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Test to evaluate countercurrent chromatographs Liquid stationary phase retention and chromatographic resolution

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Abstract

Countercurrent chromatography (CCC) is a liquid chromatography (LC) technique with a special column able to retain a liquid stationary phase while the liquid mobile phase is pumped through. The coil planet centrifuge machines are made of open tube wound on spools. A simple test is proposed. The methanol–water (90:10, v/v)–heptane biphasic system is used with heptane as the mobile phase in the ascending or tail-to-head mode. The methanol–water stationary phase retention volume is measured at different flow-rates and rotor rotation speeds. After every machine equilibration, an alkylbenzene mixture is injected and the retention factors, peak efficiencies and resolution factors are measured or calculated for each solute. The wealth of information contained in the data set obtained is demonstrated. Four coil planet centrifuge machines of very different characteristics and one hydrostatic CCC machine with channels and ducts were submitted to the test. It was shown that the S_f , stationary retention factor, obtained with these machines was linearly dependent on the square root of F , the mobile phase flow-rate [Q. Du, C. Wu, G. Qian, P. Wu, Y. Ito, J. Chromatogr. A 835 (1999) 231–235]. It is shown that the slopes of the S_f versus $F^{1/2}$ lines could be related to a minimum rotor rotation, ω_{mini} , necessary to obtain the hydrodynamic equilibrium. The S_f and F parameters give the mobile phase linear velocity, u . It is shown that u is proportional to the square root of ω , the rotor rotation speed. The slope and intercept of the latter relationship also result in an ω_{mini} value coherent with the first one. With the peak efficiencies and chromatographic resolution factors obtained for toluene and hexylbenzene, the parameters: number of plates per tubing turn, machine volume for one plate, and tubing length for one plate, were calculated and compared for the five machines. The internal diameter of the tubing used is shown to be a critical parameter acting on the machine volume and number of tubing turns. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

In countercurrent chromatography (CCC), the stationary phase and the mobile phase are two immiscible liquid phases [1]. Centrifugal fields are

needed to hold the liquid stationary phase when the mobile phase is pushed through it [2]. Due to the liquid nature of the stationary phase, CCC is a liquid chromatography (LC) technique that uses special columns. Indeed, the CCC machines are just “columns”. However, in chromatography, the column is the heart of the separation process. It is then of paramount importance to know it exactly.

In LC, there are several kinds of columns, ion-

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exchange, gel permeation and bonded silica columns. The C_{18} bonded silica columns are the most popular ones used in classical reversed-phase LC. All chromatographers know that two C_{18} columns with the same length and internal diameter may give very different results with the same sample, the same LC system, mobile phase and hardware [3,4]. The differing behaviors of the C_{18} columns have numerous causes that can be due to: (1) differences in the silica physicochemical properties such as particle diameter, surface area, pore size and pore volume, (2) differences in the silica chemical properties such as its purity, metal content and residual silanol density, (3) differences in the C_{18} bonding density and nature (monomeric or polymeric), (4) special treatment such as a short-chain bonding following the C_{18} bonding (end capping treatment), and (5) the packing procedure [3]. This is the reason why a lot of tests are used to evaluate classical LC columns. The plate number, the band asymmetry factor, the column permeability (pressure drop), the selectivity and the bonded-phase surface concentration are examined using standardized tests [4].

The CCC columns have a very different nature. They can be made of a coiled open tube for the hydrodynamic machines [1,2] or channels connected by ducts for the hydrostatic machines [5]. The liquid stationary phase is stable only as long as the centrifugal field exists, i.e., the CCC column exists as long as the machine rotor is running. If a given biphasic liquid system is used, the mobile phase and stationary phase chemical nature are exactly the same in two different CCC machines, that is to say, two different CCC columns. The same sample mixture injected into the two CCC columns will produce two differing chromatograms because: (1) the machine efficiency may differ, changing the peak sharpness, and (2) the stationary liquid phase volume retained in the machines may also vary, changing the peak position. The latter parameter can be measured experimentally which allows a total prediction of peak position.

In describing a practical approach to CCC, we defined the only three active parameters that the operator can tune: the mobile phase flow-rate, the rotation speed of the machine rotor, and the temperature [6]. The evaluation of the quality of a CCC machine should cover two points: its ability to retain

a large volume of liquid stationary phase and its efficiency in mixing the mobile and stationary phase to produce a fast solute exchange between phases. In this work, we propose a simple test using an easy-to-prepare and use biphasic liquid system: the heptane–methanol–water system, and a set of stable and easy-to-detect solutes: the alkylbenzene homologous series. We will use the test to evaluate five different CCC machines acting on the rotor rotation speed and the mobile phase flow-rate only. A simple and classical procedure will be followed to obtain the liquid stationary phase retention and the chromatographic resolution parameters. The wealth of information contained in the data obtained performing the test experiments will be exposed. The results will be used to appraise the five CCC machines and make a comparison between them.

2. Experimental

2.1. The CCC machines

Four CCC machines were coil planet centrifuges of the hydrodynamic type J^1 with different internal volumes and coil numbers as listed in Table 1. The fifth machine was a hydrostatic machine with channels and ducts. The SFCC machine was built and sold from 1988 to 1992 by the Societe Francaise de Chromato Colonnes (Paris, France) with interrupted production in 1992. It is a sturdy machine able to withstand a rotor rotation of 1500 rpm. The Kromaton 2 and the two prototypes were built by SEAB (Villejuif, France). The two prototypes were designed to have a similar and small volume with different kinds of PTFE tubing (length, internal diameter and number of turns, see Table 1) coiled in one spiral. The maximum rotation speed of these machines was 500 rpm. The HPCPC 1000 hydrostatic machine was built by Sanki Engineering, sold by J.M. Science (Grand Island, NY, USA). In the case of this hydrostatic machine, an equivalent tubing length of 15 m, 2.7 mm I.D., was estimated assuming the channels were equivalent to a cylindrical tube of equal volume. The ducts (individual volume 14.3 μ l, total volume 15 ml) were not included in the computation of this equivalent length since they contain the mobile phase only, so they do

Table 1
Technical characteristics of the CCC machines evaluated

| Apparatus | Volume (ml) | Spool | | | | Tubing | | |
|------------------|-------------|----------------|---------------------|-------------------|---------------|------------------|-----------------|-----------------|
| | | Number | Average radius (cm) | Rotor radius (cm) | β Ratio | Diameter (mm) | Length (m) | Number of turns |
| SFCC 2000 | 156 | 3 (multilayer) | 3.4 | 6.0 | 0.56 | 1.65 | 73 | 400 |
| Kromaton 2 | 94 | 2 (multilayer) | 10 | 15 | 0.67 | 2.6 | 15.5 | 28 |
| Prototype 1 | 29 | 1 (spiral) | 11.9 | 15.4 | 0.77 | 2.6 | 5.1 | 7.5 |
| Prototype 2 | 25.5 | 1 (spiral) | 10.3 | 15.4 | 0.67 | 1.65 | 12 | 18.5 |
| Sanki HPCPC 1000 | 101 | Hydrostatic | – | 7.8 | – | 2.7 ^a | 15 ^b | 1060 channels |

^a Equivalent tube diameter for a channel cross section of 5.9 mm².

^b Equivalent tubing length, channel volume = 81 μ l, total volume of 1060 channels = 86 ml.

not participate in the separation process. These equivalent values have no physical foundation. They are given for comparison purposes.

The chromatographic hardware was classical high-performance liquid chromatography (HPLC) material. A Shimadzu LC10-AS pump was used with a Shimadzu SPD 10 UV detector and a Model CR6 integrator (Touzart & Matignon, Courtaboeuf, France). A Rheodyne valve with a 20- μ l loop was used to inject the solutes.

2.2. The biphasic liquid system

The heptane–methanol–water system was selected because its phase diagram shows tie-lines converging toward the heptane apex [5,6]. It means that any biphasic composition in the diagram separates into two phases: a heavy phase made of methanol, water and small amount of heptane and a light phase of almost pure heptane (with traces of methanol). The convenience of this system is that it is possible to easily prepare more mobile phase during a run. If the heptane phase is the mobile phase, this solvent can be added directly to the reservoir. If the heavy phase is the mobile phase, the corresponding methanol–water mixture is prepared, saturated in heptane, degassed and cooled to room temperature before direct use. In the phase diagram, the selected composition was methanol–water (90:10, v/v) to which heptane was added. At 20°C, this produces a light phase of 99.9% (v/v) heptane and a denser phase whose composition is methanol–water (85.4:9.5)–5.1% (v/v) heptane. This composition was selected because the two phases form a biphasic system easily retained by a CCC machine of either type, hydro-

static or hydrodynamic and also because the two liquid phases have a polarity difference well adapted to the alkylbenzene test solutes [7]. It is pointed out that the liquid system proposed for the testing of CCC machines was selected for its easy and reproducible preparation, high hydrophobicity producing a fast phase separation and high availability and moderate toxicity of its components. Although there are no objections to use it for real-world separations, other, less hydrophobic, biphasic liquid systems will have more practical potency in sample separations or purifications. Methanol and heptane were supplied by SDS (Peypin, France). Water was deionized and distilled before use.

2.3. The test solutes

Alkylbenzenes are easily detectable solutes commonly used to check the properties of C₁₈ columns in HPLC. Their partition coefficients in the heptane–methanol–water system were extensively studied [7]. Toluene and hexylbenzene were obtained from Fluka (Sigma–Aldrich Group, St. Quentin Fallavier, France) and detected at 254 nm. Their methanol–heptane partition coefficients are 0.33 \pm 0.02 and 0.064 \pm 0.006, respectively, in the methanol–water (90:10, v/v)–heptane biphasic liquid system at 22°C. The corresponding heptane–methanol partition coefficients are the inverse values, which are 3.0 \pm 0.2 for toluene and 15.6 \pm 1.5 for hexylbenzene.

2.4. The procedure

The partition coefficients of the toluene and hexylbenzene test solutes show that the denser phase

should be the stationary phase and the heptane phase should be the mobile phase. The tail-to-head mode will be used with the coil planet CCC machines and the hydrostatic machine will be run in the similar ascending mode.

The selected machine is first filled with the denser methanolic phase without rotation. Then, the machine rotor is started at the desired rpm. A cylinder is put at the machine outlet to collect the displaced denser phase. The pump is rinsed by the heptane phase, set at the desired flow-rate and started in the tail-to-head direction. As long as the liquid phase equilibrium is progressing in the machine, only the denser methanolic phase exits by the machine outlet. When the machine is completely equilibrated, the mobile heptane phase starts to be seen at the outlet and an upper liquid layer forms in the graduated cylinder. The volume of the displaced denser stationary phase corresponds to the heptane mobile phase in the CCC machine is denoted V_M . The stationary phase volume, V_S , is simply:

$$V_S = V_T - V_M \quad (1)$$

and the stationary phase retention factor, Sf is expressed by:

$$Sf = V_S / V_T \quad (2)$$

Next, 20 μ l of a toluene+hexylbenzene solution is injected and the chromatogram is developed.

To test another set of conditions (a different flow-rate or different rotor rotation), the CCC machine is emptied using compressed nitrogen, the phase volumes are recovered and compared with the values obtained by Eqs. (1) and (2). In all cases, only ~90% of the original volume was recovered. The missing 10% are located in droplets wetting the tubing wall and not displaced by a gas flow. They are displaced by the filling of the machine by the methanolic stationary phase for the next measurement.

3. Results and discussion

3.1. Parameters measured

As indicated in Section 2.4, the stationary phase

volumes, V_S , retained for different mobile phase flow-rates and machine rotor rotation speeds were measured.

This V_S value allowed one to compute the Sf retention factor:

$$Sf = V_S / V_C \quad (3)$$

which in turn led to the mobile phase linear velocity:

$$u = \frac{4F}{\pi d^2(1 - Sf)} \quad (4)$$

with the flow-rate, F , in ml/s [= (ml/min)/60] and the tube internal diameter, d , in cm to obtain the mobile phase linear velocity, u , in cm/s.

Once the machine was equilibrated, the test mixture of toluene and hexylbenzene was injected and the respective retention volumes, V_R , and peak efficiencies, N , were measured. The resolution factor, R_s , was calculated using:

$$R_s = \frac{V_{\text{toluene}} - V_{\text{hexylbenzene}}}{\frac{W_{\text{toluene}} + W_{\text{hexylbenzene}}}{2}} \quad (5)$$

where W is the peak width at base ($=4\sigma=4V/\sqrt{N}$).

Three to five different rotation speeds were tested at three to four different flow-rates for each of the five machines generating more than 60 experiments and 500 data values. As a typical example, Table 2 lists the data obtained in the case of the SFCC 2000 hydrodynamic machine. The full set of data can be requested from the authors (berthod@univ-lyon1.fr) for download.

3.2. Stationary phase retention and flow-rate

3.2.1. The square root of flow-rate relationship

In a recent work, Du et al. found, for three coil planet hydrodynamic CCC machines and 15 different liquid systems, a linear relationship between the square root of the mobile phase flow-rate and the stationary phase retention factor [8]:

$$Sf = A - BF^{1/2} \quad (6)$$

Table 3 shows that we confirmed this relationship for the four different hydrodynamic CCC machines we tested with the heptane–water–methanol system, but not for the hydrostatic machine. The fluid

Table 2
Chromatographic data obtained with the SFCC 2000 CCC machine

| Flow-rate (ml/min) | Rotor speed (rpm) | V_s (ml) | Sf | Sf calculated (Eq. (11)) | u^a (cm/s) | Toluene | | Hexylbenzene | | R_s^b |
|-----------------------|----------------------|---------------|------|-----------------------------|-----------------|---------------|----------------|---------------|----------------|---------|
| | | | | | | V_r (ml) | N (plate) | V_r (ml) | N (plate) | |
| 1 | 500 | 125 | 0.80 | 0.79 | 3.71 | 72.3 | 310 | 39.0 | 540 | 2.9 |
| 2 | 500 | 97 | 0.62 | 0.66 | 4.55 | 91.0 | 410 | 65.2 | 1450 | 2.1 |
| 3 | 500 | 67.5 | 0.43 | 0.56 | 5.26 | 110.8 | 1060 | 92.8 | 3000 | 1.8 |
| 1 | 600 | 133 | 0.85 | 0.84 | 4.87 | 66.9 | 270 | 31.5 | 280 | 3.0 |
| 2 | 600 | 111.3 | 0.71 | 0.75 | 6.42 | 81.4 | 250 | 51.8 | 850 | 2.1 |
| 3 | 600 | 102 | 0.65 | 0.69 | 7.63 | 87.7 | 470 | 60.5 | 1030 | 2.3 |
| 4 | 600 | 77 | 0.49 | 0.63 | 8.66 | 104.4 | 840 | 83.9 | 1950 | 1.9 |
| 1 | 700 | 134.8 | 0.86 | 0.87 | 6.00 | 65.7 | 300 | 29.8 | 600 | 3.6 |
| 2 | 700 | 123 | 0.79 | 0.80 | 8.11 | 73.6 | 400 | 40.9 | 680 | 3.1 |
| 3 | 700 | 114 | 0.73 | 0.76 | 9.75 | 79.6 | 390 | 49.3 | 680 | 2.6 |
| 4 | 700 | 104 | 0.67 | 0.72 | 11.14 | 86.3 | 580 | 58.7 | 1200 | 2.6 |
| 1 | 800 | 137.3 | 0.88 | 0.88 | 7.09 | 64.0 | 240 | 27.5 | 300 | 3.2 |
| 2 | 800 | 128 | 0.82 | 0.84 | 9.76 | 70.2 | 270 | 36.2 | 370 | 2.8 |
| 3 | 800 | 124 | 0.79 | 0.80 | 11.82 | 72.9 | 270 | 39.9 | 480 | 2.6 |
| 4 | 800 | 110.5 | 0.71 | 0.76 | 13.56 | 82.0 | 420 | 52.6 | 710 | 2.5 |

^a u = Mobile phase linear velocity (cm/s).

^b R_s = Toluene/hexylbenzene resolution factor.

behavior in the hydrodynamic and hydrostatic machines is very different [1,2,5,6]. It is not surprising to obtain the results of Table 3. In the following, the Sanki hydrostatic machine will be submitted to the same test as the four hydrodynamic machines, but the differences in the results obtained will not be further commented on: they are due to the differing flowing mechanisms between the liquid phases and the very different centrifugal fields in the two types of CCC machines.

3.2.2. The “flush away” flow-rate

Eq. (6) quantifies an observation noted earlier by the CCC chromatographers: the phase retention decreases when the flow-rate is high. However, the Sf decrease is not linear with the mobile phase flow-rate as found in one case [9]. Eq. (6) shows that there is a maximum flow-rate, $F_{Sf=0}$, for which no stationary phase is left in the machine, it is all flushed away:

$$F_{Sf=0} = (A/B)^2 \quad (7)$$

Table 3 lists the “flush away” flow-rates, $F_{Sf=0}$, and clearly shows the theoretical character of this

value. Flow-rates as high as 940 ml/min in a 94-ml machine or 370 ml/min in a 29-ml machine would produce 0.1 min and 0.08 min dead-times, respectively. It is doubtful that any liquid–liquid equilibrium could take place under such conditions. Practically, we were not able to obtain a stable liquid equilibrium at 3 ml/min and 400 rpm with the SFCC 2000 machine when its “flush away” $F_{Sf=0}$ value is 4.8 ml/min (Table 3). The mobile phase was continuously displacing some stationary phase without reaching an equilibrium as described in a previous work [10]. We did not test flow-rates higher than 5 ml/min because a pressure higher than 5 bar or 70 p.s.i. could disrupt the PTFE tubes of our hydrodynamic machines (1 p.s.i. = 6894.76 Pa). Du et al. used a 44 ml/min flow-rate, producing a 0.30 Sf value at 900 rpm with the chloroform–water system [8]. The corresponding “flush away” $F_{Sf=0}$ value was 120 ml/min, a three-times higher flow-rate.

The $F_{Sf=0}$ parameter can be considered as indicative of the strength of the mobile–stationary phase equilibrium obtained in a given machine, with a particular liquid system and centrifugal field. A high $F_{Sf=0}$ value, more than 20-times higher than the operating flow-rate, tells that the stationary phase

Table 3
Regression parameters of the $Sf = A - BF^{1/2}$ lines

| Apparatus | Rotor speed (ω , rpm) | A | B | r^2 | $F_{Sf=0}^a$ (ml) | ω_{mini} for $F_{Sf=0}=0^c$ (rpm) |
|---------------------------------|----------------------------------|------|-------|-------------------|----------------------|--|
| SFCC 2000 | 400 | 1.22 | 0.56 | 0.990 | 4.8 | 430±20 |
| | 500 | 1.11 | 0.32 | 0.991 | 12 | |
| | 600 | 1.04 | 0.20 | 0.993 | 27 | |
| | 700 | 1.02 | 0.15 | 0.993 | 46 | |
| | 800 | 1.01 | 0.12 | 0.994 | 71 | |
| Kromaton 2 | 300 | 0.90 | 0.080 | 0.983 | 126 | 150±50 |
| | 400 | 0.85 | 0.035 | 0.991 | 590 | |
| | 500 | 0.86 | 0.028 | 0.991 | 940 | |
| Prototype 1 | 300 | 0.93 | 0.082 | 0.862 | 130 | 220±60 |
| | 400 | 0.94 | 0.070 | 0.956 | 180 | |
| | 500 | 0.93 | 0.048 | 0.941 | 370 | |
| Prototype 2 | 300 | 0.99 | 0.25 | 0.998 | 16 | 220±60 |
| | 400 | 0.95 | 0.14 | 0.991 | 46 | |
| | 500 | 0.96 | 0.11 | 0.990 | 76 | |
| Sanki HPCPC 1000 hydrostatic | 400 | 0.87 | 0.15 | n.s. ^b | 33 | Not applicable |
| | 500 | 0.94 | 0.20 | 0.983 | 22 | |
| | 600 | 0.99 | 0.24 | 0.786 | 17 | |
| | 700 | 0.86 | 0.11 | 0.856 | 61 | |
| | 800 | 0.83 | 0.065 | 0.833 | 160 | |

^a $F_{Sf=0} = (A/B)^2$, the “flush away” flow-rate, see text.

^b n.s.: Non significant, only two flow-rates were done at this rotation speed giving a non significant 1.00 r^2 coefficient.

^c See text and Fig. 1.

retention will be high and stable (easy injection). A $F_{Sf=0}$ flow-rate less than 10-times higher than the working flow-rate means that the stationary phase is not tightly held, or the Sf value will be mediocre, or stationary phase loss will be observed when injecting the sample.

3.3. Stationary phase retention and centrifugal field

3.3.1. A minimum rotation speed ω_{mini}

The observation of the Table 3 coefficients shows that there is a strong correlation between the rotor rotation speed and the “flush away” flow-rate, the A and B parameters. Fig. 1 shows the plot of the “flush away” flow-rate versus the square of the rotation speed. For the SFCC 2000 machine, the “flush away” flow-rate is linearly proportional to the square of the rotor rotation speed for the higher rpm values. This point is difficult to confirm with the other three

machines that do not spin faster than 500 rpm. The linear relationship between the maximum flow-rate and ω^2 makes sense since the strength of the centrifugal field is proportional to ω^2 . Although it is

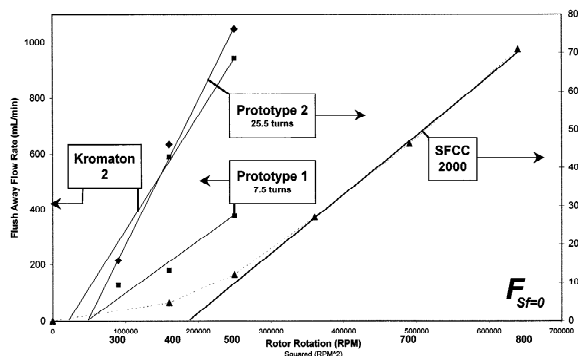


Fig. 1. The “flush away” flow-rate, $F_{Sf=0} = (A/B)^2$ versus ω^2 , the rotor rotation speed, curves. The arrows point toward the relevant ordinate.

not possible to firmly establish this relationship for the other three machines, the lines connecting the “flush away” flow-rate for the higher rotation speeds were drawn in Fig. 1. These lines cut the ordinate at a rotation speed that can be defined as the lowest rotation speed (=minimum centrifugal field) necessary to obtain a stable equilibrium between the mobile and stationary liquid phases. These ω_{mini} values are listed in Table 3. They should be related to the Ito β ratio (average radius of the tubing in the spools over the distance between the spool axis and the rotor axis) since a high β ratio is related to a high centrifugal field at the same rotation speed [1,2]. Indeed, the ω_{mini} value of the SFCC 2000 machine is the highest, 430 rpm, when this machine has the lowest β value, 0.56. The β and ω_{mini} values of the other three machines are close and the inaccuracy of their ω_{mini} values is too high to make any conclusions.

3.3.2. Linear mobile phase velocity and rotation speed

Eq. (4) shows that the linear speed of the mobile phase inside the tubing, u , is related to the flow-rate and the Sf phase retention factor. Since Sf and ω are interdependent, the possible relationship between u and ω should be studied. The mobile phase velocity, u , was calculated using Eq. (4) for every experiment (Table 1). Then, the u values were plotted versus ω at constant flow-rate. Curves were obtained. A second-order relationship between u and ω is evaluated. It is actually the second-order relationship between ω and u that turned out to be the most appropriate. The plots of u versus the square root of ω , at a constant flow-rate, were linear. Table 4 lists the C and D coefficients and regression coefficients of the u versus $\omega^{1/2}$ lines:

$$u = C\omega^{1/2} + D \quad (8)$$

Table 4
Regression parameters of the $u = C\omega^{1/2} + D$ lines

| Apparatus | Flow-rate (ml/min) | C | D | r^2 | Relationship of C and D with F^a | u_{mini} for Sf=0 ^b (cm/s) | ω_{mini} for Sf=0 ^c (rpm) |
|------------------------------|--------------------|--------|--------|-------|--|--|--|
| SFCC 2000 | 1 | 0.571 | -9.1 | 0.999 | $C = 0.724F^{1/2} - 0.16$ | 0.78 | 300 |
| | 2 | 0.850 | -14.4 | 0.999 | $r^2 = 0.999$ | 1.56 | 340 |
| | 3 | 1.10 | -19.4 | 0.999 | $D = -14F^{1/2} + 5.1$ | 2.34 | 390 |
| | 4 | 1.29 | -23.0 | 0.999 | $r^2 = 0.998$ | 3.12 | 410 |
| Kromaton 2 | 1 | 0.059 | 3.22 | 0.656 | $C = 0.714F^{1/2} - 0.69$ | 0.78 | - |
| | 2 | 0.269 | 2.58 | 0.964 | $r^2 = 0.979$ | 1.56 | - |
| | 3 | 0.516 | 0.841 | 0.999 | $D = -4.6F^{1/2} + 8.35$ | 2.34 | 8 |
| | 4 | 0.778 | -1.44 | 0.999 | $r^2 = 0.894$ | 3.12 | 34 |
| Prototype 1 | 1 | 0.089 | 0.019 | 0.996 | $C = 0.238F^{1/2} - 0.15$ | 0.314 | 11 |
| | 2 | 0.175 | -0.510 | 0.997 | $r^2 = 0.996$ | 0.63 | 42 |
| | 3 | 0.254 | -1.09 | 0.989 | $D = -1.7F^{1/2} + 1.8$ | 0.94 | 64 |
| | 4 | 0.326 | -1.68 | 0.982 | $r^2 = 0.984$ | 1.26 | 81 |
| Prototype 2 | 1 | 0.436 | -4.57 | 0.999 | $C = 0.733F^{1/2} - 0.30$ | 0.78 | 150 |
| | 2 | 0.731 | -8.36 | 0.999 | $r^2 = 0.999$ | 1.56 | 184 |
| | 3 | 0.966 | -11.4 | 0.999 | $D = -9.5F^{1/2} + 5.0$ | 2.34 | 202 |
| | 4 | 1.17 | -14.1 | 0.998 | $r^2 = 0.999$ | 3.12 | 217 |
| Sanki HPCPC 1000 hydrostatic | 1 | 0.0216 | -0.624 | 0.884 | $C = 0.351F^{1/2} - 0.38$ | 0.29 | 1800 |
| | 2 | 0.0618 | -0.355 | 0.730 | $r^2 = 0.814$ | 0.58 | 230 |
| | 3 | 0.170 | 1.82 | 0.834 | $D = 7.28F^{1/2} - 9.2$ | 0.87 | - |
| | 4 | 0.391 | 7.15 | 0.995 | $r^2 = 0.756$ | 1.16 | - |

^a In the form: $C = EF^{1/2} + G$ and $D = HF^{1/2} + I$, the constants E , G , H and I are used in Eqs. (10) and (11).

^b See text and Fig. 1.

^c ω value for which Eq. (8) gives u_{mini} , compare with Table 3 corresponding value.

With the notable exception of the Kromaton 2 machine at 1 ml/min, the regression coefficients of the u versus $\omega^{1/2}$ lines for the four hydrodynamic machines were higher than 0.98. For the SFCC 2000 and Prototype 2, the r^2 values were higher than 0.999. The Eq. (8) relationship means that the average kinetic energy of an element of liquid mobile phase, $1/2mu^2$, is linearly proportional to ω , the rotor rotation speed. Physically the mobile phase linear velocity cannot be lower than a minimum value, u_{mini} , that is the linear speed in the open tube without any stationary phase in it ($Sf=0$). The u_{mini} value depends on the mobile phase flow-rate and the tube diameter (Eq. (4) with $Sf=0$). If there is a minimum value of u , then there is also a minimum value of ω (Eq. (8)). The u_{mini} values are listed in Table 4 along with the corresponding theoretical minimum rotation, ω_{mini} , speeds calculated using:

$$\omega_{\text{mini}} = [(u_{\text{mini}} - D)/C]^2 \quad (9)$$

Comparing the SFCC 2000 and the Prototype 2 machines for which the regression coefficients of the u versus $\omega^{1/2}$ were the best, the ω_{mini} values obtained correspond closely to the ω_{mini} values obtained studying the flow-rates (Eqs. (6) and (7) and Fig. 1). For the two other machines with poorer regression coefficients, the concordance of the two ω_{mini} values is lower.

3.3.3. Looping the loop

Table 4 shows that the C and D parameters of Eq. (8) depend on the flow-rate at which they were obtained. A linear relationship between both values and the square root of the flow-rate was established. The equations and regression coefficients are listed in a column of Table 4. This last finding allows one to loop the loop. The velocity of the mobile phase inside the tubing of a hydrodynamic coil planet centrifuge machine can be expressed as a linear combination of the two active parameters, the flow-rate, F , and the rotor rotation speed, ω . Combining Eq. (8) and the regression equations of Table 4, with E and G , the regression constants for C , and H and I , the regression constants for D , it becomes:

$$u = EF^{1/2}\omega^{1/2} + G\omega^{1/2} + HF^{1/2} + I \quad (10)$$

Eq. (4) shows that u and Sf are related. Combining Eqs. (4) and (10), it becomes:

$$Sf = 1 - \frac{4\sqrt{F}}{\pi d^2 \left(E\sqrt{\omega} + H + G\sqrt{\frac{\omega}{F}} + \frac{I}{\sqrt{F}} \right)} \quad (11)$$

Eq. (11) should be compared to Eq. (6) and Table 3. The experimental A parameters listed in Table 3 are relatively close to unity. The theoretical B value is a complex function of $F^{1/2}$ and $\omega^{1/2}$ that contains the decreases of B with the increases of ω observed in Table 3. However, B may not be constant for a large range of flow-rate values. The negative character of the H and G values may largely compensate for the positive character of the E and I values (see Table 4). Fig. 2 shows the theoretical three-dimensional diagram of the function $Sf=f(F, \omega)$ and Table 2 lists the theoretical Sf values calculated for the SFCC 2000 machine using Eq. (11). The agreement is very good for low flow-rates. A significant divergence between the experimental and calculated Sf values is observed at high flow-rate and low rotation speed. Fig. 2 gives a good idea of the stationary phase retention by the four hydrodynamic machines.

3.4. Liquid phase motion in a coil planet centrifuge

The pioneering works of Ito and co-workers on the liquid phase motion inside the tubing of a coil planet centrifuge showed that there was a succession of mixing and decantation zones when the rotation speed was high enough [1,11,12]. The mixing zones are located close to the rotor rotation central axis where the rotor rotation and the spool rotation have opposite directions. The decantation zones are located far from the central axis where the rotor and the spool rotation have identical directions and the centrifugal field is maximum [11]. The fact that one liquid phase remains stationary when the other flows through it, is not well explained or understood by the fluid mechanics. It was noted that the ω_{mini} minimum rotation speed depended on the apparatus geometry, but only trials could locate this minimum rotation speed [1,11]. The results described here clearly confirm that there is a minimum rotation speed, ω_{mini} , necessary to obtain the mixing–demixing

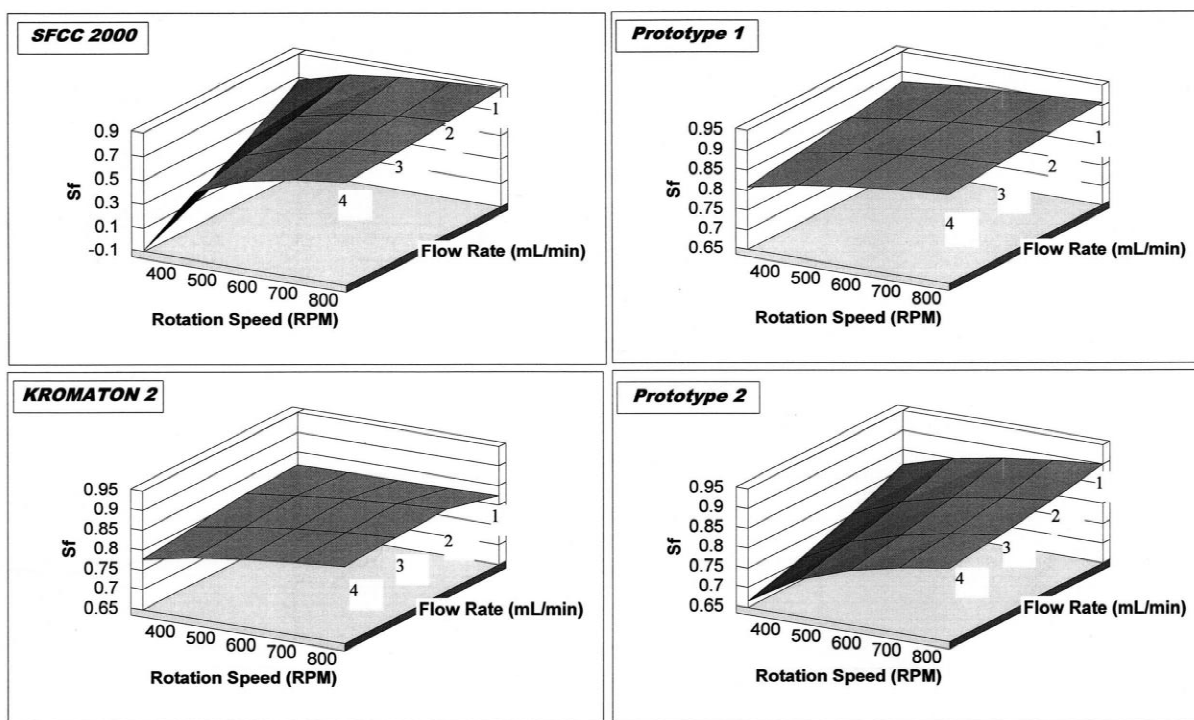


Fig. 2. Three-dimensional diagrams of the stationary phase retention parameter, S_f , versus the rotor rotation speed (rpm) and the mobile phase flow-rate (ml/min).

equilibrium. For the first time, a simple set of measurements allows to give a precise value for the ω_{mini} parameter. This minimum rotation speed is dependent on the geometrical characteristics of the machine including the rotor radius, the spool radius (i.e., the Ito β ratio), the tubing length and internal diameter. It is also likely depending on the biphasic liquid system used although this point cannot be demonstrated in this work. It may be necessary to do more fundamental studies similar to this one with different machines and different liquid systems to have a better understanding of the behavior of the two liquid phases inside the open tube of the CCC hydrodynamic coil planet centrifuge machine.

3.5. Chromatographic efficiency and resolution

It is important to retain a maximum volume of stationary phase inside the CCC column because it increases the chromatographic resolution. Indeed, Eq. (5) can be written as:

$$R_s = \frac{\sqrt{N}}{4} \cdot \left[\frac{P_2 - P_1}{\frac{V_M}{V_S} + \left(\frac{P_1 + P_2}{2} \right)} \right] \\ = \frac{\sqrt{N}}{4} \cdot \left(\frac{P_2 - P_1}{\frac{1 - S_f}{S_f} + \frac{P_1 + P_2}{2}} \right) \quad (12)$$

in which P_1 and P_2 are the partition coefficients of solutes 1 and 2, respectively. Eq. (12) shows that a small S_f value will produce a small R_s value.

3.5.1. Criteria to compare machine efficiency

The other parameter acting on the resolution factor is the peak efficiency, N . Table 2 lists the efficiencies obtained for toluene and hexylbenzene with the SFCC 2000 CCC machine. It is usual in HPLC to plot the peak efficiency, N , versus the mobile phase linear velocity, u . Fig. 3 shows such plots for the SFCC 2000 machine (Fig. 3, top and data in Table 2) and the Kromaton 2 machine (bottom). These two

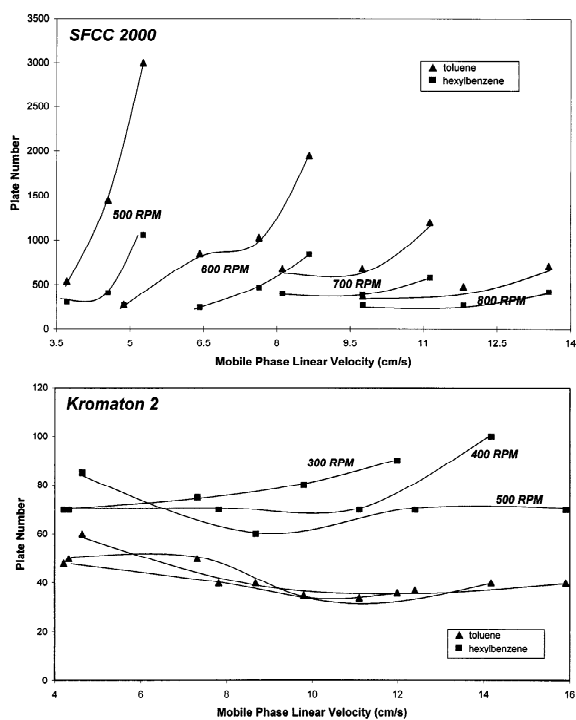


Fig. 3. The peak efficiency versus the mobile phase linear velocity. Triangles: toluene; squares: hexylbenzene.

machines show two different behaviors. The peak efficiency obtained with the SFCC 2000 machine (low β , high number of turns and small spools, Table 1) shows two opposite trends: (i) it increases when the mobile phase velocity increases due to increases in flow-rate at a constant rotation speed and (ii) it

decreases at a constant flow-rate when the mobile phase velocity increases due to a Sf increase (=the rotation speed increases). The peak efficiencies are in the 250–1000 plate range for toluene and 500–3000 plate range for hexylbenzene, the less retained peak. The three other machines with a high β value, a low number of turns and large spools, have a different $N=f(u)$ pattern. The peak efficiency is almost insensitive to the mobile phase flow-rate. For the Kromaton 2 machine and linear velocities within 4 and 16 cm/s, the toluene peak efficiency was always within 35 and 60 plates and the hexylbenzene efficiency was within 60 and 100 plates (Fig. 3, bottom). The toluene peak efficiencies were within 9 and 18 plates and 40 and 100 plates for the Prototype 1 and 2 machines, respectively. The hexylbenzene efficiencies were more than three-times higher, in the 40–100 plate and 120–250 plate range for the Prototype 1 and 2 machines, respectively. The average efficiency values are listed in Table 5.

The plate count obtained for a compound depends on its partition coefficient and also on the number of tubing turns in the CCC column. Table 5 lists the average number of plates per tubing turn for the hydrodynamic machines and the number of channels necessary to generate one plate for the hydrostatic machine. For the more retained hexylbenzene solute, the average number of plates per tubing turn is well above unity. For example, it is possible to generate 10 theoretical plates in one tubing turn of the Prototype 2 machine with hexylbenzene. This clearly shows the efficacy of the hydrodynamic Ito machines

Table 5
Evaluation of the machines efficiency

| Apparatus | Average efficiency ^a (plates) | | Number of plates per tubing turn ^b (plates/turn) | | Volume for one plate ^c (ml/plate) | | Tubing length for one (cm/plate) | |
|---------------------------------|---|--------------|--|------------------------|---|--------------|-------------------------------------|------------------|
| | Toluene | Hexylbenzene | Toluene | Hexylbenzene | Toluene | Hexylbenzene | Toluene | Hexylbenzene |
| SFCC 2000 | 460 | 1020 | 1.2 | 2.6 | 0.34 | 0.15 | 16 | 7.2 |
| Kromaton 2 | 43 | 76 | 1.5 | 2.7 | 2.2 | 1.2 | 41 | 23 |
| Prototype 1 | 13 | 66 | 1.7 | 8.8 | 2.2 | 0.44 | 32 | 6.2 |
| Prototype 2 | 57 | 180 | 3.1 | 9.7 | 0.45 | 0.14 | 21 | 6.7 |
| Sanki HPCPC 1000 hydrostatic | 100 | 180 | 0.09 plates/channel | 0.17 plates/channel | 1.0 | 0.56 | 15 ^d | 8.3 ^d |

^a See Table 2 for the full set of data for the SFCC 2000 machine.

^b See Table 1 for the number of tubing turns in each hydrodynamic machine and the hydrostatic machine contains 1060 channels.

^c See Table 1 for the machine volumes.

^d Assuming the Sanki machine contains an equivalent tubing length of 15 m, 2.7 mm I.D.

in contacting the mobile and stationary phase. For comparison, with the same solutes and liquid system, 11 and six channels of the Sanki hydrodynamic machine were necessary to generate one theoretical plate for toluene and hexylbenzene, respectively. However, if we consider the machine volumes and calculate the average volume necessary to generate a theoretical plate, the hydrostatic machine is competitive. The small spools of the SFCC machine generate a high number of plates with a reduced machine volume. The last point considered is the tubing length used to generate a plate. The Kromaton 2 machine needs a longer length of tubing to generate a theoretical plate. Possibly this is related to the large bore tube (2.6 mm I.D.) used in this machine.

3.5.2. Resolution factor

Practically, resolution is the important factor. When the resolution factor of two adjacent peaks is higher the 1.5, they are fully separated with a

baseline return between them. Fig. 4 shows the toluene–hexylbenzene resolution factors, R_s , obtained with the four hydrodynamic machines. Eq. (12) shows that the R_s factor depends on the solute partition coefficients, on the peak efficiencies and on the Sf phase retention factor. The solute partition coefficients are the same for the whole set of experiments so resolution differences are due to the two other parameters. Two different behaviors are observed. For the SFCC 2000 and the Prototype 2 machines the centrifugal field is critical. For a given rotor rotation, a rapid decrease in the resolution factor occurs when the mobile phase velocity (i.e., the flow-rate) increases. At a constant flow-rate, the resolution factor increases with the rotation speed. For the two other machines, the trend is roughly the same but the changes are not sharp and there is some overlap of the curves (Fig. 4).

Comparing Figs. 3 and 4, it is clear that the resolution factor is more sensitive to the Sf phase

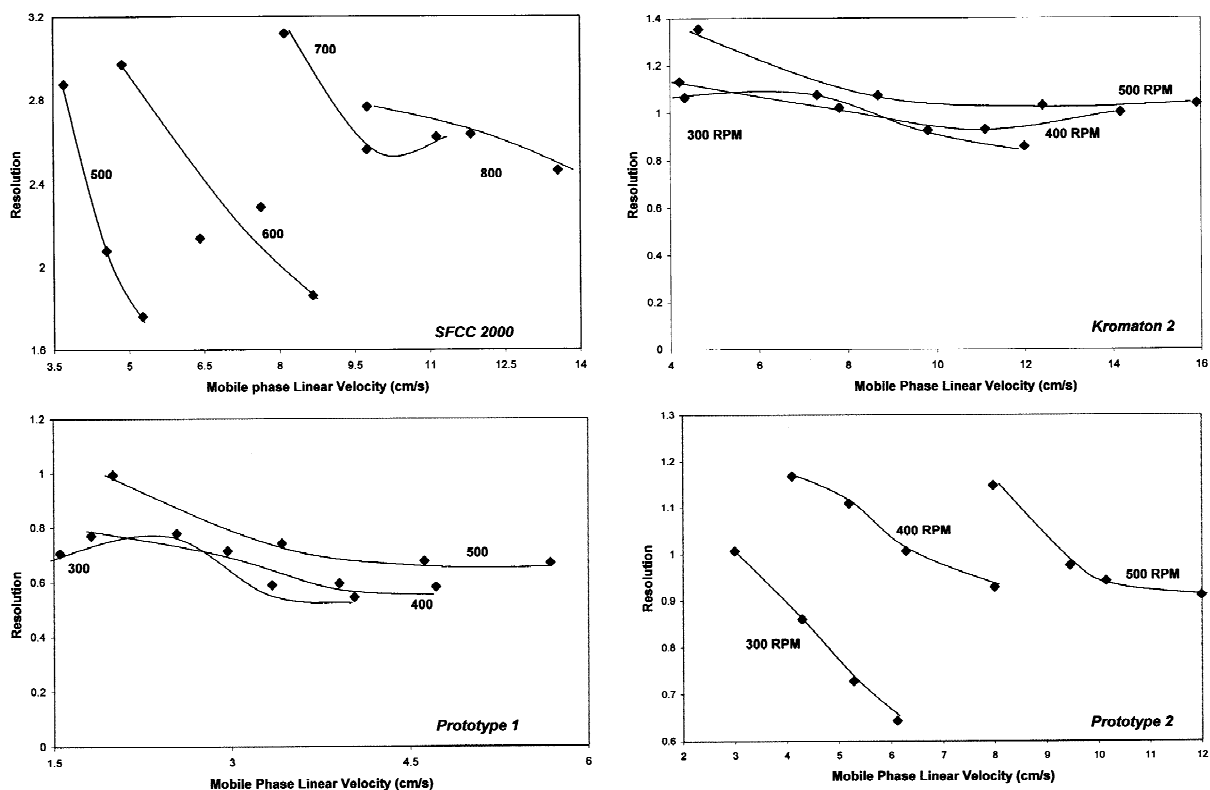


Fig. 4. The resolution factor, R_s , versus the mobile phase linear velocity for the four hydrodynamic machines.

retention factor than to the peak efficiency. Indeed, for the SFCC 2000 machine, Fig. 3 shows that the toluene efficiency doubles and the hexylbenzene efficiency is multiplied by a factor 6 at 500 rpm. The corresponding resolution variation, for the same changes in mobile phase velocity and the same 500 rpm rotation speed, is a 40% decrease from 2.9 to 1.8 due to the 50% decrease of the *Sf* ratio (Table 2). Eq. (12) shows that efficiency intervenes with its square root when *Sf* intervenes directly in the resolution factor. Then, the number of theoretical plates alone is not a reliable parameter to measure the resolution capability of a CCC machine because the stationary phase volume varies. Fig. 2 shows that the *Sf* ratio block diagrams of the SFCC 2000 and the Prototype 2 machines are similar. The *B* terms of Eq. (6) and the *C* terms of Eq. (8) are similar for the two machines and three- to nine-times higher than the corresponding terms of the two other machines (Tables 3 and 4).

3.6. Winding tubing to make a CCC hydrodynamic machine

The first parameter that is considered when making a new CCC machine is the column volume, V_T . This volume depends on the purpose of the column: a small volume will permit fast analyses, a large volume will allow preparative injections. The second parameter is the internal diameter, d , of the tubing that will be used. This diameter d gives the length of the tubing:

$$L = 4V_T / \pi d^2 \quad (13)$$

Next, the design of the machine should be considered: rotor radius, R , and spool radius, r . There are obviously geometrical constraints. It will not be possible to build a very small machine if a large volume is desired. These two radii were related by Ito who defined the β ratio (r/R) and recommended β higher than 0.6 [1,11]. An efficient machine should have a high number of tubing turns, n . The combination of these requirements leads to:

$$\beta n = 2V_T / (\pi^2 d^2 R) \approx 0.2V_T / d^2 R \quad (14)$$

Eq. (14) shows that the tubing internal diameter is

an essential parameter in the design of a CCC column. A reduced tube I.D. will produce a long length and numerous turns. However, the two machines containing a large bore tubing (Kromaton 2 and Prototype 1) showed a correct retention of the stationary phase less sensitive to the operating parameters, flow-rate and rotor rotation, than the machine made with smaller bore tubing (Fig. 2). Although we have no practical experiment to support this affirmation, it may not be wise to wind a large length of 0.5 mm 1/16 in. PTFE tubing to make a CCC column (1 in. = 2.54 cm). The number of turns will be high but the liquids may not have enough space to equilibrate properly. Furthermore surface effects may occur. If the 1.6 mm I.D. 1/8 in. PTFE tubing is a good compromise, the number of turns should be maximized reducing the rotor radius, R , as low as geometrical and steric considerations will permit.

The rotor and the spools should be well balanced because the *B* (Eq. (6)) and *C* (Eq. (8)) coefficients of this machine should be high which means that the minimum rotation speed, ω_{mini} , will be significant. To retain a large amount of stationary phase, the low diameter spools should be rotated at a high speed. If a strong centrifugal field can be generated, that is, the rotor assembly is well balanced, then it will be possible to work with elevated mobile phase flow-rates, conserving a good stationary phase retention factor (Fig. 2). This should produce useful resolution factors (Fig. 4) in a minimum time.

To conclude, we hope to have demonstrated the usefulness of the proposed test. We have shown how the measurement of the methanol–water (90:10, v/v) phase *Sf* retention factor, followed by the injection of an alkylbenzene mixture in the heptane tail-to-head mobile phase could give a wealth of information on the CCC machines tested. We suggest that a new CCC machine be submitted to the simple experimental test proposed here as a standard evaluation procedure.

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