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Publisher *Taylor & Francis*

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Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

Centrifugal Partition Chromatography: Stability of Various Biphasic Systems and Pertinence of the “Stoke's Model” to Describe the Influence of the Centrifugal Field Upon the Efficiency

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To cite this Article Foucault, A. P. , Frias, E. Camacho , Bordier, C. G. and Goffic, F. Le(1994) 'Centrifugal Partition Chromatography: Stability of Various Biphasic Systems and Pertinence of the “Stoke's Model” to Describe the Influence of the Centrifugal Field Upon the Efficiency', *Journal of Liquid Chromatography & Related Technologies*, 17: 1, 1 – 17

To link to this Article: DOI: 10.1080/10826079408013432

URL: <http://dx.doi.org/10.1080/10826079408013432>

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CENTRIFUGAL PARTITION CHROMATOGRAPHY: STABILITY OF VARIOUS BIPHASIC SYSTEMS AND PERTINENCE OF THE "STOKE'S MODEL" TO DESCRIBE THE INFLUENCE OF THE CENTRIFUGAL FIELD UPON THE EFFICIENCY

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ABSTRACT

The stability of various biphasic systems has been determined using a High Performance Centrifugal Partition Chromatograph, and its relationship with various physical parameters has been estimated. The stability seems to be related to $\frac{\gamma}{\Delta\rho}$, the ratio of the interfacial tension to that of the density difference between the two phases, and which is a parameter accounting for both the "Stoke's radius" of the mobile phase, *i.e.* the radius of droplets of mobile phase which will have the same average linear velocity, \bar{u} , in the channels than the experimentally determined value, and the "fragility" of these droplets. The average linear velocity of the mobile phase is not dependent upon the acceleration field in the range studied, which means that, using the "Stoke's model" (droplets, even if they do not exist), the higher the acceleration field, the smaller the droplets. Consequently, there is a linear relationship between the efficiency and the rotational speed, provided all other parameters are kept constant.

INTRODUCTION

Stability of the stationary phase is of first importance in Centrifugal Partition Chromatography, where it determines the utility of a biphasic system to

achieve a chromatographic purification. We have shown recently¹ that, by plotting the ratio of the minimum volume of mobile phase to the total volume of the column, we can obtain, for a given flow rate, V_m^{\min}/V_C , 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, the slope of which allows comparison of the stability of the stationary phase between instruments and biphasic systems. This slope gives the average linear velocity of the mobile phase, \bar{u} , in a channel, which must not be confused with the average velocity in the entire instrument which includes ducts and connections, where there is no stationary phase.

We have determined \bar{u} for various biphasic systems, using the High Performance Centrifugal Partition Chromatograph (HPCPC) already described², and have attempted to correlate these data with the physical data, *i.e.* the density difference $\Delta\rho$, the interfacial tension, γ , between the two phases, and the viscosities, η_{UP} and η_{LP} , of the upper and the lower phases for these biphasic systems. We found that the "Bond number"^{3,4}, B , which is a dimensionless number describing the relative importance of gravitational to surface tension forces, does not account for the CPC stability of the solvent systems, but it corresponds to the Ito classification of the so called "hydrophobic and hydrophilic systems"⁵, in countercurrent chromatography.

A better CPC classification for the solvent system stability in centrifugal partition chromatography has been presented using the parameter $\frac{\gamma}{\Delta\rho}$, which accounts for both the Bond number and the "Stoke's radius" of the mobile phase, *i.e.* the radius of droplets of mobile phase which will have the experimentally determined linear velocity \bar{u} .

Higher values of $\frac{\gamma}{\Delta\rho}$ correspond to biphasic systems with small Bond number and large droplets, resulting in a hydrodynamic system with fast and stable flow of the mobile phase into the stationary phase, while lower $\frac{\gamma}{\Delta\rho}$ corresponds to biphasic systems with a large Bond number and small droplets, yielding a hydrodynamic system with slow and fragile droplets which are easily broken during their course through the channels, thus favoring emulsification and destabilization of the stationary phase.

In the course of these studies, we discovered that, for a given biphasic system, \bar{u} was not dependent upon the rotational speed in the range ordinarily

used by chromatographers (c.a. $\omega > 500$ rpm). This means that, if we choose a very simple "Stoke's model" for the hydrodynamic behavior of the biphasic system in the HPCPC, i.e. one for which the mobile phase can be described as droplets with a "Stoke's radius", a , defined by $a = \sqrt{\frac{9 \eta_{SP} \dot{U}}{2 \Delta \rho g}}$, where η_{SP} is the viscosity of the stationary phase, and g the acceleration field, then there is a very simple relationship between that radius and the acceleration field, which is: $a^2 g = \text{Constant}$, for a given biphasic system.

As the interface between the two phases, and thus the efficiency of the chromatographic process, is directly related to the mutual dispersion of the phases, i.e. the number of droplets and their diameter, we will show that, if all other parameters are kept constant (particularly the flow rate and the ratio of the mobile phase in the CPC column), then the efficiency of the chromatographic process is linearly related to the rotational speed, i.e. to the square root of the acceleration field.

EXPERIMENTAL SECTION

Apparatus. A Series 1000 HPCPC (Sanki Laboratories, Mount Laurel, NJ, USA) was used². It is a bench top CPC (30 x 45 x 45 cm, ≈ 60 Kg); the column is a stacked circular partition disk rotor which contains 2136 channels with a total internal volume of 240 ml. The column is connected to the injector and the detector through two high pressure rotary seals containing a drilled sapphire rod passing 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 Series 1000 allows the HPCPC to be operated in either the descending or ascending mode. The HPCPC was connected to an HPLC System Gold (Beckman, San Ramon, CA, USA), including a solvent delivery pump Model 126, a diode array detector Model 168 with a semi-prep scale flow cell, and a manual sample injector. A few turns of a 1/16" steel tubing loop, immersed in a warm water bath ($\approx 40^\circ\text{C}$), was connected between the outlet of the HPCPC and the inlet of the detector, acting as a noise suppressor. Kinematic viscosities were measured at 23°C using Cannon-Fenske routine viscometers (Touzard & Matignon, Vitry/Seine, France). Interfacial tensions were measured at 23°C using the spinning drop method, with a Site 04 apparatus (Krüss, Palaiseau, France). Densities were determined by

weighing a 25 ml flask of each phase (average of 3 to 4 measurements). Solvent polarity has been estimated using the Reichardt's dye (Aldrich, catalogue number 27,244-2), following the procedure described by S. J. Gluck *et al.*⁶

Chemicals. The following solvents came from Prolabo (Paris, France) : dimethylsulfoxide, tetrahydrofuran, n-butanol, chloroform, methanol, n-propanol, heptane, acetone. The *sec*-butanol, methyl isobutyl ketone, and n-octanol came from Aldrich (Milwaukee, Wisconsin, USA). Water was de-ionized.

Procedure. Stability of the biphasic systems was estimated by varying the flow rate from 0 to 10 ml/min, or less if the backpressure in the HPCPC was reaching 6 MPa (≈ 0.86 kPSI), and measuring the volume V_m^{\min} previously defined; the rotational speed was generally 1000 or 1200 rpm, and some experiments were performed at different rotational speeds, to evaluate the influence of this parameter upon the stability of the stationary phase. All the systems were evaluated in ascending and descending mode except the system octanol / water, for which the ascending mode was not explored, because our pump was unable to pump octanol at high flow rates.

The experiment related to the efficiency was performed with 4-hydroxybenzoic acid as a marker of the void volume and diethyl phthalate as the analyte, in the descending mode using the heptane / methanol biphasic system.

RESULTS AND DISCUSSION

Twelve biphasic systems have been studied, which represent a wide range of physical and chemical properties (see Table I for abbreviations). EtOAc/Water and CHCl_3 /Water are not very useful for purification purposes, but they have a large interfacial tension; Oct/Water is used for partition coefficient determination, and its upper phase is rather viscous; HEP/MeOH is widely used as a non polar system; WDT2, 4 and 5 are a new class of medium polarity biphasic systems we introduced recently⁷, containing water, dimethylsulfoxide and tetrahydrofuran, and which show very good solvating properties; MIBK/AcO/W and CHCl_3 /MeOH/PrOH/W are medium polarity systems too, while the three butanol-containing systems are polar systems, widely used for purification of polar compounds, such as peptides.

Table I : Abbreviations used in this paper.

Abbreviations	Solvents and their volume ratio		
EtOAc/Water	Ethyl acetate Water, 50/50		
CHCl ₃ /Water	Chloroform Water, 50/50		
n-BuOH/Water	n-Butanol Water, 50/50		
sec-BuOH/Water	sec-Butanol Water, 50/50		
HEP/MeOH	Heptane Methanol, 50/50		
Oct/Water	Octanol Water, 50/50		
	Water	Dimethylsulfoxide	Tetrahydrofuran
WDT2 (see ref. 7)	11.7	26.3	62
WDT4	21.5	21.2	57.3
WDT5	24.5	16.2	59.3
BAW	n-Butanol Acetic acid Water, 40/10/50		
MIBK/AcO/W	Methylisobutyl ketone Acetone Water, 25/50/25		
CHCl ₃ /MeOH/PrOH/W	Chloroform Methanol n-Propanol Water, 29/38.7/6.5/25.8		

Table II groups the physical data for the biphasic systems, and their phase polarities, estimated through the Reichardt's $E_T N^{\delta}$. The density differences are in the range 0.07 to 0.14 g/cm³ for all the systems except those containing CHCl₃, and the interfacial tension is generally small (< 3 dyne/cm) except for three systems which show much higher values. Viscosities are in the range 0.5 to 4 cP, except for the upper phase of Oct/Water system, for which it is 7.1 cP.

Stability of the systems. As we recently reported¹, the stability of a biphasic system can be estimated by the slope of the line which links the minimum volume of mobile phase we can obtain for a given flow rate to that flow rate, or, in order to compare both phase systems and instruments, by the slope of the following equation :

$$\frac{v_m^{\min}}{V_c} = \frac{(1-d)}{\bar{u}} \frac{F}{S} + d \quad [1]$$

where v_m^{\min} , V_c , \bar{u} , F and S have been already defined, and d is the ratio of the volume of the ducts and connections to that of the column, where there is no stationary phase.

Table II : Physical and Chemical data of the Biphasic Systems

System ^(a)	$\Delta\rho$ g/cm ³	γ Dyne/cm	η cP		$E_T N$ ^(b)	
			Upper	Lower	Upper	Lower
EtOAc/Water	0.097	13.2	0.47	1.10	0.50	1
CHCl ₃ /Water	0.478	32.8	0.98	0.55	not determined	
HEP/MeOH	0.073	1.16	0.41	0.57	0.23	0.73
WDT2	0.102	0.59	0.72	2.37	0.45	0.55
WDT4	0.130	2.15	0.62	2.88	0.45	0.64
WDT5	0.115	1.26	0.72	2.60	0.48	0.67
MIBK/AcO/W	0.084	0.25	0.70	1.42	0.72	0.68
CHCl ₃ /MeOH/PROH/W	0.213	0.42	1.78	0.97	0.69	0.57
Oct/Water	0.137	8.5 ^(c)	7.10	0.80	not determined	
n-BuOH/Water	0.144	2.91	3.05	1.33	0.65	0.89
BAW	0.101	1.21	2.89	1.51	not determined	
sec-BuOH/Water	0.093	0.53	3.64	2.02	0.73	1

$\Delta\rho$ = density difference; γ = interfacial tension; η = viscosity

(a) The solvent systems are roughly sorted according to the Ito Classification in hydrophobic, intermediate and hydrophilic groups.

(b) $E_T N$ is the normalized Reichardt index, i.e. non-polar liquids have an $E_T N$ close to 0 and polar liquids have an $E_T N$ close to 1; bold numbers means the polarity is approximative, due to the insolubility of the Reichardt's dye in the corresponding phase

(c) from Handbook of Chemistry and Physics

Regression analysis for the twelve systems, in the descending and ascending modes (except for the Oct/Water system in the ascending mode) is shown in Table III, for various rotational speeds of the HPCPC column; the calculated linear velocity, \bar{u} , of the mobile phase in the channels, is shown too.

Three significant observations emerge from these results :

- We were very surprised to find that the slope of the regression line is not dependent upon the rotational speed of the HPCPC column, in the range ordinarily used by chromatographers (c.a. 500 to 2000 rpm). We tested the lower limit of this non-dependence with the system WDT4, in the descending mode, and found that it was still true at 400 rpm, while the stationary phase was no longer stable at 200 rpm. We did not check how the lower limit was system

dependent, since, as we will show below, it is always better for the chromatographer to work at higher rotational speeds.

- 2 • Even if we can describe a system as more stable or less stable, they are all stable enough to allow for chromatographic runs; for example we can calculate from the data of Table III that the retention of the stationary phase ($1 - V_m^{\min}/V_C$) will be 54% for $F = 5$ ml/min for the system $\text{CHCl}_3/\text{MeOH}/\text{PrOH}/\text{W}$ in the descending mode, and 41% for the same flow rate, for the system WDT2 in the ascending mode. For the system BAW, the retention of the stationary phase will be 57% and 40% for $F = 10$ ml/min in the descending and ascending mode, respectively. We will define as "very stable" the systems where the retention of the stationary phase is $> 60\%$ for $F = 10$ ml/min, as "less stable" those where the retention is $< 50\%$ for the same flow rate, and as "stable" the intermediates systems; dotted lines on Table III separate these categories.
- 3 • In a previous paper¹ we wrote that *d*, the ratio of the volume of ducts and connections to that of the column, should be a constant for the HPCPC since they are geometrically defined, but we see in Table III that *d* varies from 14% (WDT4 in the descending mode) to 33% ($\text{CHCl}_3/\text{MeOH}/\text{PrOH}/\text{W}$ in the descending mode) with an average of 22% for the descending mode, and 21% in the ascending mode. Using the dimensions given by the manufacturer (see experimental), we find that the ducts represent a total volume of ≈ 32 ml; adding 5 ml for the connections between disks and for the two rotary seals leads to a minimum value of $d \approx 15\%$, which is approximately the minimal value we find for the system WDT4 in the ascending mode. Higher values for *d* must be understood as a consequence of the geometry of the channels and ducts, and the way the mobile phase is injected in the channels, *i.e.* the nature of the passage of a continuous mobile phase, in the ducts, to a discontinuous mobile phase, in the channels, and of the inverse phenomenon. We must then define a "dynamic duct", which is the part of the column where there is no stationary phase, and thus no chromatographic process. The best way to measure the dynamic ducts for a CPC column and a biphasic system is to calculate the

Table III : Data for the linear regression analysis of the relationship : $\frac{v_m^{\min}}{V_c} = \frac{(1-d)}{\bar{u}} \frac{F}{S} + d$

Descending Mode : Lower Phase as Mobile Phase

System ^(a) (rot. speed , rpm)	$10^3 \frac{1-d}{\bar{u}}$ (min/cm) (10 ³ Std. error)	10 d (10 Std. error)	r ² (nb of exp.)	\bar{u} (cm/sec)	
CHCl ₃ /Water (700)	0.46 (0.05)	2.02 (0.05)	0.988 (3)	28.8	very stable
Oct/Water (1000)	0.52 (0.06)	2.23 (0.06)	0.988 (3)	24.8	
EtOAc/Water (1200)	0.64 (0.04)	2.65 (0.05)	0.984 (6)	19.0	
n-BuOH/Water (800, 1200)	0.96 (0.08)	2.13 (0.09)	0.96 (8)	13.6	
WDT5 (800, 1200, 1400)	1.1 (0.06)	2.26 (0.07)	0.96 (13)	11.7	stable
WDT4 (400, 1200, 1400)	1.1 (0.05)	2.28 (0.06)	0.99 (6)	11.9	
HEP/MeOH (1200)	1.3 (0.09)	2.3 (0.07)	0.986 (5)	9.6	less stable
BAW (1200)	1.3 (0.1)	2.1 (0.1)	0.985 (4)	9.8	
WDT2 (1000, 1400)	2.5 (0.2)	2.6 (0.1)	0.97 (9)	4.9	
sec-BuOH/Water (1200)	2.7 (0.2)	1.6 (0.1)	0.97 (10)	5.1	
MIBK/AcO/W (1000)	2.9 (0.4)	2.5 (0.5)	0.96 (4)	4.3	stable
CHCl ₃ /MeOH/PrOH/W (700)	3.16 (0.09)	1.9 (0.1)	0.997 (5)	4.3	

Ascending Mode : Upper Phase as Mobile Phase

System ^(a) (rot. speed , rpm)	$10^3 \frac{1-d}{\bar{u}}$ (min/cm) (10 ³ Std. error)	10 d (10 Std. error)	r ² (nb of exp.)	\bar{u} (cm/sec)	
CHCl ₃ /Water (700)	0.46 (0.01)	2.29 (0.01)	0.998 (3)	28.1	very stable
EtOAc/Water (1200)	1 (0.05)	1.48 (0.05)	0.990 (6)	14.2	stable
n-BuOH/Water (800, 1200)	1.2 (0.05)	1.54 (0.05)	0.990 (7)	11.8	stable
HEP/MeOH (1200)	1.5 (0.2)	1.9 (0.2)	0.988 (3)	9.0	
WDT5 (800, 1400)	1.8 (0.1)	1.8 (0.1)	0.97 (8)	7.53	
WDT4 (1200)	2.0 (0.3)	1.4 (0.4)	0.97 (3)	7.15	
sec-BuOH/Water (1200)	2.3 (0.1)	2.8 (0.2)	0.97 (8)	5.3	less stable
BAW (1200)	2.5 (0.2)	1.8 (0.2)	0.986 (5)	5.5	
CHCl ₃ /MeOH/PrOH/W (700)	2.6 (0.7)	3.3 (0.7)	0.81 (5)	4.3	stable
MIBK/AcO/W (1000)	2.8 (0.1)	2.9 (0.2)	0.988 (7)	4.3	deficient pumping
WDT2 (1400)	5.0 (0.6)	1.7 (0.5)	0.95 (5)	2.8	
Octanol/Water					

(a) Systems are sorted in order of decreasing stabilities, and the dotted lines make the separations between very stable, stable and less stable systems (see text).

$V_c = 240$ ml; $S = 0.059$ cm²; F from 1 to 10 ml/min.

slope and intercept of equation [1] by regression analysis. From our results, we find a dynamic duct of $\approx 22\%$ for most of the biphasic systems, the worst being 33% for the system $\text{CHCl}_3/\text{MeOH}/\text{PrOH}/\text{W}$ in the ascending mode. 22% instead of 15% corresponds to a layer of pure mobile phase of $\approx 8 \mu\text{l}$ in each channel, which is $\approx 88 \mu\text{l}$.

Correlation between the stability and the physical parameters. The exact nature of the flow of mobile phase into the stationary phase in the channels of the HPCPC column is still a matter of controversy, and a direct visualization of that flow will be very useful, as it has been already done for the Ito CCC apparatus⁸

As is usual in other fields (e.g. sedimentation of proteins in a centrifuge), we will adopt a very simple model to describe the hydrodynamic of the flow in a channel. We will imagine the mobile phase in a channel as droplets with an average radius, "a", corresponding to Stoke's Law. For the average linear velocity \bar{u} , we experimentally found :

$$\bar{u} = \frac{2 a^2 \Delta \rho g}{9 \eta_{\text{SP}}} \quad [2]$$

where $g = \omega^2 R$, R being the average radius of the HPCPC centrifuge, and ω the rotational speed, $\Delta \rho$ the density difference between the two phases and η_{SP} the viscosity of the stationary phase, which can be the upper or the lower one, depending upon the chosen mode.

The independence, for a given biphasic system and a given mode, of the average linear velocity of the mobile phase, \bar{u} , toward the acceleration field, g , leads to the simple relationship :

$$a^2 g = A \quad [3]$$

A being a constant for a specific system and mode; this means that the higher the acceleration field, the smaller will be the radius of the droplets of mobile phase in a channel. Comparing various biphasic systems, A will characterize the dispersion of the mobile phase in the stationary phase, since a smaller value of A means that, for a given acceleration field, the continuous mobile phase coming from the duct will break into many smaller droplets, while a larger value of A means it will break into few larger droplets. "A" may be called the dispersion term; its dimension is $\text{L}^3 \text{T}^{-2}$. From [2] and [3], we get :

$$A = \frac{9 \bar{u} \eta_{SP}}{2 \Delta \rho} \quad [4]$$

As the dimension of A is the same as that of the ratio $\frac{\gamma}{\Delta \rho}$ (i.e. L³T⁻²) where γ is the interfacial tension, we can then write the following equation :

$$A = B \frac{\gamma}{\Delta \rho} \quad [5]$$

where B is a dimensionless number. From [3] and [5] it becomes :

$$B = \frac{\alpha^2 g \Delta \rho}{\gamma} \quad [6]$$

Equation [6] can be used to compare B to the numerous dimensionless numbers we can find in the literature, and thus find out if it has already been described : B was defined in 1928 by W.N. Bond *et al.*³, and is called the Bond number⁴. It characterizes the relative importance of gravitational to surface-tension forces, and accounts for the fragility of a droplet. "A droplet of liquid in motion through another liquid differs in its behavior from a solid sphere in that it may (a) be deformed, (b) have a circulation set up within itself by the shearing effect of the relative motion of the two fluids. These effects upset the stability of the drop, causing it to oscillate about the spherical shape and eventually to burst into fragments or, at least, into smaller drops"⁴. From [4] and [5] we get :

$$B = \frac{9 \bar{u} \eta_{SP}}{2 \gamma} \quad [7]$$

Like A, B is not dependent upon the acceleration field. Table IV gives the values of A and B calculated with equation [4] and [7], for the twelve solvent systems, in the descending and ascending modes; systems are sorted in order of decreasing stability in the descending mode. Figure 1 shows the twelve systems with the Bond number corresponding to the descending mode, and sorted in order of increasing Bond number. From Table IV and Figure 1, we can conclude that B does not account for the stability of the systems in centrifugal partition chromatography, but it corresponds to the Ito classification of the so-called "hydrophobic and hydrophilic systems"⁵, which accounts for the hydrodynamic behavior of solvents systems in the Ito CCC apparatus. From Table IV, it seems that the stabilities of the solvent systems are related to both

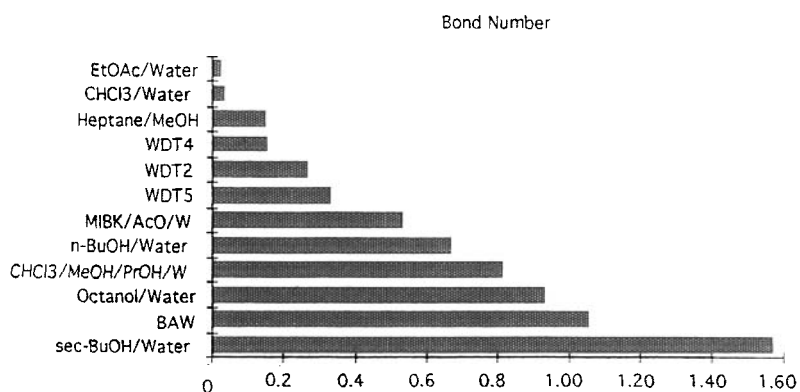


Figure 1 The twelve systems and their Bond numbers, corresponding to the descending mode.

Table IV : The dispersion term, A, and the Bond number, B.

System	Descending mode		Ascending mode	
	A	B	A	B
CHCl ₃ /Water	2.66	0.04	1.46	0.02
Octanol/Water	57.87	0.93	•	•
EtOAc/Water	4.14	0.03	7.28	0.05
n-BuOH/Water	13.54	0.67	4.70	0.23
WDT5	3.67	0.33	7.62	0.69
WDT4	2.56	0.15	7.15	0.43
Heptane/MeOH	2.42	0.15	3.18	0.20
BAW	12.64	1.06	3.69	0.31
WDT2	1.58	0.27	2.90	0.50
sec-BuOH/Water	9.04	1.57	5.14	0.90
MIBK/AcO/W	1.61	0.53	3.27	1.08
CHCl ₃ /MeOH/PrOH/W	1.61	0.82	0.88	0.45

The systems are sorted according to their decreasing stability in the descending mode.

A in $\text{cm}^3\text{sec}^{-2}$, B dimensionless

the dispersion of the mobile phase (A) and to the fragility of the droplets (B) : higher values of A (large droplets) combined with lower values of B (less fragility) result in very stable systems (e.g. CHCl₃/Water, EtOAc/Water); lower values of A (small droplets) combined with higher values of B (more fragility) yield less stable systems (e.g. MIBK/AcO/W, CHCl₃/MeOH/PrOH/W).

Table V shows the twelve systems sorted according to $\frac{A}{B} = \frac{\gamma}{\Delta\rho}$, a parameter which accounts both for the dispersion of the mobile phase and for the fragility of the droplets; Figure 2 shows the correlation between this parameter and the average linear velocities of the mobile phases in the channels. It can be seen from these data that $\frac{\gamma}{\Delta\rho}$ varies in the same way as the average linear velocities we experimentally determined.

This correlation could be interpreted as follows :

1. $\frac{\gamma}{\Delta\rho}$ small = A small and B large

Small and unstable droplets moving slowly, and easily broken into smaller ones, leading to some possible emulsification, dragging the stationary phase out of the column. These systems can be used at low flow rate, in order to minimize emulsification and keep a sufficient amount of stationary phase in the column.

2. $\frac{\gamma}{\Delta\rho}$ large = A large and B small

Large and very stable droplets moving fast; if so, rapid mass transfer between mobile and stationary phase is not favored for these systems, which should display poor chromatographic efficiencies.

3. $\frac{\gamma}{\Delta\rho}$ medium = A and B medium

This is the common case, and is the best suited for chromatographic applications; the droplets are small enough to allow for a reasonable rate of mass transfer between the two phases, and fast enough to keep a large amount of stationary phase in the column. Some emulsified layer may be present in the channels, like described by D. Armstrong *et al.*⁹, but this has no negative repercussion upon the stability of the stationary phase.

Efficiency and Rotational Speed. The improvement of the resolution between peaks by increasing the rotational speed of the CPC column has been recognized since the early beginnings of CPC¹⁰, even if the reason for this phenomenon was never systematically explored. Using our very simple model

Table V : Comparison of the parameter $\frac{\gamma}{\Delta\rho}$ and the experimental average linear velocities of the mobile phase, in descending and ascending mode.

System	$\frac{\gamma}{\Delta\rho}$	\bar{u} DM	\bar{u} AM
EtOAc/Water	136.51	19.0	14.2
CHCl ₃ /Water	68.62	28.8	28.1
Octanol/Water	62.04	24.8	
n-BuOH/Water	20.21	14.2	11.3
WDT4	16.61	11.9	7.2
Heptane/MeOH	15.96	9.6	9.0
BAW	11.97	9.8	5.5
WDT5	10.97	13.1	7.5
WDT2	5.84	4.9	2.8
sec-BuOH/Water	5.74	5.1	5.3
MIBK/AcO/W	3.02	4.3	4.3
CHCl ₃ /MeOH/PrOH/W	1.97	4.3	4.3

\bar{u} in cm/sec, $\frac{\gamma}{\Delta\rho}$ in cm³ sec⁻²

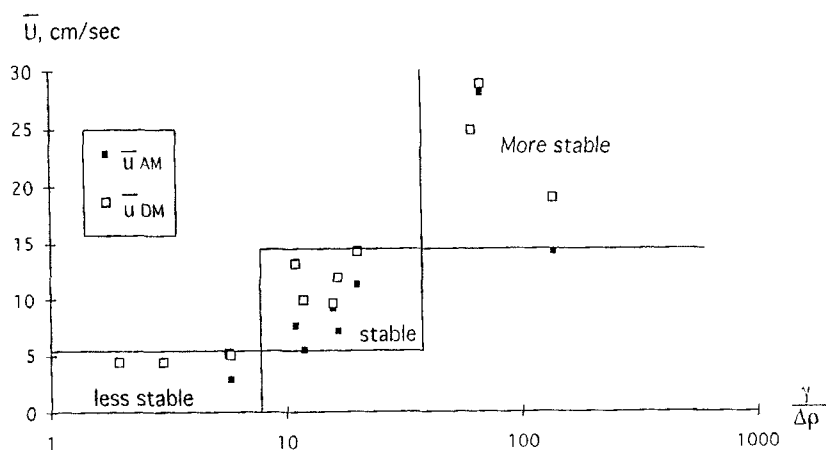


Figure 2 Correlation between the average linear velocity, \bar{u} , of the mobile phase in the channels of the HPCPC, and the parameter $\frac{\gamma}{\Delta\rho}$.

\bar{u}_{AM} = average linear velocity in the ascending mode

\bar{u}_{DM} = average linear velocity in the descending mode

to describe the hydrodynamics of the flow in a channel, *i.e.* the "Stoke's model", with droplets simulating spheres with a radius, "a", corresponding to Stoke's law, for the average linear velocity \bar{u} , we can express the interfacial area between the two phases as a function of the acceleration field as follows :

from equation [1], the volume of mobile phase in the channel (not in "dynamic" ducts and connections) is :

$$V_m^{\min} = V_m^{\min} - V_{\text{ducts}} = \frac{(1-d)}{\bar{u}} \frac{F}{S} V_C \quad [8]$$

Since the droplets have a surface $s = 4 \pi a^2$, a volume $v = 4/3 \pi a^3$, then the number of droplets in the channels is $n = V_m^{\min} / v$, and the interfacial area between the two phases in the channels is :

$$I = n s = V_m^{\min} \frac{3}{a} \quad [9]$$

From [3], [4], [8], and [9], we obtain :

$$I = (1-d) V_C \frac{F}{S} \frac{27}{2} \frac{\eta_{SP}}{\Delta\rho} A^{-3/2} g^{1/2} \quad [10]$$

where all terms are constant for a given system and a mode, except for F and g.

For a given biphasic system and a given flow rate, then the efficiency should be directly related to the interfacial area :

$$N \approx I \approx g^{1/2} \quad [11]$$

where N is the number of theoretical plates for a given peak.

We have tested equation [11] with the system n-Heptane/Methanol in the descending mode, the rotational speed being varied between 700 and 2000 rpm. The flow rate was kept constant and equal to 7 ml/min, and the volume of mobile phase, V_m , was 120 ± 4 ml, which is 27 ml more than the calculated V_m^{\min} , in order to keep this volume as constant as possible throughout the experiments, as it has been shown that this parameter has a strong influence upon the efficiency¹. V_m was determined using 4-hydroxybenzoic acid (non retained solute), and diethyl phthalate was the analyte, with a partition coefficient of 0.27 ± 0.01 . The number of plates has been estimated by using both the width at half height and at the base of the

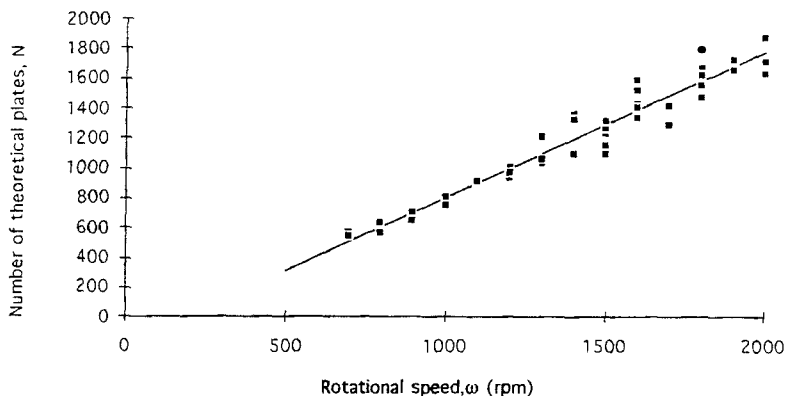


Figure 3 Relationship between N , the number of theoretical plates, and ω , the rotational speed of the HPCPC column, all other parameters being kept constant.

peak, and runs where the two values were not in close agreement were rejected. Runs where V_m was not constant were also rejected too. 59 out of 77 injections were taken into consideration.

Figure 3 shows the results of this investigation; as predicted by equations [10] and [11], based on the "Stoke's model", N is proportional to $g^{1/2}$, i.e. to the rotational speed ω , in the range ordinarily used by the chromatographer (500 to 2000 rpm).

Regression analysis leads to the equation :

$$N = 0.98 (0.03) \omega - 175 (40)$$

$$n = 59 \quad r^2 = 0.95 \quad s = 87 \quad \mathcal{F} = 1126$$

where n is the number of experiments, r the correlation coefficient, s the standard deviation, and \mathcal{F} the Fisher's test parameter (ω in rpm).

We conclude from these results that, even if it has not been proven that the mobile phase flows as droplets in the stationary phase, in the channels of the HPCPC column, we can use the simple "Stoke's model" to account for the dependence of the efficiency upon the rotational speed. The upper limit of this relationship should be reached when N approximates the number of physical plates, i.e. the number of channels, 2136 with our instrument. Figure 4 allows the

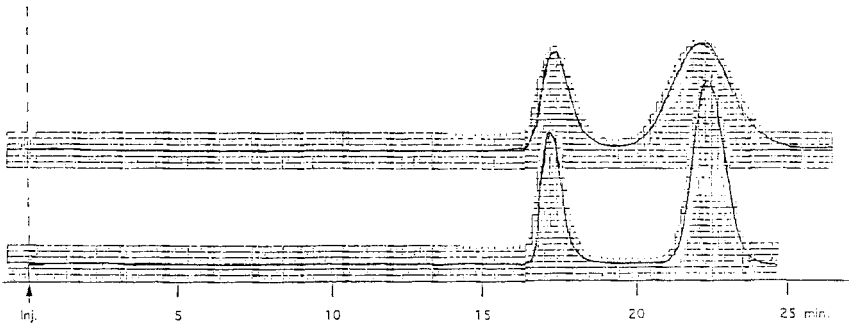


Figure 4 Comparison of HPCPC chromatograms obtained at 700 and 2000 rpm, all other parameters being kept constant.
 Solvent system : Heptane / Methanol, descending mode
 Unweighted but identical quantities injected
 Flow rate : 7 ml/min; $\frac{v_m}{V_c} = 0.5$ Upper : 700 rpm; lower : 2000 rpm

comparison of two chromatograms obtained at 700 and 2000 rpm, all other parameters being kept constant.

CONCLUSION

The "Stoke's model", which is very simple and simulates the mobile phase to droplets with an average radius calculated by using the Stoke's law and the average linear velocity deduced from the regression analysis of the dependence of the volume of the mobile phase in the CPC column with its flow rate, gave us a simple parameter ($\frac{\gamma}{\Delta\rho}$) to estimate the stability of a biphasic system in centrifugal partition chromatography, and is fully compatible with the evolution of the efficiency of the CPC column with the acceleration field. The best results will be obtained for higher rotational speed, whatever the system is, not because the retention of the stationary phase is higher, but because the dispersion of the mobile phase in the stationary phase becomes better.

The back pressure, which is a non-chromatographic parameter, and mainly due to hydrostatic pressure ($\Delta P \approx \Delta\rho g$)⁹ will be the major obstacle to achieve the best performance of a CPC apparatus. Instruments must be able to

work at high pressure, in order to use them with any biphasic system at higher rotational speeds to obtain as many theoretical plates as the column can yield.

ACKNOWLEDGMENT

C. Bocard and H. Seris, Institut Français du Pétrole, are gratefully acknowledged for allowing us to use their spinning drop apparatus and get the interfacial tension of our systems; their hospitality was greatly appreciated.

We thank Vegatec for the loan of an HPLC System Gold.

We thank Sanki Laboratories for the loan of a Series 1000 HPCPC.

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Received: August 17, 1993

Accepted: August 30, 1993