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Efficiency and Resolution in Countercurrent Chromatography

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EFFICIENCY AND RESOLUTION IN COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

Efficiency and resolution have been studied with a CCC apparatus classified by Y. Ito¹ as a HDES type J, and known as the HSCCC or the Multi-layer Coil Separator Extractor. It has been shown that the efficiency decreases when increasing the linear velocity of the mobile phase in the column, which can be done by increasing the flow rate, or by decreasing the volume of the mobile phase in the column. The two ways of changing the linear velocity are not equivalent, since, at constant linear velocity, the efficiency increases when the volume of the mobile phase is well balanced with that of stationary phase. For each flow rate there is a minimum value for the volume of the mobile phase, and the relationship is linear. Working under these conditions, changing both the flow rate and the volume of the mobile phase leads to an efficiency which goes through a minimum at medium flow rates.

The resolution does not show this minimum : it is best at low flow rates and low volumes of mobile phase, and its variations are more important at low flow rates. This means that, if the resolution is good at moderate flow rates, it will remain good at higher flow rates. Combining three equations, it has been possible to predict the resolution when working with the minimum volume of mobile phase for a given flow rate.

INTRODUCTION

Factors acting on the efficiency and resolution in CCC are numerous and often interdependent; many of them are relevant to the CCC device, such as the nature of the gravity field (HSES or HDES, type I, J, L, etc.)², or the shape of the column (channels or coiled tube). Other factors are more relevant to the way the CCC devices are used, such as the rotational speed, the ratio of mobile to stationary phase, the flow rate, the physical properties of the biphasic system.

The influence of these parameters can be clearly understood if each of them is varied separately; however, papers in the field are not numerous. A complete study of van Deemter plots has been published by D.W. Armstrong *et al.*³. They used an instrument based on HSES (the Sanki CPC, model LLN), and showed that van Deemter-type plots exhibit maxima rather than minima. Therefore, the best efficiency is obtained at high or low flow rates. Resolution and efficiency have been studied by W.D. Conway *et al.*⁴ with a slowly rotating non planetary device (HDES), and they concluded that resolution is favored by a large ratio of the stationary phase, and the column efficiency is independent of that ratio.

The role of the flow rate and of the ratio of stationary phase have not been yet systematically studied for the classical HSCCC, which is a HDES type J, planetary instrument, like the multilayer coil planet centrifuge. We have undertaken systematic variations of these parameters with the HSCCC, choosing a simple biphasic system (hexane, 1% water in methanol, already used by D.W. Armstrong *et al.* to conduct their study with the CPC). We have shown that, for a given column and rotational speed, the best resolution is obtained for a low flow rate (0.2 ml/min) and a small volume of the mobile phase (9% of the volume of the column). Efficiency goes through a minimum located at a flow rate of ≈ 3 ml/min and a volume of the mobile phase of $\approx 20\%$ of the total volume); these experimental conditions are similar to those used by most people working with a CCC device.

EXPERIMENTAL

HSCCC studies were carried out at ca. 25°C with an Ito multilayer coil separator extractor (P.C. Inc., Potomac, MD, USA). The column was made of a 1.6 mm I.D. teflon tube, 61.4 m long, wound on the original holder; the total volume

was 143 ml for 152 turns. All data were obtained with a constant rotational speed, ω , checked by stroboscopic observations, and fixed at 710 rpm \pm 2%. A Valco valve was used (VICI Valco, Houston, TX, USA), with a 500 μ l sample loop. The pump was a Waters model 501 (Milford, MA, USA). Two kinds of tubing were used for inlet and outlet of the CCC column :

1) for small flow rates ($F < 2$ ml/min), two capillaries were used, made of 0.125 mm I.D. teflon tubing; the extra column volume was \approx 150 μ l

2) for higher flow rates ($F \geq 2$ ml/min), two 1 mm I.D. PTFE tubings were used, and the extra column volume was \approx 1.9 ml (not including the UV cell)

The outlet flow tube was partly enclosed in the oven of a temperature control system (column heater and temperature control module (TCM) (Waters, Milford, MA, USA), maintaining a temperature of 35 $^{\circ}$ C ($T_{\text{experiment}} + 10^{\circ}$ C), yielding a noiseless baseline for the detector, an Isco model UA5 with a 300 μ l, 5 mm path length UC cell (ISCO, Lincoln, NE, USA).

A fraction collector (Isco model 328) was used to check for the accuracy and precision of the flow rates ($F < 2$ ml/min); higher flow rates were randomly checked with a graduate cylinder and a chronometer.

n-Hexane and methanol, analytical reagent grade, came from Prolabo (Paris, France).

4-Hydroxybenzoic acid was from Jansen (Geel, Belgium), diethyl phthalate was from Aldrich (Milwaukee, WI, USA), dipropyl phthalate, ethyl and butyl phenylacetate were kindly provided by the laboratory of organic chemistry of our institute.

In all the experiments, the upper phase (hexane rich) was the stationary phase, and the lower phase (methanol rich) was pumped from the head to the tail. In this reversed phase operating mode (the apolar solvent being the stationary phase), 4 hydroxybenzoic acid ($K=10^{-2}$, checked by spectrophotometry) was taken as the non retained compound whose duration of passage was the column dead time t_0 , leading to a direct estimation of the volume of the mobile phase V_m , and of that of stationary phase V_s , with $V_m + V_s = V_c$, V_c being the volume of the column (143 ml). 4-Hydroxybenzoic acid was always injected with the test solutes; the relative error generated by this method has been corrected throughout the study (actual $V_m = \frac{\text{measured } V_m - 10^{-2} V_c}{1 - 10^{-2}}$).

The partition coefficients of diethyl phthalate (peak 1), dipropyl phthalate (peak 2), ethyl phenylacetate (peak 3), and butyl phenylacetate (peak 4) have been estimated from experiments for which the volume of the mobile phase was <50% of the total volume, in order to get a correct precision. They were as follow :

	$K = \frac{C_{(\text{hexane rich stat. phase})}}{C_{(\text{methanol rich mob. phase})}}$	n	s
peak 1 diethyl phthalate	0.261	30	0.015
peak 2 dipropyl phthalate	0.431	23	0.028
peak 3 ethyl phenylacetate	0.585	31	0.032
peak 4 butyl phenylacetate	0.955	32	0.052

where n is the number of measurements, and s the standard deviation.

The separation factors were then ($\alpha_j = K_j / K_i$)

$$\alpha_{1,2} = 1.65$$

$$\alpha_{1,3} = 2.23$$

$$\alpha_{2,3} = 1.35$$

$$\alpha_{3,4} = 1.65$$

In all the experiments, the efficiency has been estimated for each peak (assuming Gaussian shape), by the classic equation :

$$N = 5.54 \left(\frac{V_r}{\delta_{0.5}} \right)^2 \quad (1)$$

and sometimes by :

$$N = 4 \left(\frac{V_r}{\delta_{0.6}} \right)^2 \quad (2)$$

$\delta_{0.5}$ and $\delta_{0.6}$ being the peak width at 0.5 or 0.6 x the peak height (with the same units as V_r).

The resolution between two peaks has been estimated with the classical equation :

$$R_s = 2 \frac{V_{r2} - V_{r1}}{\omega_1 + \omega_2} \quad (3)$$

ω_i being the base width of peak i , directly measured, or estimated through $\delta_{0,5}$, and V_{ri} the retention volume corresponding to peak i .

RESULTS AND DISCUSSION

FILLING THE COIL: DEFINITION OF V_m^{\min}

Different procedures are employed to allow the volume ratio of the two phases to be chosen at will.

- A method analogous to that of B. Testa *et al.*⁵ can be used : while the CCC column is in rotation, the coil is first filled with the mobile phase (lower phase), and then a desired volume of the stationary phase is added to the column by displacing the mobile phase⁶ ;

- When the common way of filling the CCC column is used, *i.e.* filling first with the stationary phase, then displacing with the mobile phase, we get, if the injection is performed without delay, the minimum volume of mobile phase, V_m^{\min} , which can be obtained for the flow rate F which has been established. If bleeding occurs (*i.e.* slow decrease of the stationary phase content, V_s , with time, and which is more important for larger flow rates), then further injections are made with the actual value of $V_m < V_m^{\min}$, and estimated *via* the t_0 of the 4 hydroxybenzoic acid. From that point, if the flow rate is decreased, the next injection will be performed for $V_m < V_m^{\min}$ corresponding to the actual flow rate. Figure 1 is the map of all the couples F, V_m we tested with our system.

We tested our system for flow rates ranging from 0.1 to 9.9 ml/min, and V_m ranging from ≈ 10 ml to 143 ml (≈ 7 to 100% V_c), with approximately 70 injections.

Regression analysis for the experimental points corresponding to the conditions V_m^{\min} led to a straight line, $V_m^{\min} = CF + D$:

$$V_m^{\min} = 5.51 (\pm 0.11) F + 9.58 (\pm 0.35) \quad (4)$$

$$n = 16 \quad r^2 = 0.994 \quad s = 1.60 \quad \mathcal{F} = 2481$$

where n is the number of observations, r the correlation coefficient, s the standard deviation and \mathcal{F} the Fisher's test parameter (V_m^{\min} in ml, F in ml/min).

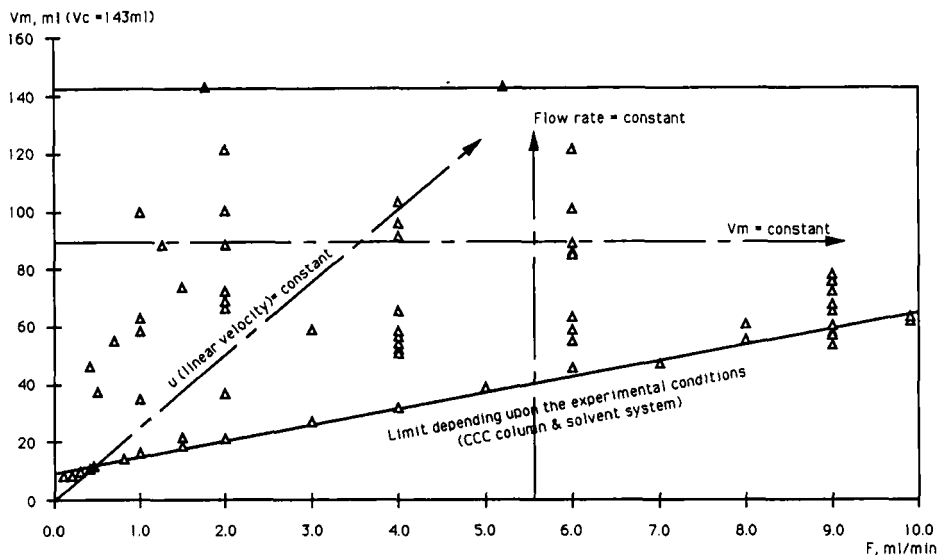


Figure 1 Map of the experimental points, with the various ways to vary the parameters, and the experimental limit $V_m^{\min} = CF + D$

The slope (in min^{-1}) is characteristic of the stability of the stationary phase in the CCC column: the lower it is, the larger will be the amount of stationary phase for higher flow rates. The intercept (in ml), is the extrapolation of V_m^{\min} at 0 flow rate; it includes the extra column volume, but is larger: for our experiments the extra column volume was $150 \mu\text{l}$ for the capillaries and 1.9 ml for the standard tubes; it gives the largest amount of stationary phase (93.3% of V_c) which can be obtained with our conditions and our biphasic system. The slope and intercept are both functions of the rotational speed and of the properties of the biphasic system which is used (viscosity, density difference). Even if we can emphasize that the slope should increase with the viscosity, and decrease with the density difference and with the rotational speed, further studies have to be done to clearly understand how the relationship between F and V_m^{\min} is influenced by these parameters. It must be noted that most people using a CCC device work on this straight line, since they generally use the standard method to fill their instrument, and usually they inject a sample while equilibrating the column with the mobile phase.

EFFICIENCY VERSUS V_m AND F :

Stricto sensus, the "Van Deemter type plot" is the relationship between the plate height, H , and the flow rate, F , or the linear velocity of the mobile phase, u , for a given value of V_m , since, in HPLC, this value is set up by the solid matrix and cannot be varied. In CCC, various Van Deemter type plots can be drawn for various values of V_m as represented by a horizontal line in Figure 1. For CCC, other types of plots can be drawn :

- Following a vertical line (Fig. 1), we will get the variation of H at a given flow rate while varying V_m .
- Following a straight line going through the origin, we will get the variation of H at a given linear velocity, u , of the mobile phase ($u = \frac{F L}{V_m}$, L being the length of the column).

Figure 2 to 4 show these three types of variations for different values for F , V_m , and u .

From Figures 2 and 3 it can be seen that the plate height increases when the flow rate increases at constant V_m , and decreases when V_m increases at constant flow rate, which means that, in any case, the plate height increases with the linear velocity of the mobile phase. The higher the velocity (800 or 1100 cm/min), the higher the plate height will be (12 to 14 cm). For these two types of variations, there is no maximum or minimum for H ; its variation seems more important at small volume of the mobile phase when F is varied, and at small value of F when V_m is varied.

From these results, it seems that efficiency is better for lower flow rates and higher volumes of the mobile phase, which means for lower linear velocities. If the linear velocity was the only parameter governing the efficiency, there should be no variation for H in Figure 4 (an horizontal line for each panel); this seems to be true for lower value of u ($75 < u < 90$ cm/min), while H decreases markedly for higher value ($730 < u < 790$ cm/min). This means that the variation of H at constant linear velocity reflects the importance of the ratio of the volume of the two phases in the CCC column more than that of the flow rate : a better mass transfer may occur when the two phases are present in similar ratio in the column. Finally, and if the resolution is not involved, it means that to work with high linear velocities (*i.e.* short time for analysis), it is better to work with higher flow rate while increasing the volume of the mobile phase (*i.e.* $V_m > V_m^{\min}$).

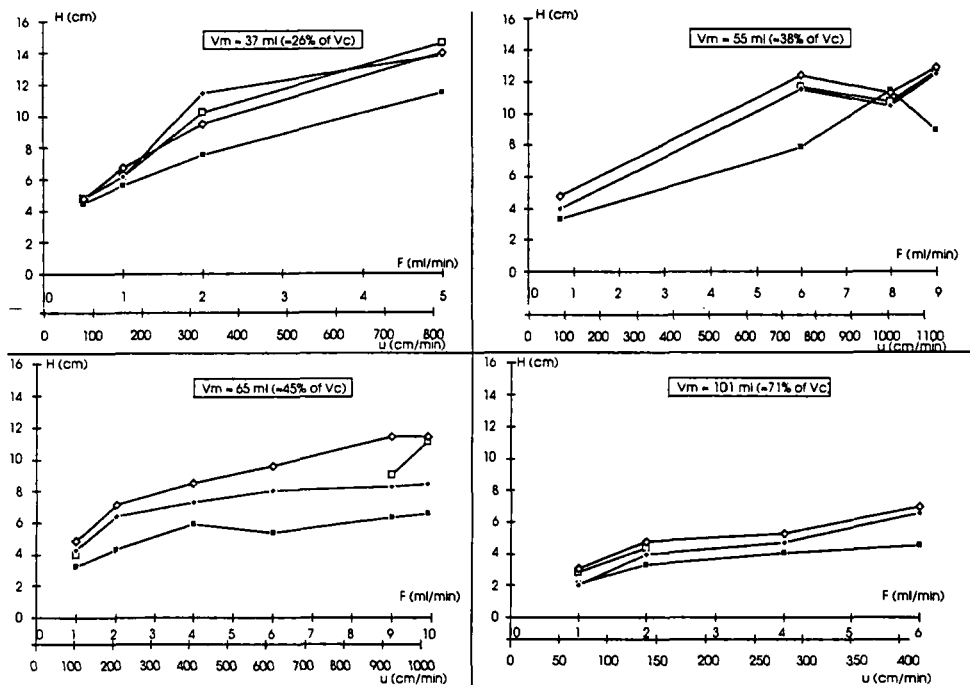


Figure 2

Variation of the plate height, H , with the flow rate, at constant volume of the mobile phase, V_m . The volume of the column is $V_c = 143$ ml. X scale is in flow rate or in linear velocity units, which are equivalent

since $u = \frac{F L}{V_m}$, L being the length of the column (6140 cm)

--■-- H (peak 1) □-- H (peak 2) - - - H (peak 3) ◊-- H (peak 4)

Now, we need to know how the efficiency varies when one continues to work with the minimum volume of the mobile phase for a given flow rate, *i.e.* working at V_m^{\min} . Figure 5 gives the variations of the plate height with F , V_m and u when we describe the straight line $F = f(V_m^{\min})$ which appears in Figure 1.

The observed curves reflect the combination of the effects of both an increase of the flow rate and of the volume of the stationary phase, which have opposite effects.

Starting from no flow rate, the plate height starts decreasing a little bit, then increases markedly with a maximum around 2 ml/min. For a flow rate in the

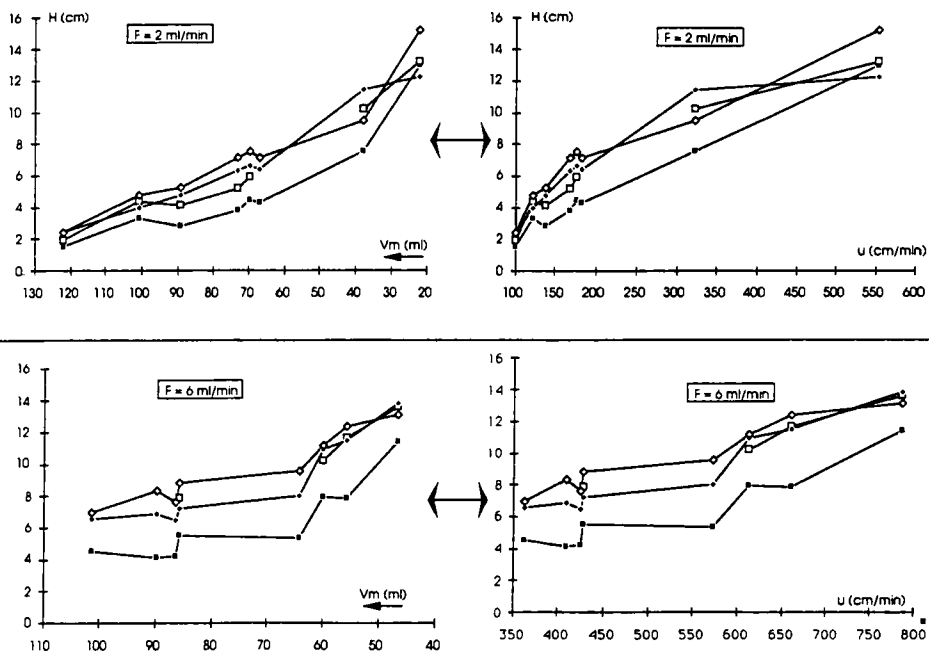


Figure 3 Variation of the plate height, H , with the volume of the mobile phase, V_m , at constant flow rate, F . X axis is in volume units on the left (Increasing from right to left), and in linear velocity units on the right.
 -■- H (peak 1) -□- H (peak 2) -◆- H (peak 3) -◇- H (peak 4)

range 2 to 7 ml/min, the efficiency of the column is minimum, with a plate height of around 15 cm (three theoretical plates per turn of the coil). For higher flow rate, the plate height decreases, and we observe the same efficiency at 1 and 9 ml/min, i.e. $H \approx 7$ cm, or 6 theoretical plates per turn of the coil.

This means that, if we want to work at V_m^{min} conditions (and this is the way most people do), it is more efficient to work at low flow rates, or high flow rates. Except for very low flow rates ($F < 0.2 \text{ ml/min}$), these experimental points are very similar to those obtained by D. Armstrong *et al.* with an HSES apparatus (the Sanki CPC™, model LLN), and confirm that CCC must be regarded as a unique chromatographic system since, with the standard conditions (i.e. working at

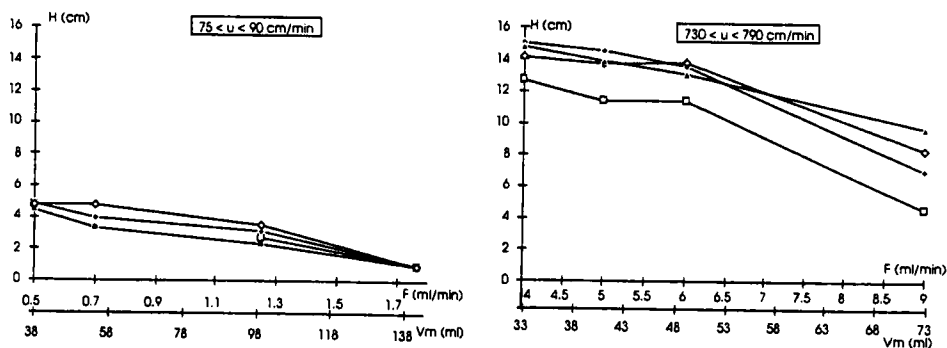


Figure 4 Variation of the plate height, H , with F or V_m at constant linear velocity of the mobile phase.

V_m^{\min}), the pseudo Van Deemter curves (pseudo since the volume of the mobile phase is not a constant) show maxima rather than minima.

To get a complete understanding of how the efficiency is related to both F and V_m , we can now plot, on the map F/V_m , the couples F, V_m where we find the same efficiency for a given peak, and thus define the curves of isoefficiency for our CCC device. Figure 6 shows the example obtained with peak 3: the worst efficiency is obtained along the straight line $V_m^{\min} = f(F)$, between 3 and 7 ml/min ($H \approx 14$ cm).

Starting from this "ledge", the plate height decreases whatever the direction of the variation; this means that, as far as the resolution is not concerned, we get the same efficiency (c.a. $H=8$ cm) for $V_m = 14.5$ ml and $F = 0.8$ ml/min (3 hours to elute a compound with a partition coefficient, $K = 1$), and for $V_m = 61$ ml and $F = 8$ ml/min (18 min to elute the same compound). On Figure 7 is the comparison of the two chromatograms; the resolution is different, but the efficiency is the same, for analysis time of 180 min and 20 min, respectively.

It must be pointed out that the efficiency remains good for very low flow rate, which means that the longitudinal diffusion of solutes in the coil which constitutes the column have little influence on the band broadening in CCC. If we consider around 1000 theoretical plates at 0.2 ml/min, with our column (152 turns, 61.4 m long), it means that ≈ 0.15 turn, or 6.1 cm, must be regarded as a single

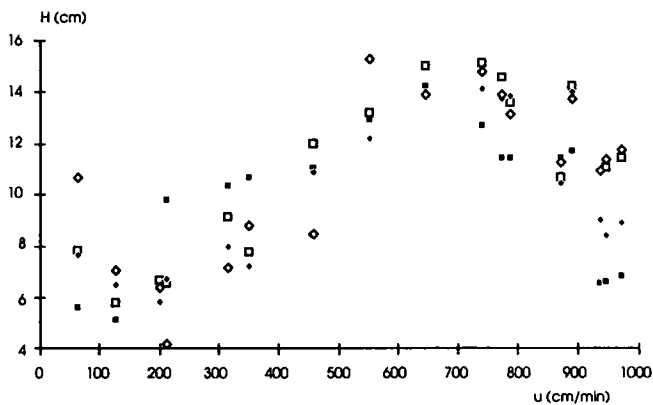
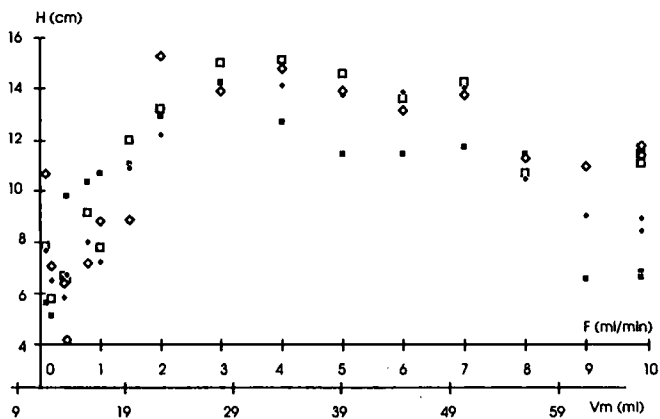


Figure 5 Variation of the plate height, H , with the parameters F , V_m , and u , for the experimental conditions corresponding to the minimum volume of the mobile phase, V_m^{\min} , compatible with the actual flow rate, F .

V_m^{\min} (ml) and F (ml/min) are dependant, with the relation :

$$V_m^{\min} = 5.51 F + 9.58.$$

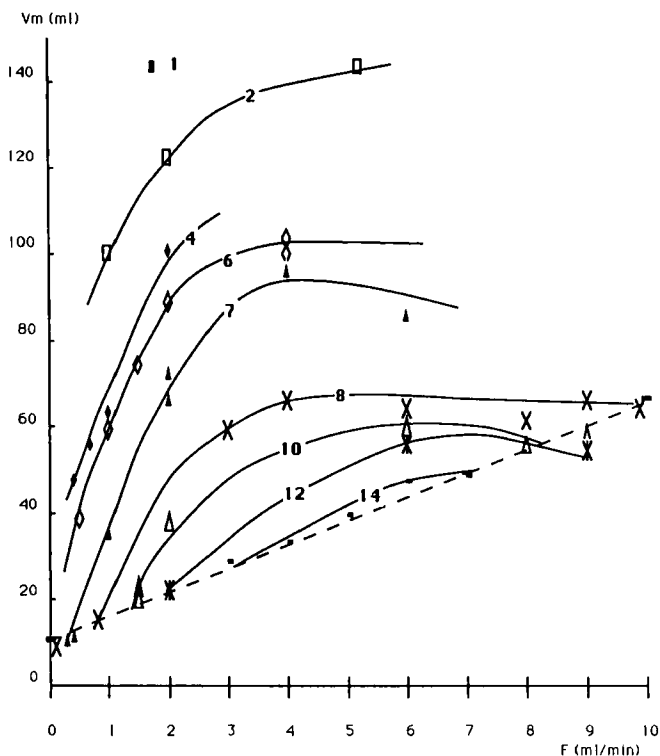


Figure 6 Isoefficiency curves corresponding to the peak 3. The coordinates for a point define the conditions (F and V_m) which has been used for the injection. The plate height (in cm) is indicated on the corresponding curve.

channel where the partition process occurs, and from where solutes cannot easily diffuse longitudinally.

This "channel", generated by the variable gravity field occurring from the two-axis gyration mechanism, is similar to the physical channel which is found in the HSES device from which D. Armstrong found that efficiency was increased while decreasing the flow rate.

From these similarities we can try to compare the experimental points corresponding to the conditions V_m^{\min} , to a model similar to that proposed by D. Armstrong³, and which can be written :

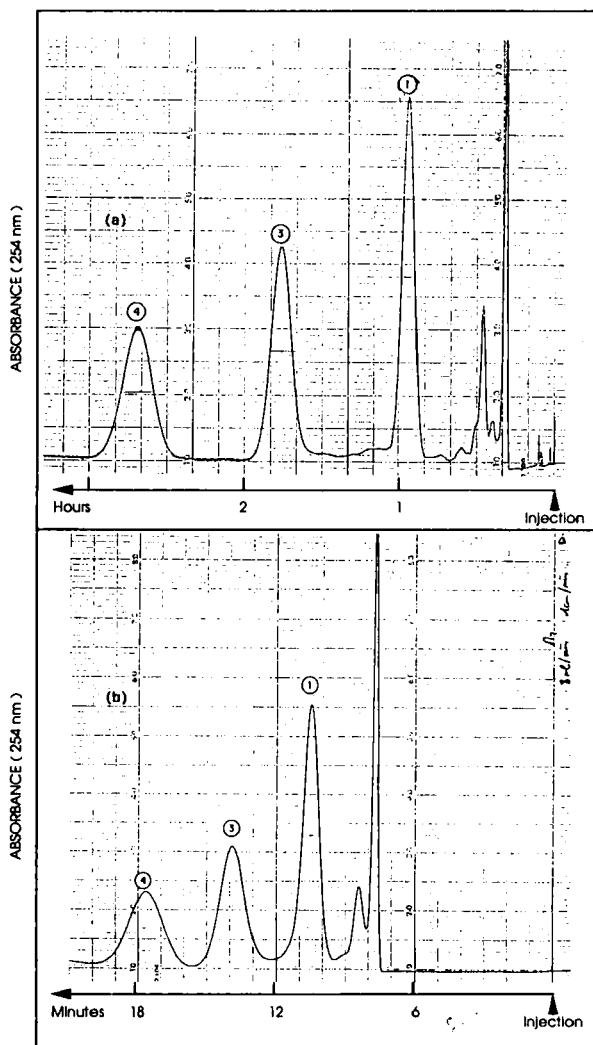


Figure 7

Comparison of two chromatograms showing the same efficiency for two very different experimental conditions :

- a / The flow rate is 0.8 ml/min, and the volume of the mobile phase is 14.5 ml (10% of the volume of the column, V_C)
- b / The flow rate is 8 ml/min, and the volume of the mobile phase is 61 ml (43% of the volume of the column, V_C)

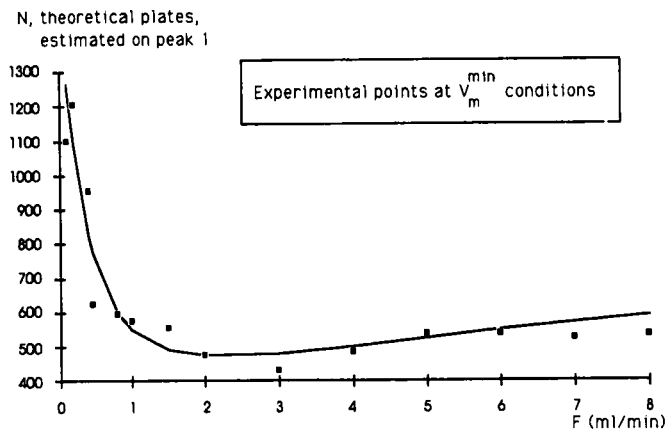


Figure 8 Comparison between experimental efficiency observed on peak 1, with the conditions V_m^{\min} , and the predicted efficiency using the Armstrong's equation : $N = N_0 \exp\left(-\frac{A}{F} + B F^b\right)$

$$N = N_0 \left(1 - \exp\left(-\frac{A}{F} + B F^b\right) \right) \quad (5)$$

N_0 , A , B and b being constants (related to the apparatus, the solvent system, and the solutes), and N the number of theoretical plates. Figure 8 gives an example of simulation for peak 1, and the constants for the other peaks have been calculated as follow :

N° peak	A (ml/min)	B ($\text{min}^{0.5} \text{ml}^{-0.5}$)	b	N_0
1	0.35	0.2	0.5	1300
2	0.45	0.15	0.5	1200
3	0.55	0.15	0.5	1200
4	0.5	0.11	0.5	1500

Except for very low flow rates ($F < 0.2$ ml/min), it seems that the Armstrong's equation fits our results well, and can be used to further estimate the resolution as a function of the flow rate.

Variation of the resolution with V_m and F .

More important than the efficiency will be the variations of the resolution between two peaks with the flow rate and the volume of the mobile phase.

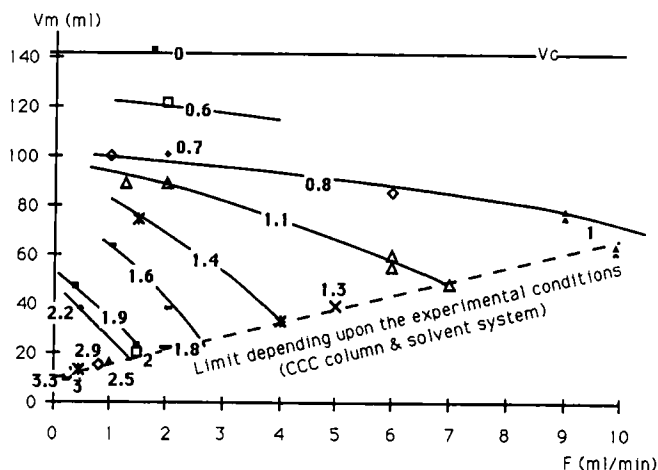


Figure 9 Isoresolution curves for peaks 1 and 2. The coordinates for a point define the conditions (F and V_m) which have been used for the injection. The value for the resolution is indicated on the corresponding curve.

For two very close compounds, and after selecting the most suitable solvent system, optimizing the experimental conditions to get the highest resolution, R_s , is the only way to achieve success.

As we did for H, we can plot R_s on a map with the coordinates F , V_m , and we can link the experimental points for which we find the same resolution for two given peaks; thus we define the curves of Isoresolution for our CCC device (Figure 9 to 11).

Isoresolution curves clearly indicate that the best resolution is obtained at very low flow rate and smaller volumes of mobile phase: 0.2 ml/min and 7% of the total volume for V_m . Starting from this point, R_s decreases sharply with F and V_m , after while the variation is less pronounced. For example, for peaks 1 and 2, R_s varies from 3.3 to 1.4 when F increases from 0.2 to 4 ml/min, then only from 1.4 to 1 when F increases from 4 to 10 ml/min. From the shape of the Isoresolution curves, we see that it is possible to get the same resolution for a small volume and a high flow rate ($R_s = 1.1$ for $V_m = 50$ ml and $F = 7$ ml/min, on Figure 9), and for a higher volume and a smaller flow rate ($R_s = 1.1$ for $V_m = 90$ ml and $F = 1.5$ ml/min, same Figure).

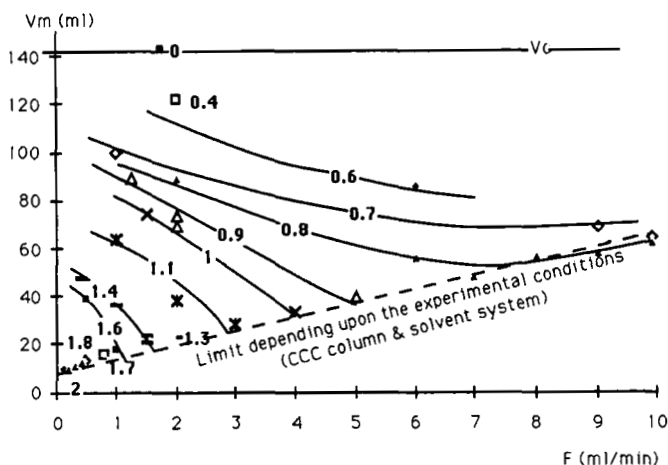


Figure 10 Isoresolution curves for peaks 2 and 3. The coordinates for a point define the conditions (F and V_m) which have been used for the injection. The value for the resolution is indicated on the corresponding curve.

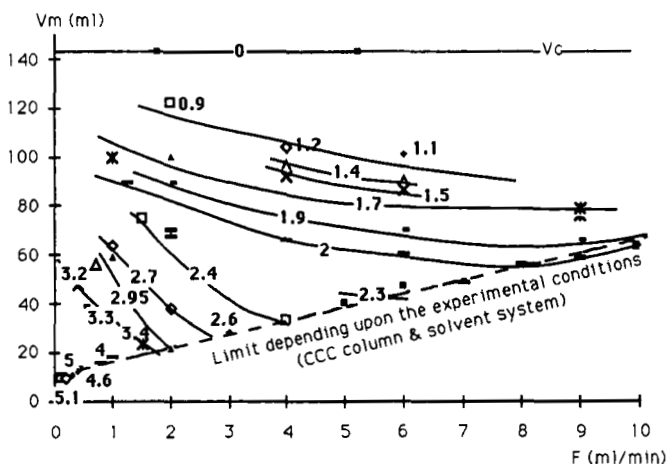


Figure 11 Isoresolution curves for peaks 1 and 3. The coordinates for a point define the conditions (F and V_m) which have been used for the injection. The value for the resolution is indicated on the corresponding curve.

Figure 12 gives the variation of the resolution along the straight line $V_m^{\min} = f(F)$. Except for $F < 0.2$ ml/min⁷, the resolution decreases when the flow rate is increased. The variations are more accentuated at low flow rates : for example, the value for R_s for peaks 2 and 3, which is 2 at 0.4 ml/min, is only 0.99 at 4 ml/min, and then varies slowly, to be 0.8 at 9 ml/min. Figure 13 compares the three corresponding chromatograms.

Equation for the resolution, at V_m^{\min} conditions.

From the classical equations :

$$R_s = 2 \frac{V_{r2} - V_{r1}}{\omega_1 + \omega_2} \quad (3)$$

and
$$N = 16 \left(\frac{V_r}{\omega} \right)^2 \quad (6)$$

and assuming that the number of theoretical plates is the same for two close peaks (and this is nearly true for CCC, see Figures 2 to 5), we can rewrite equation (3) as follows :

$$R_s = \frac{1}{2} \sqrt{N} (\alpha - 1) \frac{K_1}{K_1 (\alpha + 1) + 2 V_m / V_s} \quad (7)$$

For the experimental points corresponding to the conditions V_m^{\min} , we have shown that V_m and N can be derived from the flow rate, F (equations (4) and (5)), so that in this particular case, the resolution can be calculated directly from the partition coefficients, the selectivity factor, and the flow rate :

$$R_s = \frac{1}{2} \sqrt{N_0 \left(1 - \exp\left(-\frac{A}{F} + B F^b\right)\right)} (\alpha - 1) \frac{K_1}{K_1 (\alpha + 1) + 2 \frac{C F + D}{V_c - C F - D}} \quad (8)$$

With our experimental conditions, we have $b = 0.5$, $C = 5.51$ min⁻¹, $D = 9.58$ ml, A between 0.35 and 0.55 ml/min and B between 0.11 and 0.2, and N_0 between 1200 and 1500, depending upon peaks of interest. We have tested this equation and the resulting curves are shown on Figure 12. The correlation is rather good, except for $F < 0.2$ ml/min (see note 7), and for the resolution between peaks 1 and 3 at high flow rate, where the measured resolution is better than the predicted one.

We have now to understand how A , B , b , C and D are related to the experimental conditions and to the physico-chemical properties of the solvent system.

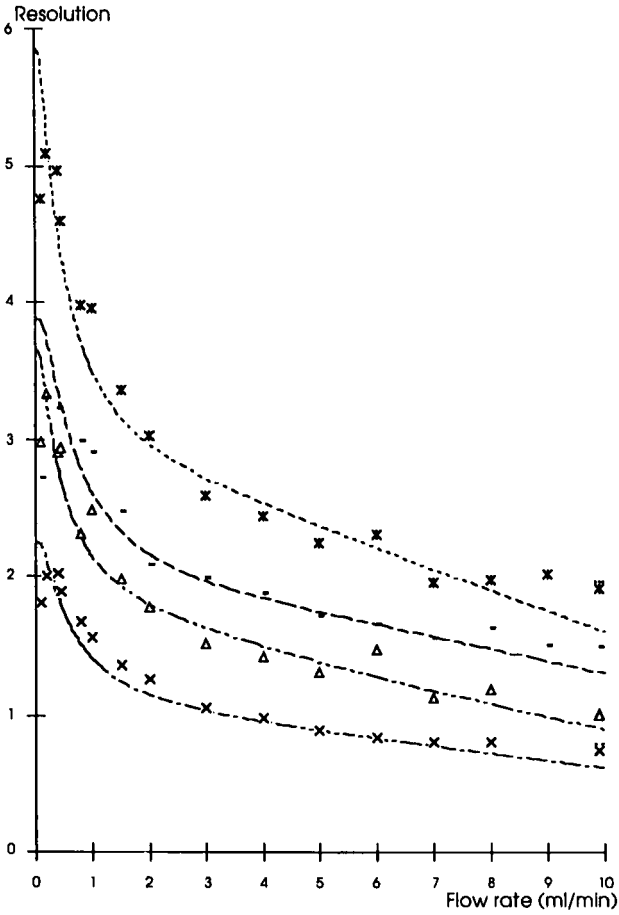


Figure 12 Variation of the resolution with the flow rate, when working at V_m^{\min} conditions. Comparison of the experimental points with the curves calculated with equation (8).
 x : resolution between peaks 1 and 3
 - : resolution between peaks 3 and 4
 Δ : resolution between peaks 1 and 2
 x : resolution between peaks 2 and 3

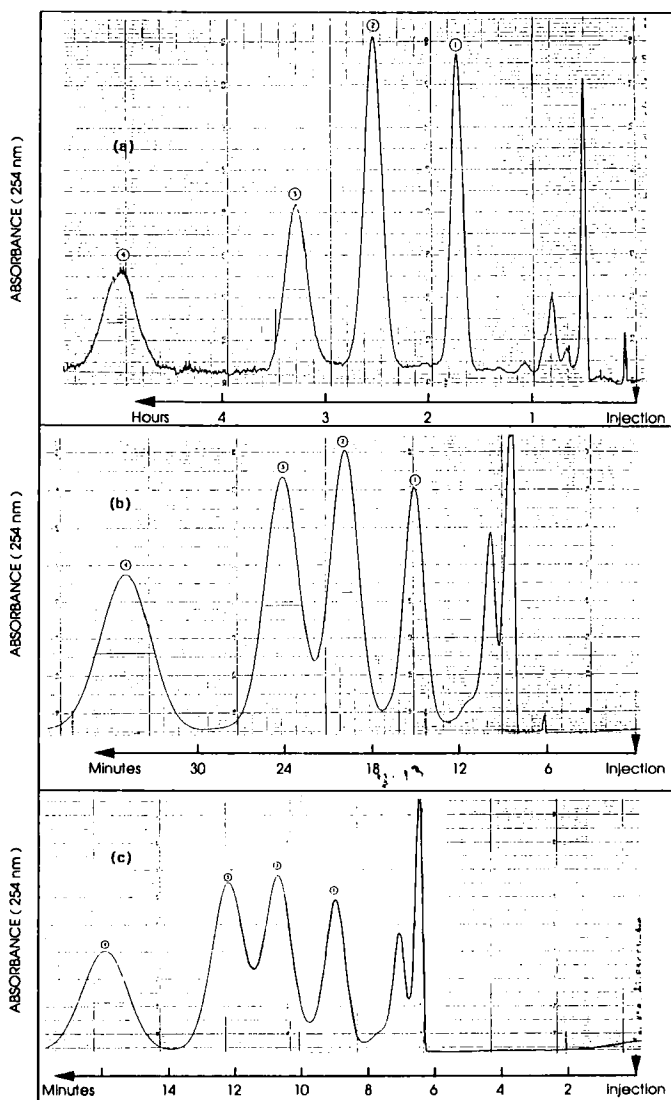


Figure 13 Evolution of the resolution when working at V_m^{\min} conditions
 a/ $F = 0.4$ ml/min, $V_m = 9\%$ of the volume of the column, V_C .
 b/ $F = 4$ ml/min, $V_m = 23\%$ of V_C .
 c/ $F = 9$ ml/min, $V_m = 41\%$ of V_C .

CONCLUSION

We have shown that the efficiency decreases when the linear velocity of the mobile phase increases. Contrary to what we find in HPLC, there are two ways to increase the linear velocity in CCC, *i.e.* increasing the flow rate, or decreasing the volume of the mobile phase. There is a common way to use a CCC column, which is to fill it with the minimum volume of mobile phase compatible with the actual flow rate, and we have shown that there is a linear relation between this volume, V_m^{\min} , and the flow rate, F . Working with these conditions leads to an efficiency going through a minimum for medium flow rates, and reflecting the two opposite effects of increasing both F and V_m^{\min} . In this particular case, the efficiency can be calculated using an Armstrong's equation, the constants for this equation being a function of the design of the CCC device, the experimental conditions, the physical properties of the solvent system and the nature of the solutes.

We have shown that the resolution in CCC is maximum for low flow rates and low volume of the mobile phase. The resolution decreases markedly when increasing the flow rate for lower values, and then the decrease is less pronounced. Combining three equations, we have found a good correlation between the experimental points and the calculated curves giving the resolution as a function of the flow rate when the CCC column is used with the minimum volume of mobile phase for the actual flow rate.

From our result, we conclude that, when the resolution is critical, the CCC column has to be used at low flow rate (*i.e.* 0.2 ml/min for our instrument) and low volume of the mobile phase (*i.e.* 7% of the volume of the column for our instrument). For that point, and for two compounds with a partition coefficient close to 0.6 and a selectivity factor of 1.2, we can calculate a resolution of 1.1 with only 758 plates in our instrument; for an HPLC column, assuming that the ratio of the mobile phase is 90% of the free volume of the HPLC column, it would need 10,000 plates to get a resolution of only 0.3 (125,000 plates to get a resolution of 1.1). This makes CCC very attractive in the near future.

We have now to understand how the various constants found in the equations are related to the design of the CCC column, the experimental conditions, and the physical properties of the solvent system.

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