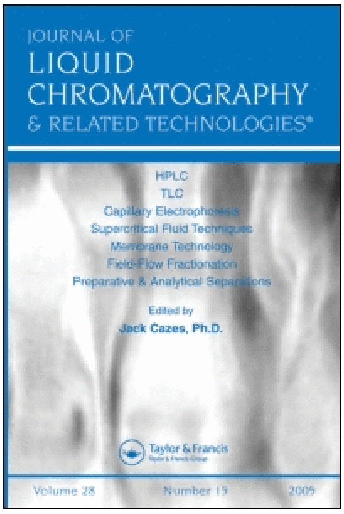

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COUNTERCURRENT CHROMATOGRAPHY WITH A NEW CENTRIFUGAL PARTITION CHROMATOGRAPHIC SYSTEM

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ABSTRACT

Efficiency and resolution have been studied with a new centrifugal partition chromatograph, the series 1000 HPCPC™ system, classified by Y. Ito[1] as a HSES non-planetary with rotary seals. It has been shown that, in the range 1-10 ml/min, the efficiency is not directly dependent upon the flow rate, but increases markedly with the volume ratio of the mobile phase in the column. For each flow rate there is a minimum value for the volume of the mobile phase, and the relationship is linear; working within these conditions, changing the flow rate and the volume of the mobile phase leads to an efficiency which increases with the flow rate from 1 to 20 ml/min. This increase in efficiency, which goes through a decrease of the ratio of the stationary phase in the column, results in resolution for two peaks which is roughly constant over a broad range of flow rates.

INTRODUCTION

In the course of our studies of efficiency and resolution in countercurrent chromatography (CCC)[2], we tested a new centrifugal partition chromatograph, called the Series 1000 HPCPC (HPCPC thorough this paper), which is a bench top chromatograph with a stacked circular disk-type column engraved with 2136 channels for a total volume of 240 ml (Figure 1). We operated this instrument with conditions similar to those used with its parent, the CPC model LLN[3], *i.e.*, a rotational speed of 1300 or 1400 rpm, a working pressure of about 6 MPa (≈ 900 psi), and we studied the efficiency of the instrument with the same simple biphasic system Hexane, 4% Water in Methanol[4] and the same test samples we already used to investigate the Ito multilayer coil separator extractor.

By independently varying the flow rate and the ratio of the mobile phase in the column, we have shown that the efficiency is mainly related to the mobile phase ratio, and not to the flow rate; efficiency increases when the ratio of the mobile phase increases. Since, for two close peaks, the resolution varies as $\sqrt{N}$ and $\left(\frac{V_m}{V_s}\right)^{-1}$, within a broad range for the flow rate and for the ratio of the mobile phase in the HPCPC, the resolution remains roughly constant.

EXPERIMENTAL

According to the nomenclature of Y. Ito[1] the new centrifugal partition chromatograph, called the Series 1000 HPCPC , Figure 1 (Sanki Laboratories, Mount Laurel, NJ, USA), is a HSES non-planetary with rotary seals. It is a bench top CPC (30 x 45 x 45 cm, ≈ 60 Kg), and the column is a stacked circular disk rotor which contains 2136 channels for a total of 240 ml. The column is connected to the injector and the detector through two rotary seals made of a all drilled sapphire rod going through two toroidal seals similar to those used with HPLC pump pistons. The partition disks are engraved with 1.5 x 0.28 x 0.21 cm channels connected in series by 1.5 x 0.1 x 0.1 cm ducts. A 4 port valve included in the serie 1000 allows the HPCPC to be operated in either the descending or ascending mode. A former CPC, the model LLN[3] was also used; the column in the LLN consists of 6 rectangular cartridges of 400 channels each. The channels are 1.24 x 0.24 x 0.11 cm, linked with 1.24 x 0.09 x 0.11 cm ducts. In order to run the two instruments with the same average centrifugal field ($\bar{G} = \omega^2 \bar{R}$), the HPCPC was rotated at 1400 rpm, and the CPC model LLN at 1100 rpm, giving a centrifugal field of ≈ 180 **G**; an additional set of data has been obtained for the HPCPC rotating at about 1300 rpm. For all the experiments the pressure drop was about 6 MPa (≈ 900 psi).

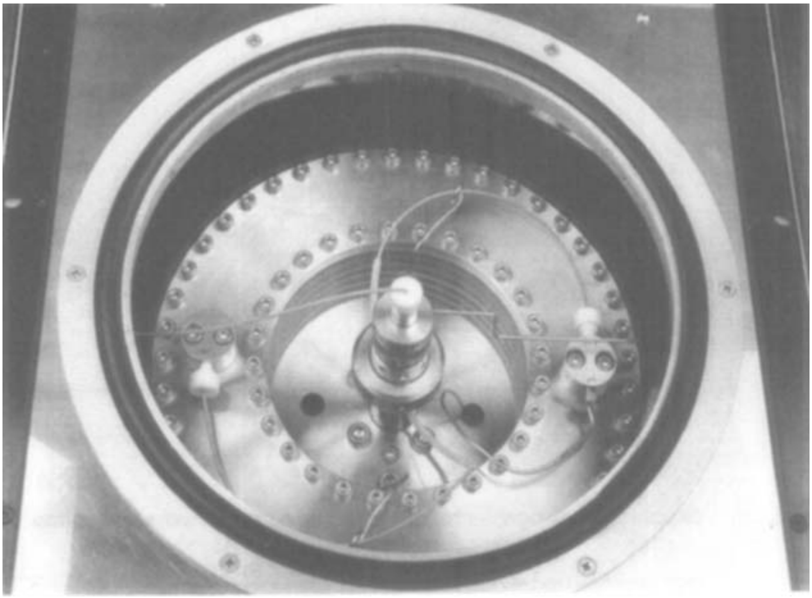

Figure 1 : ***Photograph of the Series 1000 HPCPC system, showing the upper rotary seal and the top of the stacked partition disk column. The overall dimensions are ≈ 30 x 45 x 45 cm and the weight is around 60 Kg.***

n-Hexane and Methanol were HPLC or ACS grade (Aldrich Chem. Co., Milwaukee, WI). The simple system n-Hexane, 4% water in Methanol, was used with the lower phase as the mobile phase, in the descending mode.

4-Hydroxybenzoic acid (non-retained compound), Diethyl phthalate (peak 1), Dipropyl phthalate (peak 2), Ethyl phenylacetate (peak 3), and Butyl phenylacetate (peak 4), were the same as in reference 2, where their partition coefficients, and the way to calculate the efficiency for a given peak, the resolution between two peaks, and the definition of V_m^{min}, the minimum volume of mobile phase which can be obtained for a given flow rate, will be found.

RESULTS AND DISCUSSION

Figure 2 is a graph of all the plots F *vs* Vm/Vc we studied with the HPCPC and the CPC model LLN, Vc being the total volume of the column, Vm the volume of the

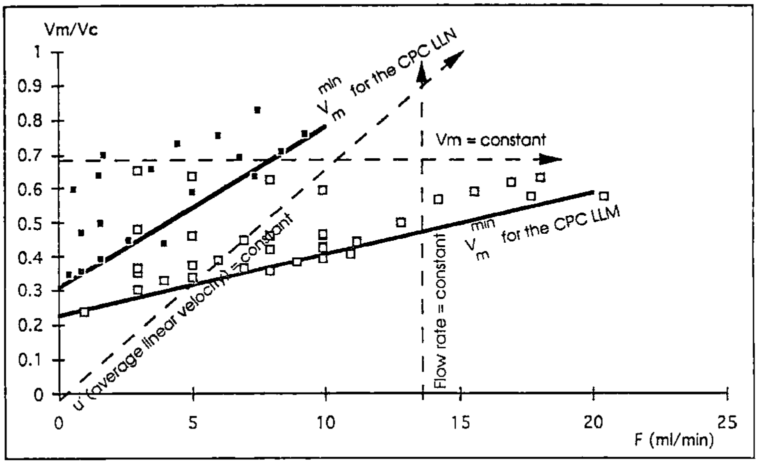

Figure 2 : Graph of the experimental points, with the various ways to vary the parameters, and the experimental points $\frac{V_m^{min}}{V_c} = aF + b$, for the HPCPC and the CPC model LLN.

□ : experiments with the HPCPC

■ : experiments with the CPC model LLN

u' is the average velocity in the column (channels + ducts), calculated by $u' = \frac{FL}{V_m}$, L being the length of the column. It is an average between the linear velocity in the ducts, imposed by F, and the linear velocity in the channels, imposed by the centrifugal field and the properties of the biphasic system.

mobile phase in the column, and F the flow rate. For the two devices, we found that there is a linear relationship between the flow rate and the minimum volume of mobile phase we can obtain for the actual flow rate, V_m^{min}, as we already observed for the Ito CCC column[2]. Regression analysis for these points led to the following equations :

CPC model LLN : $$\frac{V_m^{min}}{V_c} = 0.047\ (\pm 0.003)\ F + 0.31\ (\pm 0.017)$$

$n = 9$ $R^2 = 0.968$ $s = 0.029$ $\mathcal{F} = 239$

HPCPC: $$\frac{V_m^{min}}{V_c} = 0.018\,(\pm 0.001)\,F + 0.228\,(\pm 0.012)$$

$$n = 8 \qquad R^2 = 0.976 \qquad s = 0.019 \qquad \mathcal{F} = 286$$

where n is the number of observations, R the correlation coefficient, s the standard deviation, and $\mathcal{F}$ the Fisher's test parameter.

The intercept is the extrapolation of $\frac{V_m^{min}}{V_c}$ at F = 0 ml/min; for the two CPC this corresponds to the volume of the connecting ducts and capillaries between the partition channels and cartridges or disks (≈ 30% of the total volume for the CPC model LLN, and 23% for the HPCPC). The intercept should not be dependent upon the nature of the biphasic system nor upon the rotational speed.

The slope (in min cm^{-3}) is characteristic of the stability of the stationary phase for a given column, but, as we will show later, it does not allow comparison between columns since it does not take its cross section into account. The slope is a function of the rotational speed and of the properties of the biphasic system which is used (viscosity, density difference). As already stated[2], even if we can emphasize that the slope should increase with the viscosity of the stationary phase and decrease with the density difference and the rotational speed, further experiments have to be performed to clearly understand how the relationship between F and V_m^{min} is influenced by these parameters.

EFFICIENCY VERSUS V_m *AND* F:

We already define various ways to study the relationship between the efficiency, the flow rate, and the volume ratio of the mobile phase in the column :

_ Van Deemter plots (H = f(F)) can be drawn by variation of the flow rate, at constant Vm/Vc , as represented by a horizontal line in Figure 2 (H is the plate height).

_ Following a vertical line, we will get the variation of H at a given flow rate, while varying Vm/Vc .

_ Following a straight line going through the origin, we will get the variation of H at a given average velocity, u' of the mobile phase ($u' = \frac{F\,L}{V_m}$, L being the length of the column); experiments conducted at constant u' will have the same t_o , *i.e.*, retention time for a non-retained solute.

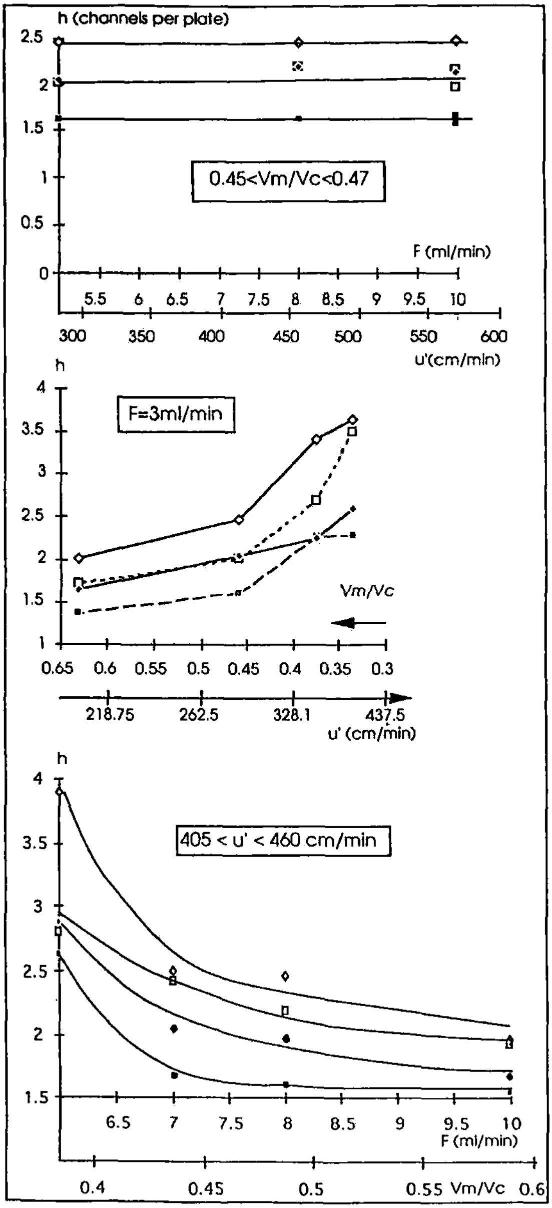
h (channels per plate)
2.5
2
1.5
1
0.5
0
0.45<Vm/Vc<0.47
F (ml/min)
5.5 6 6.5 7 7.5 8 8.5 9 9.5 10
300 350 400 450 500 550 600
u'(cm/min)
h
4
3.5
3
2.5
2
1.5
1
F=3ml/min
Vm/Vc
0.65 0.6 0.55 0.5 0.45 0.4 0.35 0.3
218.75 262.5 328.1 437.5
u' (cm/min)
h
4
3.5
3
2.5
2
1.5
405 < u' < 460 cm/min
6.5 7 7.5 8 8.5 9 9.5 10
F (ml/min)
0.4 0.45 0.5 0.55 Vm/Vc 0.6

Figure 3 shows these three types of variations of h, the reduced plate height or the number of channels per plate, for different values for F, Vm/Vc , and u', for the HPCPC (h = 2136/N, N being the number of theoretical plates).

It can be seen that, in the range studied, the efficiency does not vary with the flow rate, but increases sharply (*i.e.* h decreases) when the ratio of the mobile phase increases. The variation observed at constant average velocity is then mainly due to the dependence of h upon Vm/Vc . This result is very different of that observed with the Ito CCC[2], where the plate height increased with the flow rate at constant Vm. This may be explained by the fact that the velocity of the mobile phase within a channel should not depend upon the flow rate, but upon the centrifugal field and the properties of the biphasic system, which are constant in the present study; put another way, the exchange between the two phases should be favored by a better balance in the channel, *i.e.* around Vm/Vc = 0.5; if we assume that it is an emulsion layer in each channel, as described by D. Armstrong *et al.*[5], its depth and homogeneity should be Vm/Vs-dependant (Vs = volume of the stationary phase). We already noted, with the Ito CCC column, that the parameter Vm or Vm/Vc was more important than F for its effect upon efficiency[2].

Now we need to know how the efficiency varies when one continues to work with the minimum volume of mobile phase for a given flow rate, *i.e.* working at V_m^{min} conditions. Figure 4 gives the variations of h with F and V_m^{min} when we describe the straight line $F = f(V_m^{min})$ which appears on Figure 2.

It can be seen that the efficiency increases (h decreases) without exhibiting a maximum for F in the range 1-21 ml/min; the discontinuity observed for F < 10 ml/min and F > 10 ml/min is due to the 100 rpm variation of the rotational

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Figure 3 : Variation of the reduced plate height, h (number of channels per plate), for the HPCPC.
upper : the volume of the mobile phase is constant and the flow rate is varied.
center : the flow rate is constant and the volume of the mobile phase is varied.
lower : both the flow rate and the volume of the mobile phase are varied, so that the elution time for a non retained solute is kept constant (the average velocity of the mobile phase is constant).
■ : h_1 □ : h_2 ◆ : h_3 ◇ : h_4

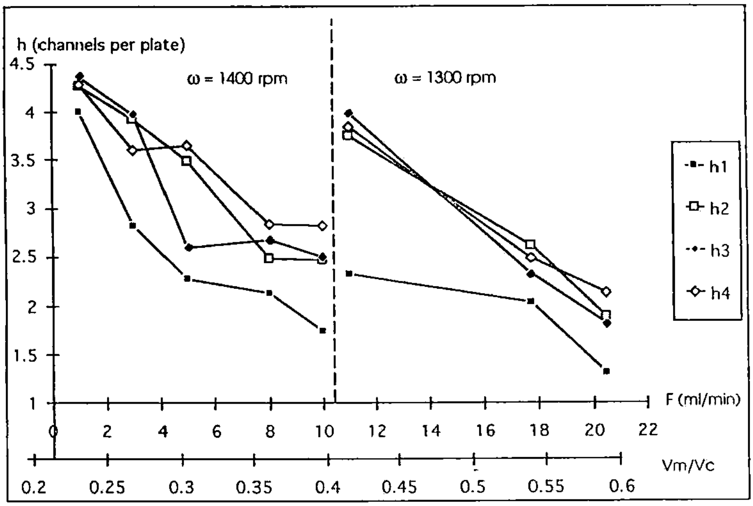

Figure 4 : Variation of the reduced plate height, h (number of channels per plate), for the HPCPC, with the flow rate and the volume of the mobile phase in the column, for the experimental conditions corresponding to the minimum volume of the mobile phase, V_m^{min}, compatible with the actual flow rate, F. V_m^{min} and F are dependent, with the relationship :

$$\frac{V_m^{min}}{Vc} = 0.018\,F + 0.228 \quad \text{(F in ml/min)}$$

speed ($\frac{\Delta G}{G} \approx 13\%$) for these two sets of experiments, and highlights the importance of operating at constant G upon the other parameters when studying CCC.

The absence of a maximum (or if any it should be for F < 1 ml/min) is different from that which we observe for the Ito CCC[2] and from the results obtained by D. Armstrong *et al.* [5] for the CPC model LLN (we did the same experiments with the CPC model LLN and observed the same results as Armstrong when working at V_m^{min} conditions, observing a maximum for h for a flow rate around 2 ml/min).

We concluded that, if we want to work at V_m^{min} conditions (and this is the way most people do), it is always more efficient to work at higher flow rate. It is very possible that a maximum for h occurs for flow rate down to 1 ml/min or less, and it

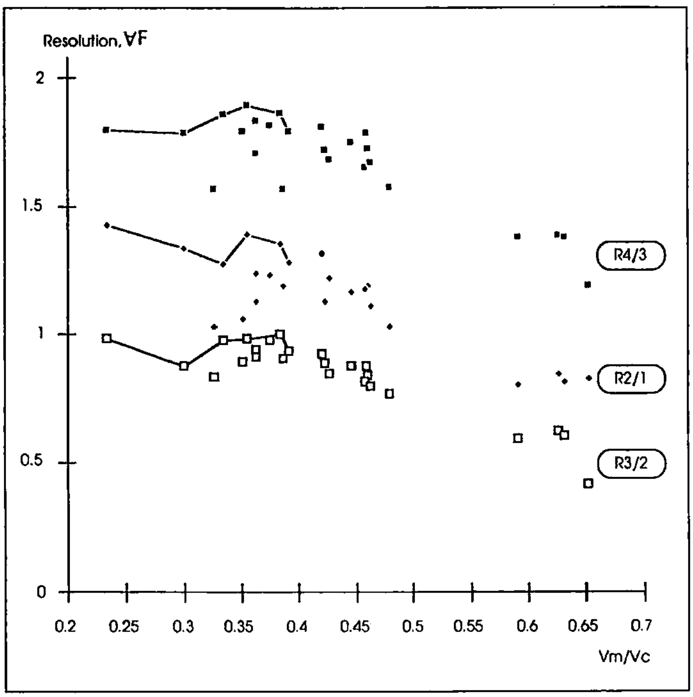

Figure 5 : Variation of the resolution with the volume of the mobile phase in the column for the HPCPC, whatever the flow rate, in the range 1 to 10 ml/min. The linked plots correspond to the experiments at V_m^{min} conditions, for 1 to 10 ml/min.

will be useful to verify that point for theory, but it is of limited importance for practical applications, since working with these flow rates is time consuming.

VARIATIONS OF THE RESOLUTION WITH Vm AND F :

More important than the efficiency will be the variations of the resolution between peaks with the flow rate and with the ratio of the mobile phase in the column. We already know that the resolution between two close peaks is related to the efficiency and to the ratio of the mobile to stationary phase by the familiar

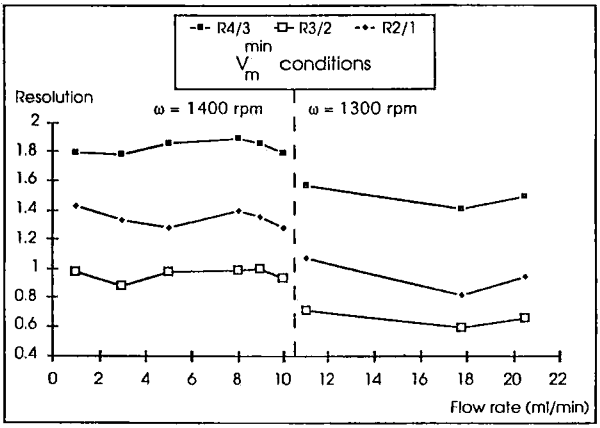

Figure 6 : Variation of the resolution with the flow rate when working at V_m^{min} conditions, for two sets of experiments at 1400 and 1300 rpm.

resolution equation, which can be written using the partition coefficient instead of the capacity factor[2] :

$$Rs = \frac{1}{2}\sqrt{N}(\alpha - 1)\frac{K_1}{K_1(\alpha + 1) + 2\,V_m/V_s} \qquad (1)$$

We have shown that the efficiency increases with the volume of the mobile phase, and does not depend upon the flow rate in the range studied; this related variation of N and Vm which have antagonist actions upon Rs, should result in a small variation of Rs in a broad range of the studied parameters, F and Vm/Vc .

Figure 5 shows the resolution observed between peaks plotted against Vm/Vc, whatever the flow rate (*i.e.* $Rs = f(\frac{Vm}{Vc}, \forall F (1 \text{ to } 10 \text{ ml/min})))^6$ which has been used to run the chromatogram. The linked plots correspond to the experiments at V_m^{min} conditions. It can be seen that, in the range Vm/Vc = 0.25 to 0.5, the resolution is roughly constant or decreases slowly; for Vm/Vc > 0.5 the decrease is more pronounced. This means that, in a large part of the graph Vm/Vc , F, of Figure 2, the resolution does not vary substantially with these parameters. Interesting is the resolution we get when working at V_m^{min} conditions.

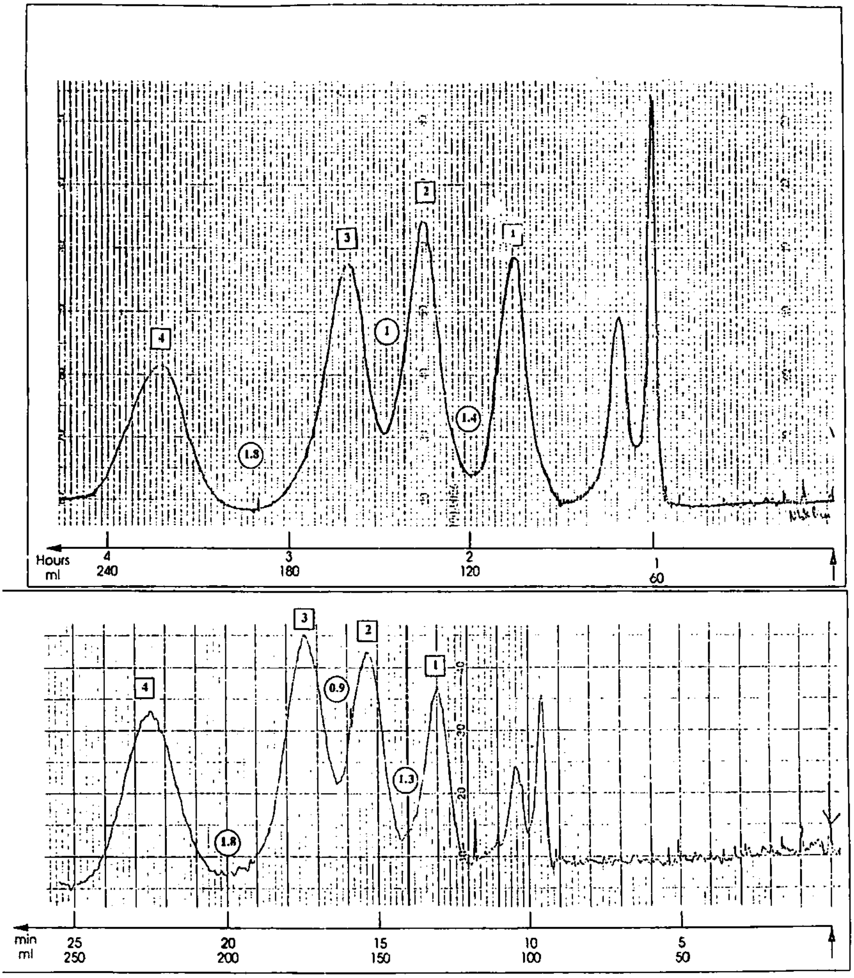

Figure 7 : Similarity of two chromatograms which were run at V_m^{min} conditions for F = 1 and F = 10 ml/min with the HPCPC.

Column : HPCPC, with 2136 channels for a total volume of 240 ml.

Biphasic system : Hexane, 4% Water in Methanol, with the lower phase as mobile phase, in the descending mode.

Solutes : ☐ Peak identification; see Experimental

○ Resolution between peaks

upper : $\frac{Vm}{Vc} = 0.23$, F = 1 ml/min   lower : $\frac{Vm}{Vc} = 0.39$, F = 10 ml/min

Figure 6 shows the results obtained for two set of experiments, at 1400 and 1300 rpm. It is evident that the resolution does not vary substantially with the flow rate when working at V_m^{min} conditions. This was already noted with the CPC model LLN for flow rate ranging from 0.3 to 4 ml/min[7] . With our biphasic system and test samples, for the CPC model LLN, we observed a sharp decrease for Rs when increasing the flow rate from 0.45 to ≈ 2.5 ml/min, then the resolution is roughly constant or even increasing a little bit, finally decreasing for F > 8 ml/min; this is in accordance with the observation by D. Armstrong of a minimum for the efficiency for a flow rate around 2 ml/min.

For our experiments, the HPCPC prototype was not tested for working pressure over 6 MPa, and we did not attempt to increase the rotational speed to get more efficiency; we can conclude that, except for viscous biphasic systems where other kinetic hindrances could appear, it is best to operate the HPCPC at high flow rates, provided the ratio Vm/Vc is not too high, *c.a.* < 0.5 or 0.6. Figure 7 shows two typical chromatograms obtained for F = 1 and F = 10 ml/min, working at V_m^{min} conditions.

CONCLUSION

We have shown that the efficiency of the new HPCPC, which is a non-planetary centrifugal partition chromatograph, is much more related to the volume ratio of the mobile phase in the column than to the flow rate. When working with the minimum volume of mobile phase, for a given flow rate, which are conditions commonly used for CCC work, the combination of an increasing efficiency when the flow rate increases (and thus V_m^{min}) with the decreasing volume of the stationary phase inherent to that variation, results in a roughly constant resolution over a broad range of flow rates. The linear relationship between the flow rate and the minimum volume of mobile phase compatible with that flow rate, V_m^{min}, which depends upon the experimental conditions and upon the properties of the biphasic system, should allow one to readily predict the best set of conditions leading to fast and effective separations.

ACKNOWLEDGEMENTS

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3 A. Berthod, D.W. Armstrong, J. Liq. Chromatogr., 11, 547-566 (1988)

4 In reference 2, page 3344, a mistake was made. We should read Hexane, 4% Water in Methanol instead of Hexane, 1% Water... This system is similar to that used by D.W. Armstrong *et al.* to conduct their studies with the CPC model LLN.

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6 $\forall$ is the mathematical symbol for "whatever".

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