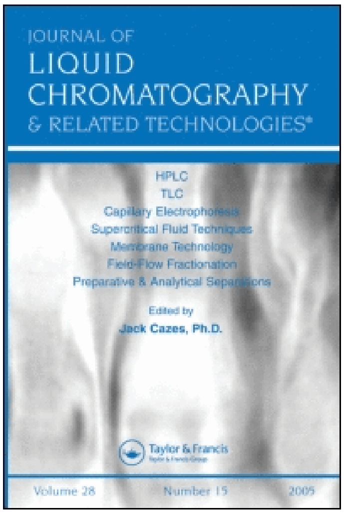

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IMPORTANCE OF THE PARAMETER Vm/Vc IN COUNTERCURRENT CHROMATOGRAPHY: TENTATIVE COMPARISON BETWEEN INSTRUMENT DESIGNS

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ABSTRACT

Results acquired with three CCC devices are compared. There is a linear relationship between the minimum volume of mobile phase, V_m^{min}, compatible with the actual flow rate, F, and that flow rate.

Vc being the volume of the column and S the cross sectional area of the channel where the chromatographic process occurs, the slope of the line $V_m^{min}/Vc = f(F/S)$ allows comparison of the stability of the stationary phase for various instruments.

This relationship leads to an estimation of the average linear velocity of the mobile phase in the channel, $\bar{u}$, namely $\frac{V_m^{min}}{V_c} = \frac{(1-d)}{\bar{u}}\frac{F}{S} + d$, d being the relative volume in the ducts and flow tubes in the column.

The efficiency is much more dependent on the relative volume of the mobile phase than it is on the flow rate for the three instruments, and it generally increases with the relative volume of the mobile phase in the column.

As a result, the resolution decreases very slowly, or not at all, when F and V_m^{min} are increased.

INTRODUCTION

Countercurrent chromatography (CCC) is now a well known technique[1], and numerous applications can be found in the literature. Among the parameters which act upon the quality of a separation, the prime importance of the nature and amplitude of the centrifugal field, and of the physical properties of the biphasic system, has been fully studied, and improvements of the instrumentation have resulted from these works[1]. Only recently, the importance of the flow rate[2], a parameter that HPLC and CCC share, and the relative volume of the mobile phase in the column [3,4], a parameter which can be varied easily in CCC but not in HPLC, have been the subject of analytical investigations.

We recently reported[3,4] that there is a linear relationship between the minimum volume of the mobile phase in a CCC column V_m^{min}, and the applied flow rate,F. In the present paper we report that the slope of this line in the plot Vm/Vc *vs.* F/S, where Vm is the volume of mobile phase in the CCC column, Vc the volume of that column, F the flow rate, and S the cross sectional area of the channel where the chromatographic process takes place, allows comparison of the stability of the stationary phase for various CCC instruments, and gives an estimate of the average velocity of the mobile phase in a channel.

By independently varying of the relative volume of the mobile phase in the column and the flow rate in the three CCC columns, we demonstrated that there is a strong relationship between the efficiency and the relative volume of the mobile phase in the column; the influence of the flow rate is not evident, except when its variation is related to the relative volume of the mobile phase, for example when working at V_m^{min} conditions; in that case the shape of the curve representing variation of the resolution with the flow rate depends upon the instrument, and the resolution generally decreases slowly or not at all when F and V_m^{min} are increased.

EXPERIMENTAL

Experiments with the Ito multilayer separator extractor (P.C. Inc., Potomac, MD, USA), the CPC model LLN and the Series 1000 HPCPC (Sanki Engineering, Nagaokakyo, Kyoto, Japan), currently available in the U.S. from Sanki Laboratories (Mount Laurel, NJ, USA) have been already reported[3,4] . All the experiments were performed with the same biphasic system, hexane, 4% water in methanol, with the methanol-rich lower phase as the mobile phase. The instruments were operated according to their specifications, *i.e.*, ≈800 RPM for the Ito CCC, and with a pressure around 6 MPa (≈900 psi) for both the CPC model LLN and the HPCPC (this corresponds to 1100 and 1400 RPM for the two instruments, respectively, which provides comparable gravitational fields).

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 3, where their partition coefficients, and the methods for calculating 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

Comparison between instruments for the stability of the stationary phase :

As previously described, the CCC column can be filled with both stationary and mobile phases by various procedures, provided that the volume of the mobile phase is equal to or larger than a minimum volume called V_m^{min}, compatible with the actual flow rate F, V_m^{min} depending upon the biphasic system and the experimental conditions. Figure 1 shows a graph of Vm/Vc , F which has been used to study the three CCC columns. The plot Vm/Vc , F is useful to understand that there is a linear relationship between V_m^{min} and F :

$$\frac{V_m^{min}}{Vc} = aF + b \qquad \text{a in min/ml, b dimensionless} \qquad (1)$$

the slope of this line could be useful to compare the stability of the stationary phase of various biphasic systems for a given instrument, but it does not allow comparison between various instruments, as it does not account for the effect of the nature of the cross section of the channel though which the flow circulates.

A more interesting relationship will be $\frac{V_m^{min}}{Vc} = f\left(\frac{F}{S}\right)$, as discussed below. Figure 2 is a schematic of the situation in a channel in a steady state equilibrium, at V_m^{min} conditions : during the elapsed time dt, the volume introduced at the top of a channel will be $\boldsymbol{\nu}_m = F\,dt$; during this elapsed time, the mobile phase which was present near the inlet has been flowing down with a linear velocity, u, which is related to the nature of the biphasic system (the density difference, $\Delta\rho$, the viscosity of the stationary phase, η_s, the interfacial tension,σ), to the nature and the amplitude of the centrifugal field, G (constant or variable), and to the design of the channel, so that it leaves a volume S dh (dh = u dt) to the incoming mobile phase, F dt, this volume, S

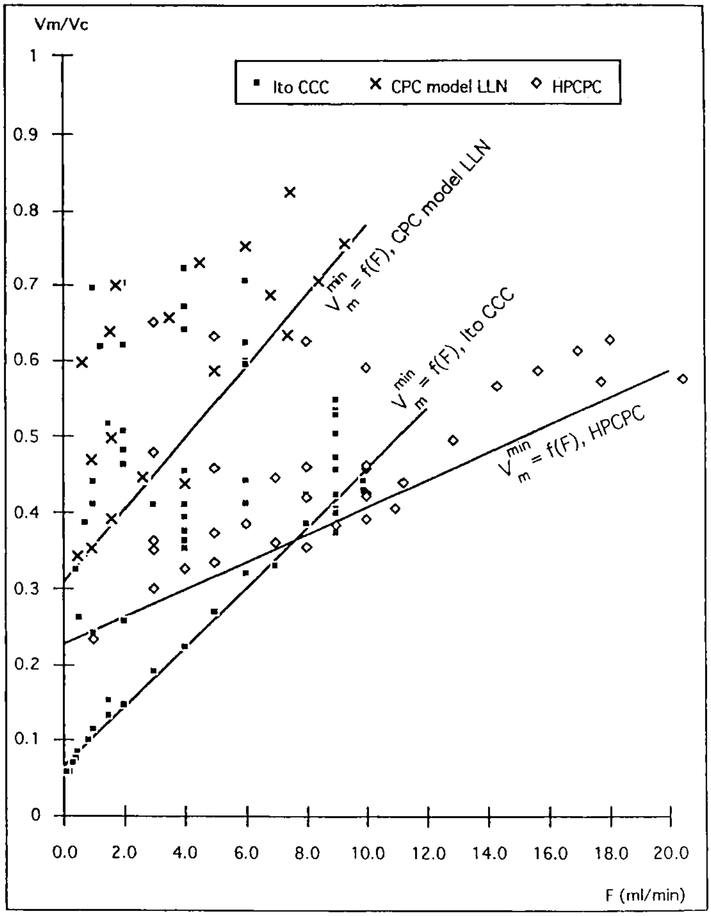
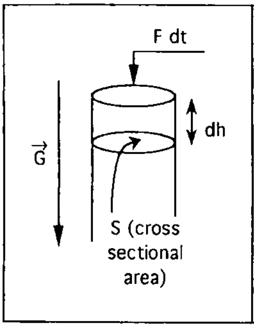


Figure 1 : Graph of the various points used to study the three CCC columns. The three lines correspond to the V_m^{min} conditions for the instruments.



$u = \frac{dh}{dt}$
(linear velocity in a channel, which is determined by various factors such as the nature of the biphasic system, the centrifugal field, and the hydro- dynamic properties of the channel)

$\nu_m = F\,dt$

$\nu_c = S\,dh$

$\nu_m + \nu_s = \nu_c$

Figure 2 : Steady state equilibrium in a channel at V_m^{min} conditions.

dh, being shared with the stationary phase in the ratio $\frac{\nu_m}{\nu_s}$ corresponding to the steady state equilibrium. The volume F dt is "sprayed out" as droplets or clouds or other form in the volume S dh, so that $\frac{F\,dt}{S\,dh}$ represents the relative volume of the mobile phase in S dh :

$$\frac{F}{S}\frac{dt}{dh} = \frac{\nu_m}{\nu_c} \tag{2}$$

If we summarize for a channel, then for all the channels,it becomes :

$$\frac{\nu_m^{min}}{\nu_c} = \frac{1}{\bar{u}}\frac{F}{S} + d' \tag{3}$$

where $\bar{u}$ is the average velocity in a channel, as it may be not constant in the whole channel[2] , and d' a constant from integration; limit conditions (for F = 0, the mobile phase is only in ducts and not in channels) lead to d' = 0. Adding the volume of the ducts, which are filled with the mobile phase only ($V_{ducts} = d\,V_c$, then $\nu_m^{min} = V_m^{min} - d\,V_c$, and $\nu_c = V_c\,(1-d)$), it becomes :

$$\frac{V_m^{min}}{V_c} = \frac{(1-d)}{\bar{u}}\frac{F}{S} + d \tag{4}$$

It should be noted that the notion of channel and duct has not been clearly defined for an Ito CCC column which comprises, simply, a coiled tube, under

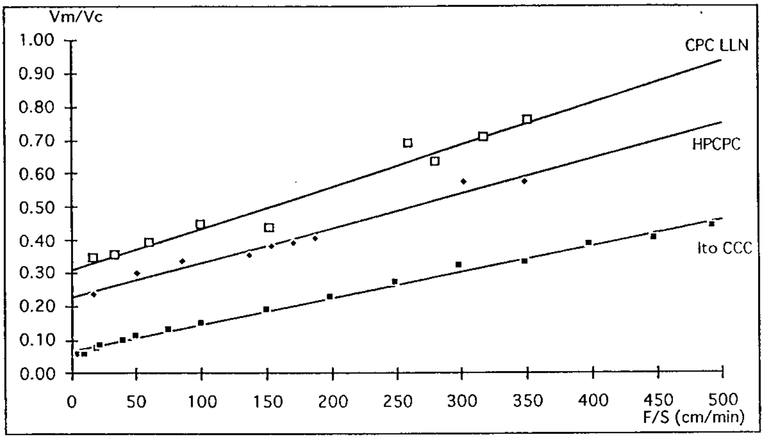


Figure 3 : Linear relationship $\frac{V_m^{min}}{V_c} = \frac{(1-d)}{\bar{u}} \frac{F}{S} + d$ for the three CCC columns.

CCC column	Vc (ml)	S (cm^2)	(1-d)/$\bar{u}$ (min/cm)	d	$\bar{u}$ (cm/sec)
Ito CCC	143	0.020	0.00079 (±0.00002)	0.066 (±0.0037)	19.7
CPC model LLN	108	0.026	0.00125 (±0.00008)	0.31 (±0.016)	9.2
HPCPC	240	0.059	0.00104 (±0.00006)	0.23 (±0.012)	12.3

planetary motion, but it may be understood as "partition volume (step) and transfer volume (step)" as in any chromatographic process.

We found experimentally that there is a linear relationship between $\frac{V_m^{min}}{Vc}$ and $\frac{F}{S}$, over a broad range of values of F, which means that $\bar{u}$ seems to be independent of F both for the Ito CCC (HDES, type J device) on one hand, and for the CPC model LLN and the HPCPC (HSES devices) on the other. Figure 3 shows the experimental results. The slope, $\frac{(1-d)}{\bar{u}}$, is an estimate of the average velocity of the mobile phase in the channels, and the higher is $\bar{u}$, the lower the variation of the volume of the stationary phase in the channels, for a variation of $\frac{F}{S}$, whatever the column is. This slope gives a good estimate of the stability of the stationary phase for various instruments, and allows comparisons between them. From Figure 3 we conclude that for the simple biphasic system hexane, 4%water in methanol, the stability of the stationary phase will be roughly the same for the Ito CCC, the CPC model LLN and the HPCPC, but the relative volume of mobile phase in the column will be different for a given $\frac{F}{S}$, because the relative volumes in the ducts and other connections, are different between these systems.

Remark :

1- the value $\bar{u}$ allows us to calculate what we could call the "Stoke radius of the mobile phase", *i.e.*, the radius of droplets of mobile phase falling in the stationary phase with the velocity $\bar{u}$ from the beginning to the end of a channel. For the HPCPC, with $\bar{u} \approx 12.3$ cm/sec, $G \approx 180\,\mathfrak{G}$, $\Delta\rho \approx 0.1$ g/cm^3 and $\eta_s \approx 0.47$ cP (ref. 5) , the droplets should have a radius $a \approx 37$ µm ($a = \sqrt{\frac{9\,\eta\,\bar{u}}{2\,G\,\Delta\rho}}$) ($\mathfrak{G} = 981$ cm/sec^2).

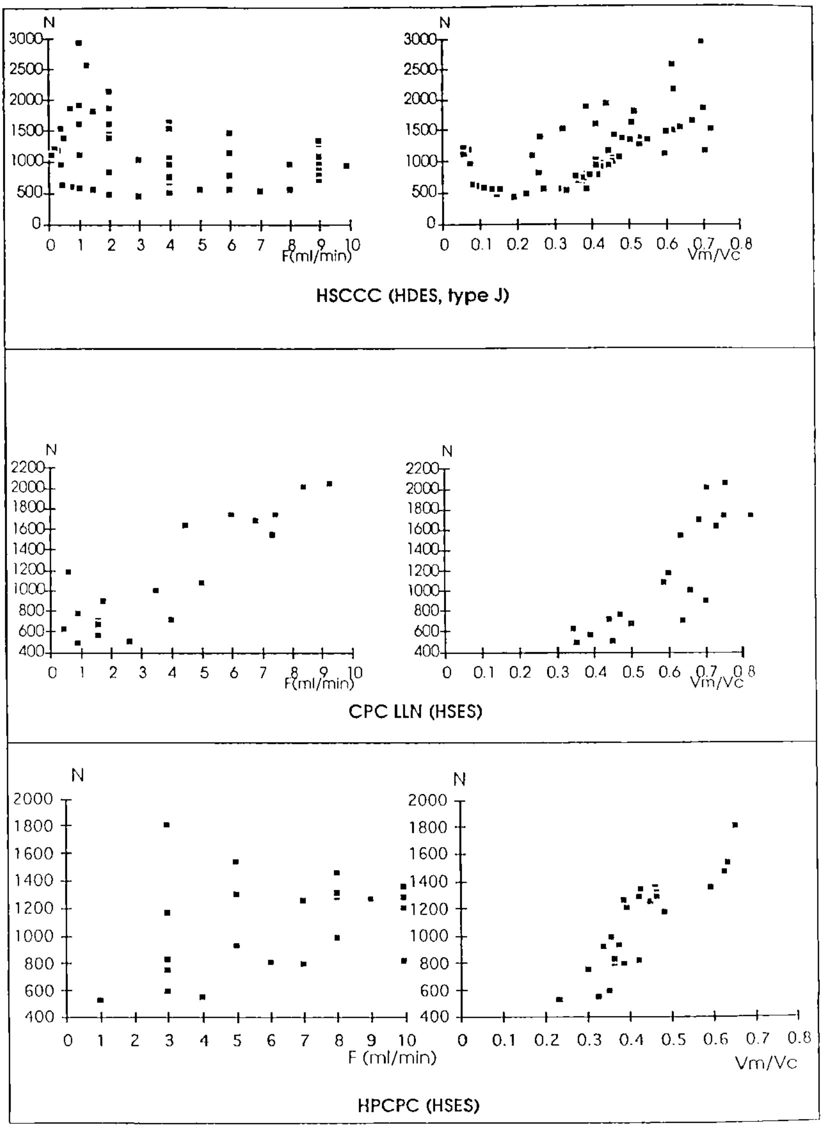
2- $\bar{u}$ should not be confused with u′ (see ref. 3 & 4), the average velocity in the CCC column (channels + ducts and connections) which is calculated from the retention time of a non-retained solute.

The slope of $\frac{V_m^{min}}{Vc} = f\left(\frac{F}{S}\right)$ should be useful to compare columns of various volumes and shapes.

Importance of Vm/Vc *upon* F *for the variation of the efficiency in Countercurrent chromatography :*

We have shown previously that there are various ways to study the evolution of efficiency and resolution in CCC, by independently varying the flow rate and the relative volume of the mobile phase in the CCC column. We highlighted, already, the important influence of the parameter Vm/Vc upon F for the variation of the efficiency with the HPCPC[4]. On Figure 4 left the efficiency (calculated on peak 1 for the three CCC columns) has been plotted against the flow rate, whatever the relative volume of the mobile phase in the column (*i.e.* $N = f(F, \forall \frac{Vm}{Vc}))^6$, and on Figure 4 right the same efficiency has been plotted against the relative volume of the mobile phase in the CCC column, whatever the flow rate (*i.e.* $N = f(\frac{Vm}{Vc}, \forall\ F)$).

The same results can be observed for the three CCC columns : the efficiency is much more dependent upon the relative volume of the mobile phase in the column than upon the flow rate. There is no apparent correlation at all between N and F, while the relationship between N and Vm/Vc appears to be a curve with a minimum for the Ito CCC column, and an increasing linear relationship for the CPC



HSCCC (HDES, type J)

CPC LLN (HSES)

HPCPC (HSES)

Figure 4 : Evolution of the efficiency (peak 1) with the flow rate, whatever the ratio of the mobile phase in the column (*i.e.* $N = f(F, \forall \frac{Vm}{Vc})$, left), and with the ratio of the mobile phase in the CCC column, whatever the flow rate (*i.e.* $N = f(\frac{Vm}{Vc}, \forall F)$, right), for the three CCC columns.

model LLN and the HPCPC. Since the efficiency is strongly correlated with the relative volume of the mobile phase, it is important to keep this parameter constant if the relation between another parameter, such as the flow rate or the rotational speed, and the efficiency, has to be found, otherwise the observed effects may not be due to the studied parameter, but to the uncontrolled variation of the relative volume of the mobile phase.

When experiments are restricted to the V_m^{min} conditions, where V_m^{min} and F are linearly dependent, one can either express the efficiency as a function of F or V_m^{min}

Since the correlation between N and Vm/Vc is not absolute, the flow rate must probably play a secondary role which has to be determined. It seems that a higher $\boldsymbol{\nu}$m/$\boldsymbol{\nu}$c in the channels favors better mass transfer between the two phases, because it increases the interface between the phases (the ratio in the channels must be understood as the actual $\boldsymbol{\nu}$m/$\boldsymbol{\nu}$c , corrected from the volume of the mobile phase in the ducts, *i.e.*, $\boldsymbol{\nu}$m = Vm - d Vc, $\boldsymbol{\nu}$c = Vc (1-d)).

Importance of Vm/Vc *for the variation of the resolution in countercurrent chromatography:*

The significant importance of Vm/Vc or Vm/Vs upon the resolution in CCC has already been reported[7,8,9], and we know that the resolution between two close peaks is related to the efficiency and to the volume ratio between the mobile and stationary phases by the relationship[3] :

$$Rs = \frac{1}{2}\sqrt{N}(\alpha - 1)\frac{K_1}{K_1(\alpha + 1) + 2\,V_m/V_s} \qquad (4)$$

where α is the separation factor (K_2 / K_1), K_1 the partition coefficient of the solute 1, and N is the number of theoretical plates.

The strong correlation between N and Vm, for the three columns, together with the supplementary term Vm/Vs which appears below the line, should result in a very strong correlation between Rs and Vm/Vc, whatever the flow rate, *i.e.*, $Rs = f\left(\frac{Vm}{Vc}, \forall F\right)$, for the three instruments.

Figure 5 shows the results obtained with the Ito CCC (top) and the HPCPC (bottom), for various flow rates in the range 0 to 10 ml/min. Plots linked together are those corresponding to the V_m^{min} conditions. Even if there are some isolated points occurring with the Ito CCC, and which confirm that the resolution is not totally

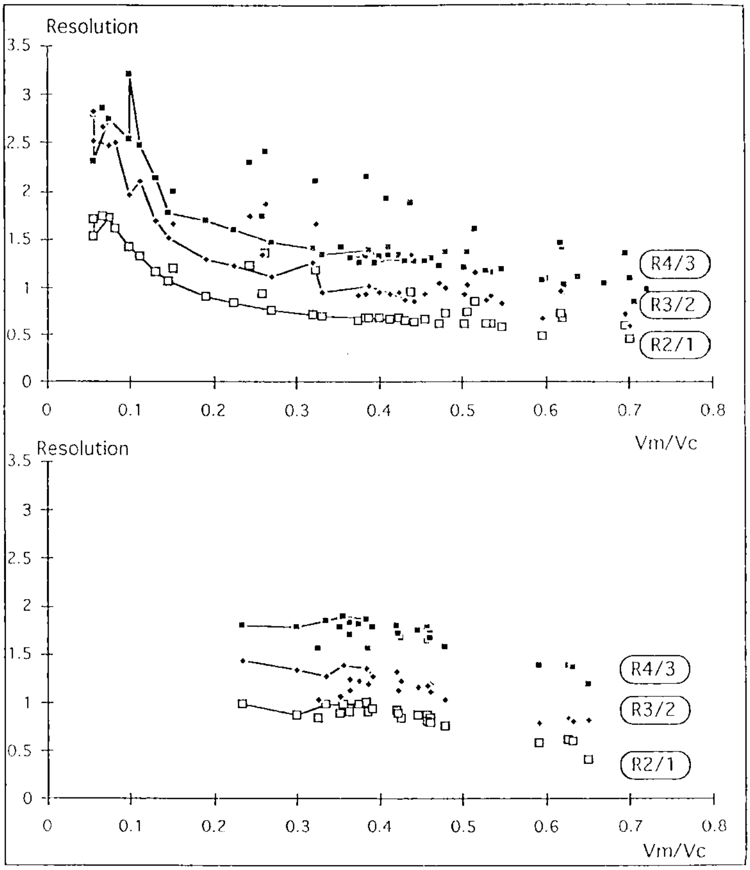


Figure 5: Variation of the resolution, Rs, with the ratio of the mobile phase in the column, Vm/Vc, whatever the flow rate, *i.e.* $Rs = f(\frac{Vm}{Vc}, \forall F)$; linked plots are those corresponding to the V_m^{min} conditions.
upper : Ito CCC column; lower : HPCPC column

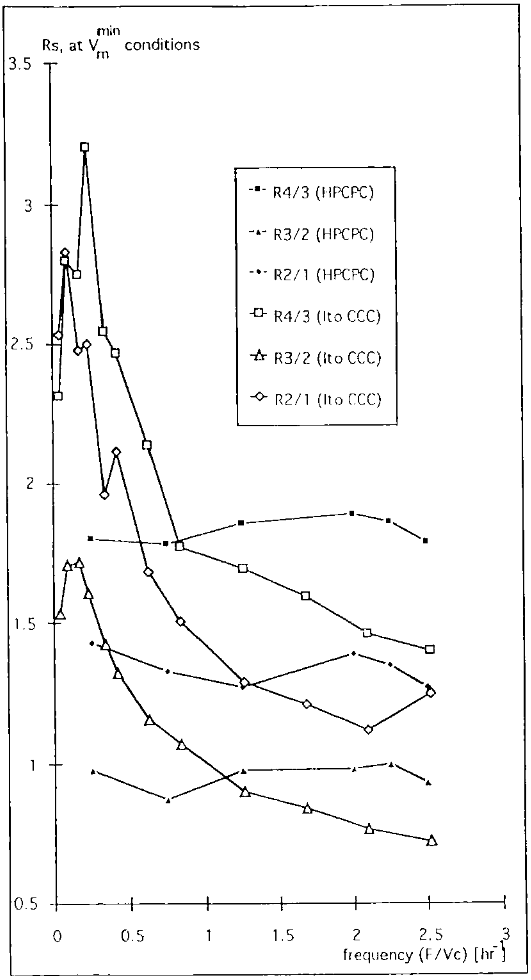


Figure 6 : Resolution at V_m^{min} conditions, plotted against the frequency of the separation (number of column volumes per hour), for the Ito CCC and the HPCPC columns. See Experimental for conditions. R4/3, R3/2, R2/1 means resolution between peaks 4 and 3, 3 and 2, 2 and 1, respectively.

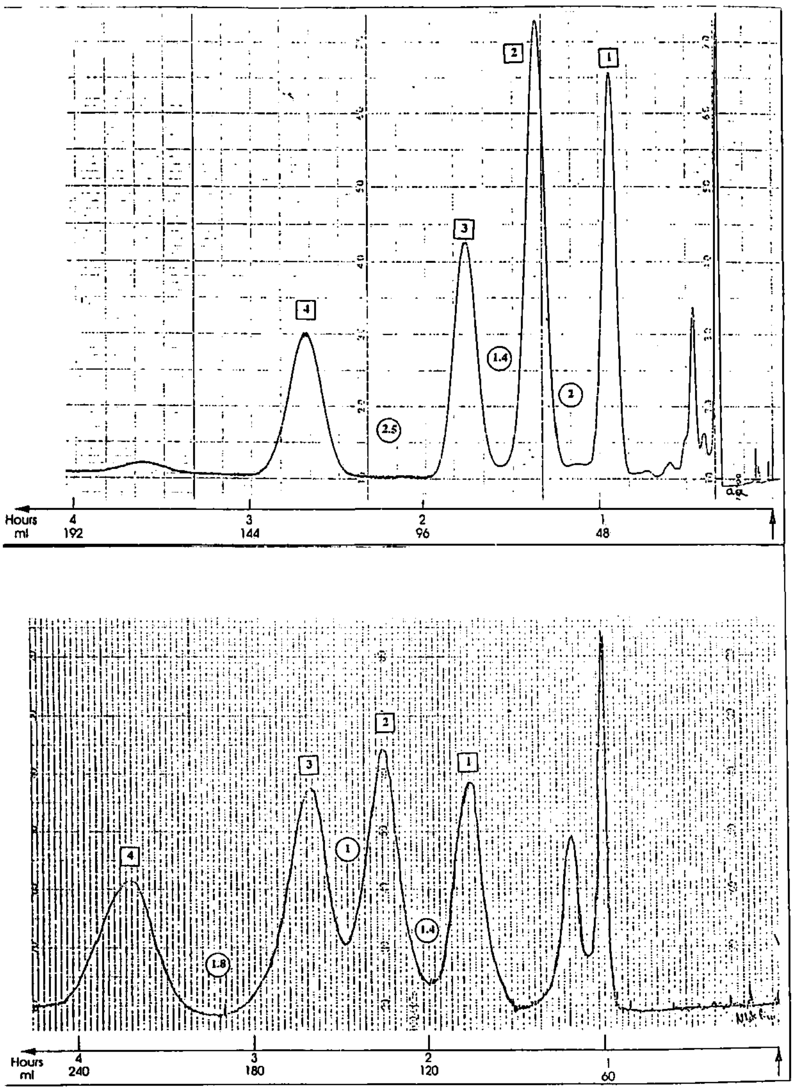


Figure 7 : Comparison between chromatograms which were run at V_m^{min} conditions and at low frequency (one run every 3 - 4 hours)

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 :	lower :
Column : Ito CCC	Column : HPCPC
Flow rate : 0.8 ml/min	Flow rate : 1 ml/min
V_m^{min} / Vc = 0.10	V_m^{min} / Vc = 0.23
F/Vc ≈ 0.33 hr^{-1}	F/Vc ≈ 0.25 hr^{-1}

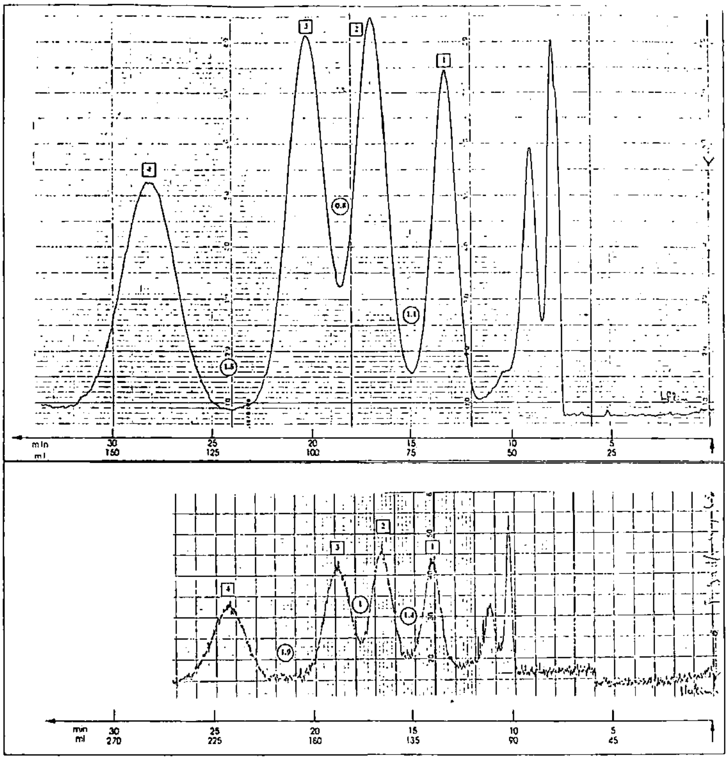


Figure 8 : Comparison between chromatograms which were run at V_m^{min} conditions and at higher frequency (one run every 25 to 30 minutes)

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 :	lower :
Column : Ito CCC	Column : HPCPC
Flow rate : 5 ml/min	Flow rate : 9 ml/min
V_m^{min} / Vc = 0.27	V_m^{min} / Vc = 0.38
F/Vc = 2.10 hr^{-1}	F/Vc = 2.25 hr^{-1}

independent of the flow rate, there is a strong correlation between Rs and Vm/Vc for the two columns, more accentuated for the HPCPC (HSES) than for the Ito CCC (HDES). As already shown[3] , resolution increases sharply for Vm/Vc < 0.2 for the Ito CCC, a value which cannot be reached with the HPCPC because of the ducts ($d = \frac{V_{ducts}}{V_c}$ = 0.228 for this device). For Vm/Vc> 0.2, the resolution slowly decreases when Vm/Vc increases, this decrease being more accentuated for the Ito CCC than for the HPCPC.

Resolution at V_m^{min} *conditions :*

Most CCC users are working at V_m^{min} conditions, to find the best combination of a small volume of mobile phase with a high flow rate, and they often adjust the composition of their biphasic system to get partition coefficients around 1 for compounds of interest. Since, for that value (K=1), the retention volume is exactly one column volume (Vr = Vm + K Vs), we found it interesting to compare the resolution given by instruments for a given number of column volumes per hour, *i.e.*, the frequency of the experiment. On Figure 6 are plotted the resolutions given by the Ito CCC and the HPCPC, against F/Vc, in hour^{-1} .

It clearly appears that, if the elapsed time for an experiment is not a critical factor, the Ito CCC column allows one to reach the best resolution for a low frequency (one run every five hours); this is because of the very low volume ratio of mobile phase we can reach with this instrument, at a very low flow rate.

When the elapsed time and throughput become significant (preparative & production scale), then the HPCPC allows one to acheive a better resolution because of its *quasi* independence with the flow rate at V_m^{min} conditions. We must note that resolution can be increased by increasing the rotational speed for the two instruments, so that the ordinate for the two sets of data can be varied, but the general shape will remain the same, *i.e.* a decrease of the resolution for an Ito CCC column, and relatively constant resolution for a HPCPC column. Figures 7&8 are two examples of chromatograms given by the Ito CCC and the HPCPC columns, at low frequency (one run every 3 to 4 hours), and higher frequency (one run every 25 min).

CONCLUSION

We have shown that plotting the ratio of the minimum volume of mobile phase we can obtain for a given flow rate, V_m^{min} / Vc, versus F/S, where F is the flow rate and S the section of the channel where the chromatographic process occurs, leads to a straight line for an Ito CCC planetary column (HDES, type J), and two CPC columns (HSES, type J non-planetary with rotary seals), the slope of which allows comparison between instruments for the stability of the stationary phase. This slope gives the average velocity of the mobile phase, $\bar{u}$, in a channel, which must not be confused with the average velocity of the mobile phase in the entire instrument ($u' = F\,L / V_m^{min}$), which includes ducts and connections where there is no stationary phase. Channels and ducts have not been clearly defined for an Ito CCC column, whereas they are geometrically fixed for the two CPC columns. This relationship, namely $\frac{V_m^{min}}{V_c} = \frac{(1-d)}{\bar{u}}\,\frac{F}{S} + d$, should be of interest for theoretical and practical development of CCC.

By independently varying the flow rate and the relative volume of the mobile phase, within the limits imposed by the properties of the biphasic system and the experimental conditions, we have shown that, for all the instruments, the efficiency is much more related to the relative volume of the mobile phase in the column than to the flow rate. For Vm/Vc < 0.2, which cannot be reached with the two CPC columns because of the ducts, the efficiency decreases when increasing Vm/Vc. For Vm/Vc>0.2 and for all the instruments, the efficiency increases with the relative volume of the mobile phase, which counteracts the loss of resolution which should result from that variation if the efficiency was a constant.

From the evolution of the resolution with the time needed for a separation, we conclude that it may be advantageous, when using a solvent system analogous to the one used in the present study (low interfacial tension, low viscosity), to use an Ito CCC column for analytical (small scale) purpose, where the duration is not a hindrance, and a HPCPC column for high throughput separations, because for this kind of column the resolution is constant when the flow rate is increased over a broad range.

Similar experiments have to be made with other solvent systems in order to get a complete understanding of how the evolution of the resolution with the relative volume of the mobile phase is modified by parameters such as the interfacial tension or the viscosity of the solvents.

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