possible. This way of using a CCC instrument was described for the separation of transition metal ions. Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} were separated using diethylhexyl phosphoric acid (DEHPA) and heptane as the stationary phase through a reversed micelle extraction process.

The extraction constants of the cations were determined accurately by CCC. The displacement process and collection of the stationary heptane phase allowed recovery of ion bands with purity higher than 95%. A ten-fold concentration of the cations in the organic phase can be obtained. The process is described.

The effect of the mobile phase pH and the ion concentrations are studied. This use of CCC allows both the deionization of charged aqueous phases and the separation and recovery of the water-contained cations by concentrating them in the heptane phase.

INTRODUCTION

In many applications, countercurrent chromatography (CCC) can be defined as a liquid chromatography technique with a liquid stationary phase. Most often, there are no countercurrent flowing phases in CCC.[1] The problem that slows the CCC development in research and industrial applications is that it is very difficult to keep a liquid phase stationary when another liquid phase is pushed through it. Dedicated, and somewhat complicated, instruments should be designed.[2] The liquid phase retention is difficult, depending on various parameters such as density differences,[3] viscosity and interfacial tension,[4] mutual solubilities,[5] rotation speed of the machine's centrifuge rotor,[6] and the apparatus design.[1,2,7] The parameters listed depends on the machine used, or are linked to the biphasic liquid system used.[8]

The efforts made to use a liquid stationary phase are justified by the advantages of the liquid physicochemical state of the stationary phase. The first, and main, advantage is the loading capacity of a liquid phase.[1,2,7] The basis of the chromatographic process is that solute separation occurs by exchanges between the mobile and the stationary phases.

The surface area between the solid stationary phase in liquid chromatography (LC) and the liquid mobile phase is the useful zone for the solute interphase exchanges. The volume is limited by a narrow thickness. This is good for the kinetics of the process, i.e., efficiency. However, the loading capacity in LC is very limited. In CCC, the whole volume of the stationary phase can be reached by the solute. The efficiency is lower than in LC, but the loading capacity is very large.[1,2]

The other advantages of a liquid stationary phase are (i) an infinite choice of biphasic systems covering a large polarity range;[1,9] (ii) so large a choice of systems produces original selectivities compared to the classical reversed phase LC technique;[10,11] (iii) during a CCC run it is possible to reverse the phase roles: the liquid stationary phase becomes the mobile phase and *vice versa*. The dual mode use of CCC[12] leads to a big advantage; (iv) everything that is injected into a CCC column is always fully recovered. There cannot be any irreversible adsorption in CCC; (v) the direct liquid-liquid partition coefficient determination is the last cited advantage of a liquid stationary phase.[11,13]

This paper presents an original use of the liquid nature of the stationary phase in CCC. Combining the loading capability of CCC with the operating mode known as displacement chromatography,[14] it is possible to separate and/or to purify significant amounts of ions of transition metals. Reversed micelles of a complexing agent are used to arrange the ions in the order of their complexation constant values. The apparent liquid-liquid partition coefficient of the ions can be related to their complexation constants.

THEORETICAL

The use of CCC for the separation of metallic ions was pioneered by Freiser.[15-17] A transfer from the aqueous phase containing, originally, the polar ions, to the organic stationary liquid phase is required to separate the ions. Complexing agents can extract metallic ions from an aqueous phase and transfer them to an organic phase, most often through an ion exchange process.[18]

With an acidic complexing agent, the chemical reaction of the phase transfer is:

$$M^{n+}_{aq} + n\,Ha_{org} \rightleftharpoons 8\,M(A)_{n,\,org} + n\,H^{+}_{aq} \qquad (1)$$

in which the subscripts aq and org refer to the aqueous and organic phase, respectively, M^{n+} and A are the metal ion and the complexing agent, respectively. The equilibrium constant corresponding to Eq. 1 is:

$$K_{ex} = \frac{[MA_n]_{org}[H^+]^n_{aq}}{[M^{n+}]_{aq}[HA]^n_{org}} \qquad (2)$$

The K_{ex} constant is related to the apparent partition coefficient, P, of the ion that governs the CCC ion retention by

$$P = K_{ex}\left(\frac{[HA]}{[H^+]}\right)^n . \qquad (3)$$

Equation 3 shows that two different cations with different K_{ex} values have different partition coefficients. They will be separated. Also, the apparent partition coefficient is not a constant. It depends on the mobile phase pH and [HA], the stationary phase complexant concentration, according to:

$$\log P = \log K_{ex} + n \log [HA] + n\,pH \qquad (4)$$

Equation 4 shows that the CCC ion retention critically depends on the mobile phase pH. The CCC retention equation can be expressed by:

$$V_R = V_M + PV_S \tag{5}$$

in which V_R is the solute retention volume, V_M is the mobile phase volume and V_S is the stationary phase volume inside the CCC machine. P is the liquid-liquid partition coefficient of the solute, expressed as the ratio of the solute concentration in the stationary phase over the solute concentration in the mobile phase. A one-unit change in the mobile phase pH produces a n order of magnitude change in the ion apparent P value (Eq. 4). This means that a given cation can be completely extracted (V_R very large) or not retained ($V_R = V_M$ if $P \approx 0$) by the same complexing agent at different pH values. Also, the study of the CCC measured partition coefficient of an ion at different pH values allows to determine its K_{ex} constant with great accuracy .[19]

Examples of extracting agents include the organophosphorus compounds such as the di-alkyl phosphinic acids (Cyanex® compounds), the di-alkyl phosphonic acids, trialkyl phosphine oxides such as the tri-octyl (TOPO), and the di-alkyl phosphoric acids such as the diethylhexyl phosphoric acid used in this work. Quaternary alkyl ammonium salts are also used.[20]

EXPERIMENTAL

Chemicals

Diethylhexylphosphoric acid (DEHPA, $C_{16}H_{35}PO_4$, m.w. 322 g/mol, d=0.975 g/cm^3, pK_A=1.4) was obtained from E. Merck (Schuchardt, Munchen, Germany). DEHPA is not soluble in water. It can form reverse micelles in an apolar solvent such as shown by Figure 1. Water is confined in a pool bonded by the phosphoric acid heads.

The transition metal ions, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} can be located inside the core of the reverse micelles. Acetate salts of the cations were obtained from Prolabo (Paris, France) and Sigma-Aldrich (L'Isles d'Abeau, France).

The organic phase was heptane, much less toxic than hexane. It was obtained from SDS (Peypin, France). All chemicals were used as received, except water that was distilled and deionized before use. The pH of the aqueous ionic solutions was adjusted by acetic acid without any salt buffer added.

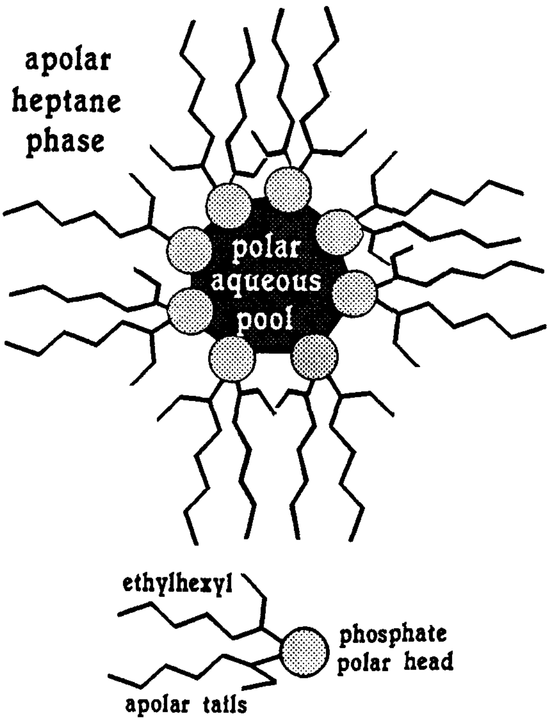


Figure 1. Surfactant molecule orientation in the reversed micelle structure. The ions are located in the water interior of the micelle where the ion-exchange process occurs.

Countercurrent Chromatograph

The CCC apparatus was the model CPHV 2000 from Société Française de Chromato Colonne, (SFCC, Shandon, Cergy-Pontoise, France). It is a coil-planet centrifuge apparatus, first designed by Ito.[21] It was fully described in a recent article.[22] The apparatus volume, V_C, was 153 mL with a total PTFE tube length of 76 m coiled on three spools. Each spool was coiled with 95 turns (5 layers of 19 turns) of 1/8" (1.6 mm i.d.) PTFE tubing.

The Ito β value is the ratio of the coil radius, r, to the spool revolution radius, R. The β ratio was 0.61 for the inner first diameter with r = 3.7 cm and R = 6 cm. It was 0.82 for the most outer visible layer with r = 4.9 cm and R = 6 cm. The average β value for this CCC apparatus was 0.72. The internal volume of one spool was 51 mL. The spools are interconnected with 1/16" PTFE tubes (0.5 mm i.d.).

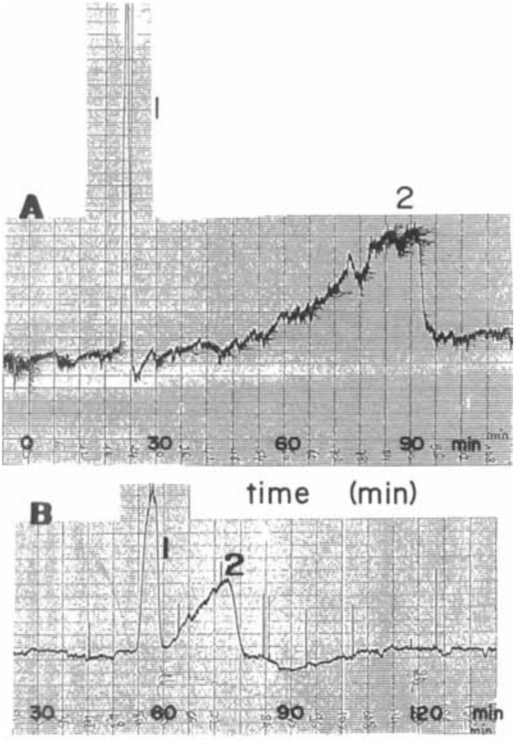


Figure 2. CCC chromatograms of transition metal ions. Rotor rotation 700 rpm; aqueous phase flow rate 3 mL/min head to tail; stationary phase heptane + DEHPA 0.5M; detection by light scattering diffusion. **A**- mobile phase pH 1.3, peak 1: Co^{2+} and Ni^{2+} (35 µmoles each), peak 2: Zn^{2+} (250 µmoles) injected in 250 µL. **B**- mobile phase pH 2.15, peak 1: Co^{2+} and Ni^{2+} (30µmoles each), peak 2: Cu^{2+} (110 µmoles) injected in 220 µL.

The whole system was housed in an air-thermostated box. The temperature was regulated at 22°C ±0.5°C. The biphasic liquid system used was heptane (+DEHPA)-water (containing cations). The heptane phase is easily retained (Sf>80%) when the mobile phase is the aqueous phase moving from the head of the apparatus to the tail (H→T).

Chromatographic Peripherals

A single pump, Shimadzu LC10AS (Touzard & Matignon, Paris, France) was used to drive both the mobile aqueous phase and the stationary organic phase.

Table 1

Retention Parameters of the Transition Metal Cations at Various pH's*

Experimental Parameters	Cation	t_R, min	V_R, mL	P_{ex}
Mobile phase pH **1.30**	Co	21.5	64	0
$V_M = 64$ mL	Ni	21.5	64	0
$V_s = 113$ mL	Cu	26	78	0.12
see Fig. 2A	Zn	91	273	1.85
Mobile phase pH **1.51**	Co	26	78	0
$V_M = 78$ mL	Ni	26	78	0
$V_s = 99$ mL	Cu	37	111	0.33
	Zn	185	555	4.8
Mobile phase pH **2.15**	Co	56	167	0
$V_M = 167$ mL	Ni	56	167	0
$V_s = 10$ mL	Cu	77	231	6.4
see Fig. 2B	Zn	>240	>720	>55
Mobile phase pH **2.55**	Co	31.5	94.5	0.26
$V_M = 66$ mL	Ni	60.5	181.5	1.04
$V_s = 111$ mL	Cu	>240	>720	>6
	Zn	>240	>720	>6

---

* Chromatographic conditions are listed in Figure 2 caption.

Detection was a problem because the ions do not absorb UV light. An evaporative light scattering detector, DDL 21 (Cunow, Eurosep, Paris, France), was used to continuously monitor the eluent. Compressed air was used as the nebulizing and drying gas. The inlet air pressure was 1.5 kg/cm^2 (20 p.s.i.). The drying chamber was set at 80°C and the photomultiplier tube voltage was 500 V.

A fraction collector, model LKB-Frac 100 (Pharmacia-Biotech, Uppsala, Sweden), allowed to collect 5 or 10 mL fractions of the mobile aqueous phase or the heptane stationary phase.

The fractions were analyzed by an Inductively Coupled Plasma-Atomic Emission Spectrometer (ICP-AES) model Optima 3000 (Perkin Elmer) with a Charge Coupled Device (CCD) detector. The plasma energy was set to 1100 W. The liquid sample was introduced in the torch using a cross-flow nebulizer

(1 mL/min liquid sample + 1 L/min argon flow rate). The argon flow rate in the torch was 15 L/min. The detection wavelengths for the Co^{2+}, Ni^{2+} and Cu^{2+} ions were, respectively, 238.892 nm, 221.647 nm and 324.754 nm. This ICP-AES spectrometer could detect concentrations as low as 10 μg/L (0.01 ppm) of each cation.

RESULTS AND DISCUSSION

Ion Separation and Extraction Constant Determination

The heptane stationary phase, containing 0.5 M DEHPA, was used to determine the extraction constants of the transition metals. The mobile phase pH was buffered at different pH values. A small decrease of the mobile phase pH was producing dramatic decreases of the cation retention volumes. Table 1 lists the retention parameters obtained at different pH values. Figure 2 shows two chromatograms actually obtained.

The results obtained show that the efficiency calculated for the retained cations (Cu^{2+} and Zn^{2+} in Figures 2-A and 2-B, respectively) is in the 300-plate range. This value is low for a hydrodynamic instrument. This means that the ion-exchange process between the two liquid phases is relatively slow. It is pointed out that the stationary phase volume was intentionally very low (V_S = 10 mL or Sf = 6%) to reduce the retention volumes in the pH 2.15 (Figure 2-B) experiment. This very low Sf value contributes greatly to the poor efficiency.

It was not possible to separate the four cations in the same run. At very low pH values (pH<2), the Zn^{2+} ion is separated from the other three cations. At pH values higher than 2.5, the Zn^{2+} and Cu^{2+}, ions are highly retained. They are not eluted from the column after 4 hours at 3 mL/min or 720 mL of mobile phase volume, although the stationary phase volume was a mere 10 mL.

Other experiments were done with DEHPA concentrations in the heptane stationary phase varying between 0.02 M and 0.5 M and mobile phase pH values varying in the 1.6-3.0 range. The log P - 2 log (DEHPA) values were plotted versus the mobile phase pH (Eq. 4). Table 2 lists the values of the slopes, intercepts, which are the log K_{ex} values (Eq. 4), and regression coefficients of the straight lines obtained. Figure 3 shows the calculated retention volumes of the four cations for mobile phase pH values between 1 and 3. It clearly shows that it was not possible to separate correctly the four cations in one run. The method allows to determine accurately the complexation or extraction constants, K_{ex}, for the studied cations.

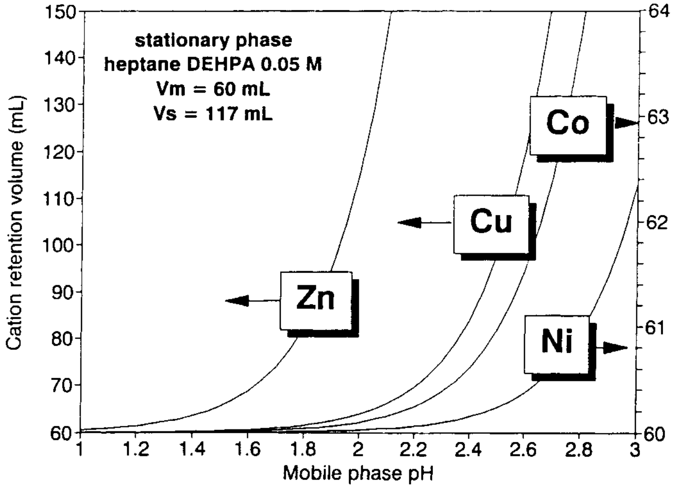


Figure 3. Retention volumes of the transition metal ions versus the mobile phase pH, calculated using the extraction constants of Table 2 and equations 3 and 5. Cu^{2+} and Zn^{2+}: left ordinate, Ni^{2+} and Co^{2+}: right ordinate.

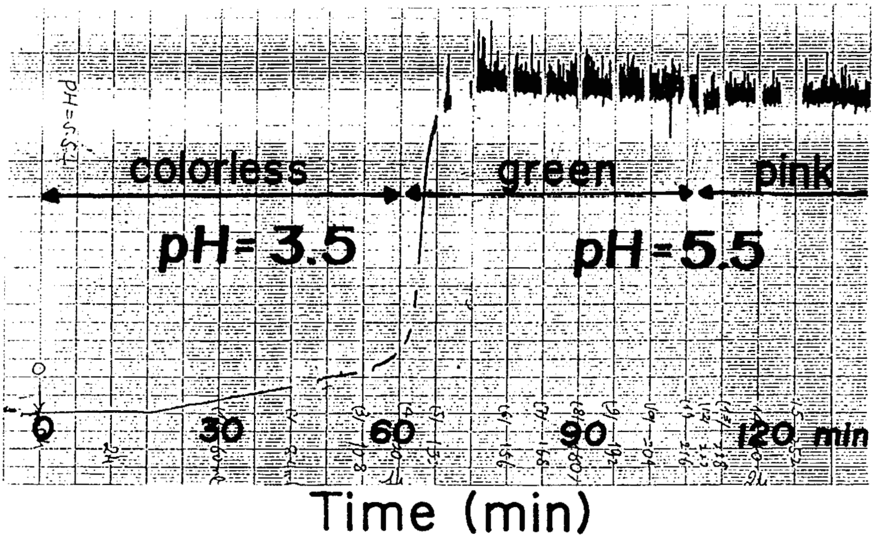
Table 2

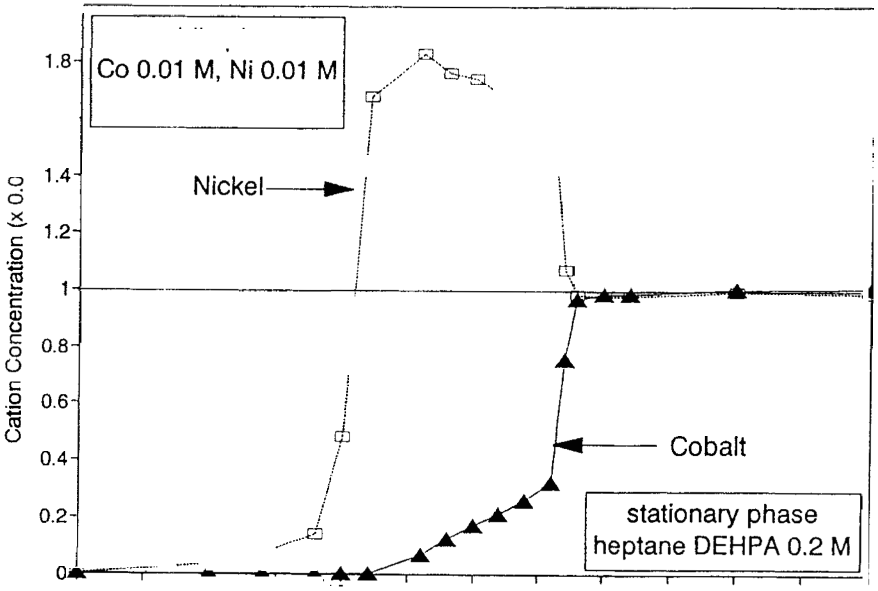
Regression Parameters of the log P - 2 log [DEHPA] = f(pH) Lines

Relative Error: 10%	Co	Ni	Cu	Zn
Slope (= n)	2.12	2.08	1.95	1.94
Intercept (= log K_{ex})	-4.5	-5.1	-2.9	-1.7
Regression Coefficient	0.984	0.986	0.991	0.994
Number of points	5	5	6	3
K_{ex}	3.3×10^{-5}	8×10^{-6}	1.3×10^{-3}	1.8×10^{-2}
Ion separation	Co/Ni	Cu/Ni	Zn/Cu	Zn/Ni
Factor: α_D	4.2	160	14	2250

Displacement Chromatography

Method: In classical displacement chromatography, the stationary phase contains an adsorbed entity, I, whose affinity constant for the stationary phase is K_I. The mobile phase contains another entity, J, which has a high affinity, K_J, for the stationary phase. The solutes, 1, 2, ... to be separated have affinities, K_1, K_2, ...


pH=5.5
colorless
green
pink
pH=3.5
pH=5.5
0
30
60
90
120 min
Time (min)


Co 0.01 M, Ni 0.01 M
Nickel
Cobalt
stationary phase
heptane DEHPA 0.2 M
Cation Concentration (x 0.0
1.8
1.4
1.2
1
0.8
0.6
0.4
0.2
0

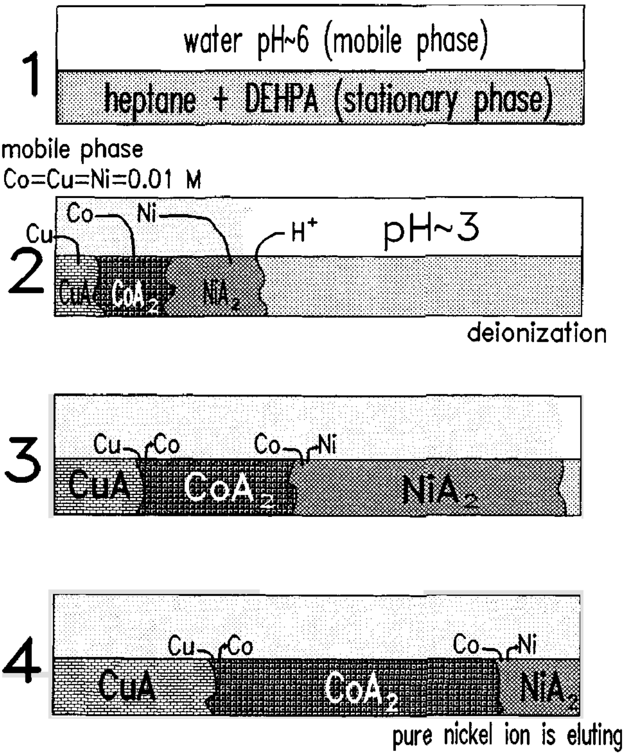


Figure 5. Separations of transition metal ions by displacement CCC. 1- The initial state of the CCC column. 2- The mobile phase contains the ions. They displace the H^+ ions. The mobile phase is deionized and acidic. 3- The ion bands form. Copper ions have the highest affinity for the stationary phase. 4- The nickel ions are eluted from the CCC column about three times more concentrated than the original 0.01M. The liquid stationary phase contains bands of the cations.

Figure 4 (left). Displacement chromatogram obtained with a one-spool CCC instrument, V_s = 20 mL heptane phase with 0.2M DEHPA, V_M = 38 mL of aqueous phase containing 0.01M of Co^{2+} and 0.01M of Ni^{2+} ions. Rotor rotation: 700 rpm; flow rate: 2 mL/min, head to tail. Top, the actual signal of the evaporative light scattering detector; the mobile phase pH values and colors are indicated. Bottom: the reconstructed chromatogram after ICP-AES analysis of the collected fractions.

whose values should be intermediate between K_I and K_J. During the process, the solutes displace I, since they have more affinity for the stationary phase. However, they are themselves displaced by the mobile phase J compound. The result is that the solutes are arranged inside the column in bands. The order of the band elution is the increasing order of the solute affinity constants.[14]

Ito introduced the use of displacement chromatography in CCC calling it: "pH-zone-refining."[23] The pH-zone-refining naming comes from the fact that the I compound in the stationary phase is an acid and the displacement J compound in the mobile phase is a base. The solutes are arranged in bands of increasing pK_A values.[24] The drawback of displacement chromatography is that the column must be regenerated in the I form after every separation. This is not a problem in CCC. The liquid stationary phase in the J form can be easily changed for a fresh phase in the I form. In this work, the stationary phase was collected for analysis.

Displacement and Ion Complexation: The I form we used is the DEHPA complexing agent (in the protonated form). We do not use a J displacer. It will be the cation with the highest affinity for the complexing agent. The cations are not injected in a limited bolus. Instead, the mobile phase contains a known concentration of transition cations so that they are introduced continuously in the CCC instrument inlet. Figure 4-A shows the actual elution profile obtained with the light scattering detector and a simple mobile phase containing only two cations: Co^{2+} and Ni^{2+} at the same M concentration (590 ppm). The effluent was collected and each fractions were analyzed by ICP-AES. Figure 4-B is the reconstructed plot of the ion concentration versus the elution volume.

The initial mobile phase pH was 5.5. Figure 4-A shows that, during one hour, (120 mL) the pH of the eluting and deionized mobile phase was 3.5. The cations were displacing the DEHPA proton from the heptane phase to the aqueous phase. Next, the nickel ion band comes out with a concentration close to 0.02 M. 0.01 M of nickel ions was in the mobile phase and the other 0.01 M comes from the nickel ions displaced from the stationary phase by the cobalt ions. A green color is noticeable. Next, the cobalt ions and the nickel ions, both at the 0.01 M concentration, are eluted out of the CCC column. The stationary phase is saturated in pure cobalt ions. It is in the J form. Figure 4 shows that 20 mL of heptane phase deionize 120 mL of aqueous phase and separate ~2.4 mmoles of nickel ions in 120 mL @ 0.02 M (green phase, Figure 4) and ~2.4 mmoles of cobalt ions complexed in the heptane phase.

The ICP-AES analysis of the phase produced the following phase concentrations: the deionized aqueous phase contained 0.3 ppm of Ni^{2+} ions and 0.2 ppm of Co^{2+} ions; the nickel ion aqueous band contained 97.4% of Ni^{2+} ions (0.018 M or 530 ppm) and only 2.6% of Co^{2+} ions ($4.8\ 10^{-4}$ M or 14 ppm); the heptane stationary phase contained Co^{2+} ions 99.3% pure at a concentration of 0.285 M

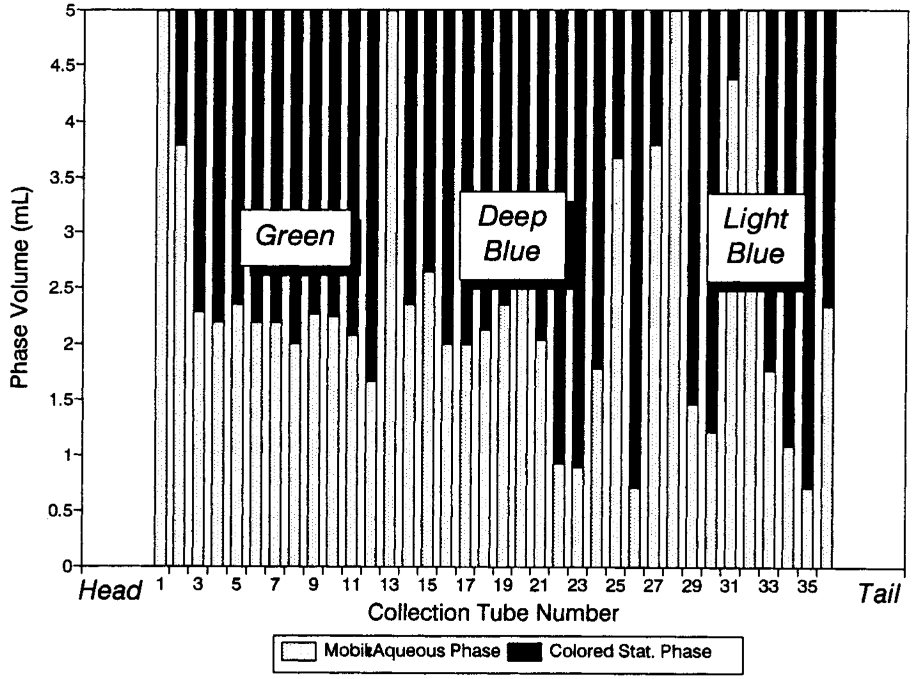


Figure 6. Representation of the 5 mL collection tubes after emptying the CCC machine. The colors correspond to the stationary heptane phase; they are due to the DEHPA complexed cations.

(16800 ppm). The deionization process has a 99.9% efficiency. The nickel band is 97.4% pure and the cobalt ion in the heptane phase is 99.3% pure with a 28 factor enrichment. The whole study of the deionization and ion extraction process was presented in our recent work.[25]

Process Description: The displacement process arranges the cations in bands of increasing K_{ex} constants. Figure 5 illustrates the steps of an experiment done with a mobile phase containing an equal concentration of Co^{2+}, Ni^{2+} and Cu^{2+} cations. Step 1: the CCC apparatus is equilibrated with water and a heptane stationary phase containing the DEHPA complexing agent. Step 2: the aqueous phase with the cation is pumped in the CCC column. The cations are complexed. They displace the hydrogen ions. The eluting mobile phase is deionized and acidic. Step 3: the cation bands form. Ni^{2+} is pushed in front by Co^{2+} which has a higher affinity for the complexing agent (Table 2). Co^{2+} himself is displaced by Cu^{2+} which forms the trailing band. This step lasts as long as the Ni^{2+} ions, pushed in the front, can find

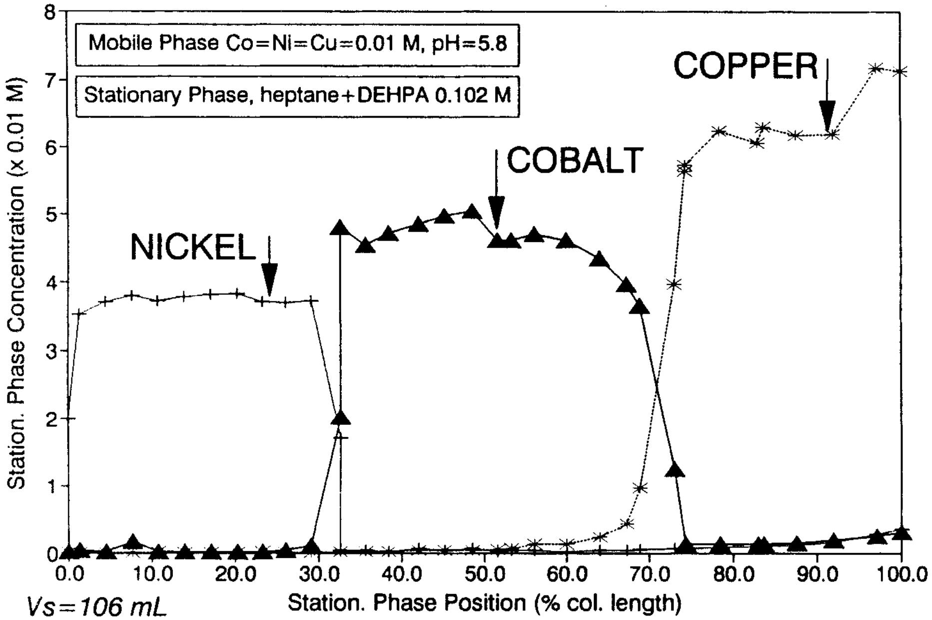


Figure 7. Reconstitution of the stationary phase composition inside the CCC instrument after ICP-AES analysis of the Figure 5 tubes. The positions of the tubes were expressed in percentage of the column length.

fresh stationary phase. Step 4: the Ni^{2+} ions reach the end of the CCC column. The mobile phase leaving the CCC machine contains only Ni^{2+} ions at a concentration close to the total initial ion concentration. As experimentally observed, the eluting mobile phase is enriched in Ni^{2+} ions.

Stationary Phase Collection: Using the unique property of CCC, i.e., the stationary phase is liquid, it is possible to collect the heptane phase with the separated cation bands. When Step 4 (Figure 5) is reached, the light scattering detector signal changes due to the Ni^{2+} ions. The mobile phase flow rate and the CCC machine are stopped. The stationary phase is pushed out of the CCC column using compressed nitrogen. Five mL fractions were collected in test tubes. The fractions contained both phases. The aqueous phase is the denser phase. The heptane phase is brightly colored by the complexed cations. Figure 6 is a representation of an actual experiment. The color of the heptane phase is indicated. The bands were sharply delimited: green nickel ion band, Tube 1 to 12; deep blue cobalt ion band, Tube 14 to 27; light blue copper ion band, Tube 29 to 36. In this experiment, the bands were separated by a small zone containing only the mobile phase (Tube 13 and Tube 28).

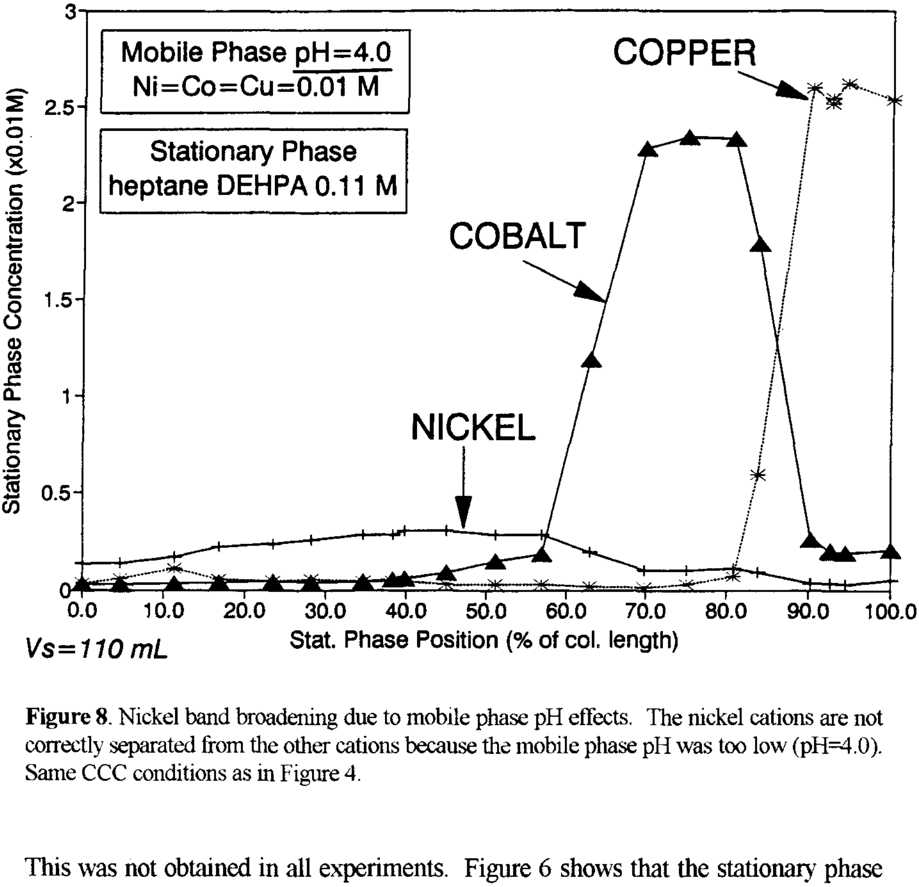
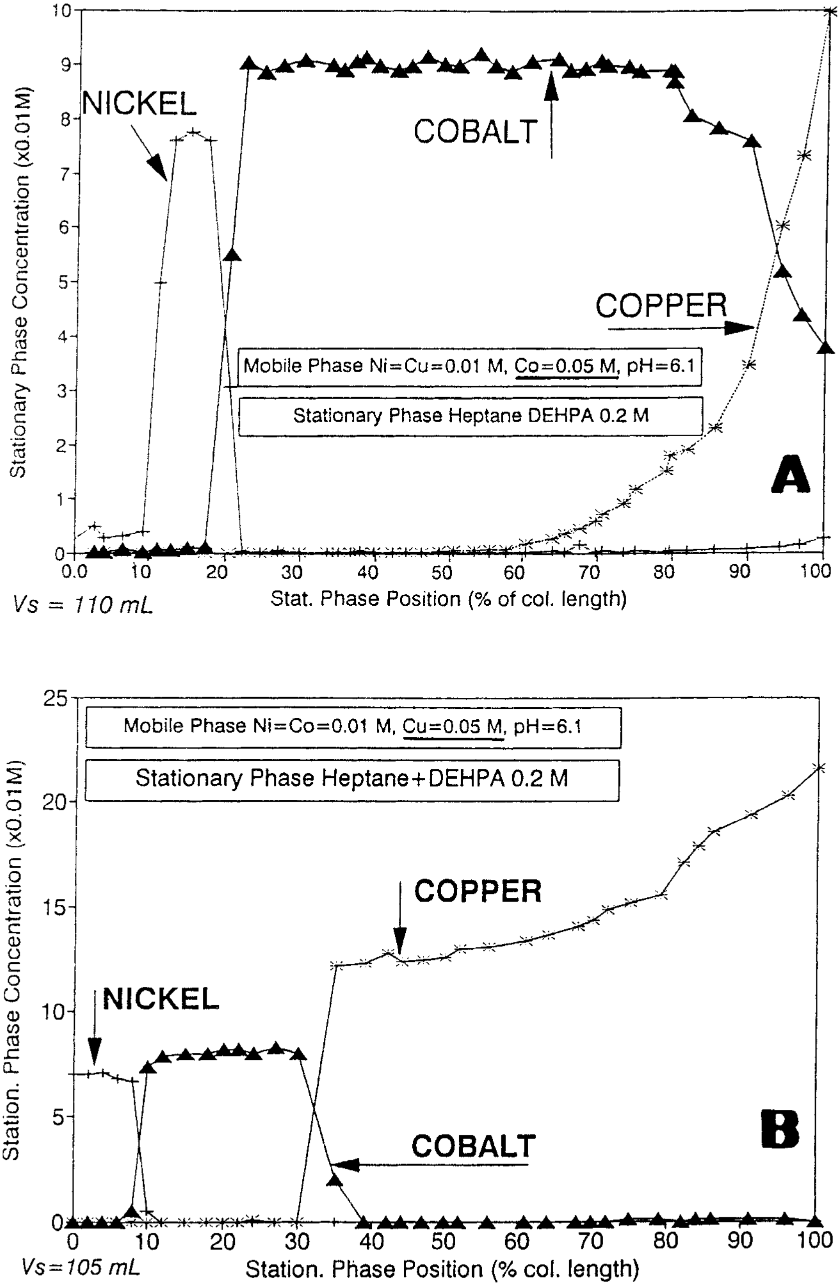


Figure 8. Nickel band broadening due to mobile phase pH effects. The nickel cations are not correctly separated from the other cations because the mobile phase pH was too low (pH=4.0). Same CCC conditions as in Figure 4.

This was not obtained in all experiments. Figure 6 shows that the stationary phase collection process does not mix up the separated bands although some broadening may occur. It is also a proof that both the aqueous and organic phases are in contact throughout the internal CCC tubing. The internal machine volume was 177 mL. It was never possible to collect this volume, only ~165-168 mL could be collected.

Band Compositions: 100 μL of the supernatant heptane phase of every tube was cut off and transferred in 10 mL of a 0.1 M HCl aqueous solution. After vigorous shaking, the heptane color disappeared. The complexed cations were back extracted into the acidic aqueous phase which was analyzed by ICP-AES. Figure 7 shows the results, arranged so that the composition of the heptane stationary phase inside the CCC machine can be visualized. The ions are clearly separated into bands whose compositions are: the nickel band is 3.7 times enriched (0.037 M or 2200 ppm) with 99.2% purity (Co^{2+}= 15 ppm, Cu^{2+}= 6 ppm). The cobalt band is 5 times enriched


10
9
8
7
6
5
4
3
2
1
0
Stationary Phase Concentration (x0.01M)
NICKEL
COBALT
COPPER
Mobile Phase Ni=Cu=0.01 M, Co=0.05 M, pH=6.1
Stationary Phase Heptane DEHPA 0.2 M
A
0.0
10
20
30
40
50
60
70
80
90
100
Vs = 110 mL
Stat. Phase Position (% of col. length)
25
20
15
10
5
0
Station. Phase Concentration (x0.01M)
Mobile Phase Ni=Co=0.01 M, Cu=0.05 M, pH=6.1
Stationary Phase Heptane+DEHPA 0.2 M
COPPER
NICKEL
COBALT
B
0
10
20
30
40
50
60
70
80
90
100
Vs=105 mL
Station. Phase Position (% col. length)

(0.046 M or 2700 ppm) with 99.1% purity (Ni^{2+}= 11 ppm, Cu^{2+}= 14 ppm). The copper band is 6.5 times enriched (0.065 M or 4130 ppm) with 95% purity (Ni^{2+}= 100 ppm, Co^{2+}= 110 ppm).

Effect of Mobile Phase pH: Eqs. 1, 3, and 4 show that the mobile phase pH can modify the apparent cation partition coefficient P. The band formation can occur only if the cations have a strong affinity for the stationary phase. Figure 8 shows the band deformation obtained with a mobile phase pH of 4. The nickel band disappeared and the cobalt band starts to broaden. The estimated apparent partition coefficients P for the nickel and the cobalt ion at pH 4 and DEHPA 0.11 M are (Eq. 3) 9 and 40, respectively. The nickel band does not form at pH 4 because the apparent affinity of the nickel cation for the DEHPA heptane phase is not high enough. The experimental minimum P value for band formation seems to be in the 100 range.

Zinc ion bands are not presented in the results. The zinc ion has the highest affinity for the DEHPA heptane phase (Table 2). It should form sharp bands. With a pH 5 mobile phase, it did. The zinc cation was displacing nicely the three other cations. However, it was forming a so strong association with the heptane-DEHPA phase that the viscosity of the liquid phase was dramatically increased. The operating pressure was increasing. The CCC machine cannot operate above 6 kg/cm^2 (85 p.s.i.). The experiment had to be stopped. Next, it was not possible to push out the liquid phases with 5 kg/cm^2 of compressed nitrogen. We had to disassemble the CCC machine, separating the three spools. Two of them were not clogged and contained heptane phase with the nickel and cobalt cation bands. The third spool was clogged. To clean it, we had to boil the coil at 100°C, pushing the contained phases with compressed nitrogen. This destroyed the bands. A heptane-DEHPA-zinc gel was obtained. The ICP-AES analysis found a majority of zinc cations in the gel, but the three other cations were also found in significant amounts. Lowering the mobile phase pH may reduce the zinc-gel formation, but the band formation of the nickel and copper cations would be precluded.

Effect of Ion Concentration: Operating lines are established in the setup of a displacement chromatography separation.[14,23] Eq. 3, which links the apparent affinity of a given cation for the heptane stationary phase, can be used to draw the operating line of an experiment.

Figure 9 (left). Band broadening due to concentration effects. **A** (top): High cobalt ion concentration. The copper cations are not correctly separated. **B** (bottom): High copper ion concentration. The three cations are correctly separated. This case corresponds to a classical displacement experiment. Same CCC conditions as in Figure 4.

In this work, we just want to show, experimentally, the effect of the ion concentration. Figure 9-A shows the bands obtained with a cobalt rich mobile phase at pH 6.1. The copper band does not form properly. The last collected tube contained 50% copper ions and 50% cobalt ions. However, the nickel band was correctly formed in the front of the large cobalt band. Artifacts due to the phase collection process cannot be excluded. The copper band should travel through the whole tubing length (76 m) pushed by compressed nitrogen. The aqueous phase (cobalt rich) is still in the tubing and some mixing may occur. Figure 9-B shows the bands obtained with a copper rich mobile phase. The bands form correctly at pH 6.1. The nickel ion band is truncated because some nickel ions were collected in the aqueous phase exiting the CCC machine. However, it should be noted that the initial concentration of the copper band was 0.125 M; it increased up to 0.23 M at the end (right part) of the band. pH effects are suspected. A 0.23 M copper concentration in a 0.2 M DEHPA heptane solution means a 1:1 complex was formed. This can be explained if a Cu(OH)-DEHPA complex is formed at pH 6.1.

CONCLUSION

A CCC instrument can be used to perform displacement chromatography, as already shown by Ito, developing the pH-zone-refining technique.[23,24] This chromatographic mode separated the solutes in band of concentration in the stationary phase. Because the stationary phase is a liquid in CCC, it is easy to stop the displacement process during the run and to collect the separated bands in the liquid stationary phase. This was shown to work with transition metal cations. It should work with other ions. DEHPA is also able to complex the rare earth cations. These ions were tried. The problem was that the bands of lanthanide ions had a high viscosity that could clog the CCC column. Another complexing agent or organic solvent should be found. The interest of this way of using a CCC machine is obvious: deionization and/or purification of cation charged waste waters can be done, as well as recovering of valuable or radioactive cations by concentration in a reduced volume of an organic phase.

REFERENCES

1. W. D. Conway, **Countercurrent Chromatography, Theory and Applications**, VCH Publishers, Weinheim, 1990.

2. N. B. Mandava, Y. Ito, eds., **Countercurrent Chromatography**, Chromatogr. Science Series, Marcel Dekker, New York, Vol. 44, 1988.

3. A. Berthod, N. Schmitt, Talanta, **40**, 1489-1498 (1993).

4. J. M. Menet, D. Thiebaut, R. Rosset, J. E. Wesfreid, M. Martin, Anal. Chem., **66**, 168-176 (1994).

5. F. Oka, H. Oka, Y. Ito, J. Chromatogr., **538**, 99-108 (1991).

6. J. M.Menet, M. C. Rolet-Menet, D. Thiebaut, in **Modern Countercurrent Chromatography**, W. D. Conway, R. J. Petroski, eds., ACS Symposium Series, ACS, Washington, 1995, Vol. 593, Ch. 3, pp. 35-46.

7. A. P. Foucault, ed., **Centrifugal Partition Chromatography**, Chromatogr. Science Series, Marcel Dekker, New York, Vol. 68, 1995.

8. A. Berthod, J. M. Deroux, M. Bully, in **Modern Countercurrent Chromato graphy**, W. D. Conway, R. J. Petroski, eds. ACS Symposium Series, ACS, Washington, 1995, Vol. 593, Ch. 2, pp. 16-34.

9. J. M. Sørenson, W. Arlt, **Liquid-Liquid Equilibrium Data Collection**, Chemistry Data Series, Dechema, Frankfurt, 1979.

10. A. P. Foucault, P. Durand, E. Camacho-Frias, F. Le Goffic, Anal. Chem., **65**, 2150-2154 (1993).

11. A. Berthod, A. I. Mallet, M. Bully, Anal. Chem., **68**, 431-436 (1996).

12. A. Berthod, C. D. Chang, D. W. Armstrong, in **Centrifugal Partition Chromatography**, A. P. Foucault, ed., Chromatogr. Science Series, Marcel Dekker, New York, 1995, Vol. 68, Ch. 1, pp. 1-23.

13. A. Berthod, in **Centrifugal Partition Chromatography**, A. P. Foucault, ed., Chromatogr. Science Series, Marcel Dekker, New York, 1995, Vol. 68, Ch. 7, pp. 167-197.

14. C. Horvath, A. Nahum, J. H. Frenz, J. Chromatogr., **218**, 365-393 (1981).

15. Y. Surakitbanharn, S. Muralidharan, H. Freiser, Anal. Chem., **63**, 1510-1516 (1991).

16. T. Araki, Y. Kubo, T. Toda, M. Takata, T. Yamashita, M. Murayama, Y. Nunogaki, Analyst, **110**, 913-916 (1985).

17. K. Akiba, in **Centrifugal Partition Chromatography**, A. P. Foucault, ed., Marcel Dekker, New York, 1995, Vol. 68, Ch. 9, pp. 219-239.

18. J. A. Marinsky, Y. Marcus, eds., **Ion Exchange and Solvent Extraction**, Marcel Dekker, New York, 1995, Vol. 12.

19. K. Akiba, in **Centrifugal Partition Chromatography**, A. P. Foucault, ed., Chromatogr. Science Series, Marcel Dekker, New York, 1995, Vol. 68, Ch. 9, pp. 219-239.

20. S. Muralidaran, H. Freiser, in **Centrifugal Partition Chromatography**, A. P. Foucault, ed., Chromatogr. Science Series, Marcel Dekker, New York, 1995 Vol. 68, Ch. 10. pp. 317-330.

21. Y. Ito, H. Oka, J. L. Slemp, J. Chromatogr., **475**, 219-227 (1991).

22. A. Berthod, M. Bully, Anal. Chem., **63**, 2508-2512 (1991).

23. Y. Ito, K. Shinomiya, H. M. Fales, A. Weisz, A. L. Scher, in **Modern Countercurrent Chromatography**, W. D. Conway, R. J. Petroski, eds., ACS Symposium Series, ACS, Washington, 1995, Vol. 593, Ch. 14, pp. 156-183.

24. A. L. Scher, Y. Ito, in **Modern Countercurrent Chromatography**, W. D. Conway, R. J. Petroski, eds., ACS Symposium Series, ACS, Washington, 1995, Vol. 593, Ch. 15, pp. 184-202.

25. A. Berthod, J. Xian, S. Alex, C. Collet-Gonnet, Can. J. Chem., **74**, 277-286 (1996).

Received March 20, 1997
Accepted June 3, 1997
Manuscript 4433