Classification of Countercurrent Chromatography Solvent Systems on the Basis of the Capillary Wavelength

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Solvent systems used for countercurrent chromatography (CCC) have been classified in three groups, defined by Ito from experimental observations of their flow behaviors in a countercurrent chromatograph. To achieve good retention of one phase of a solvent system when the other one is pumped in the column, it is necessary to know to which of these groups it belongs. As the physics of the interface of biphasic liquid/liquid systems is governed by the capillary wavelength, one expects the latter to be a key parameter for partly describing the two-phase flow inside a CCC column. Parameters derived from the capillary wavelength, the characteristic settling velocities, can also be calculated from the physicochemical properties of the phases and their interfacial tension. Their values are utilized for classification purposes and applied to previously investigated solvent systems as well as six new systems used in CCC. The classification obtained is compared with that provided by the observed flow behavior. It is found that the classification based on the characteristic settling velocity of the lighter phase into the continuous heavier one provides a reasonably reliable prediction of the experimental flow behavior in CCC.

Countercurrent chromatography (CCC) is a separation method based on the partition of solutes between two liquid phases, as they interact in a thin tube under centrifugal forces. Many different versions of CCC instruments have been developed over the last twenty years.[1] Some of them have been commercialized. In the last few years, two major CCC instrument types have received most attention due to their relatively large efficiency and speed capabilities. In these systems, the use of an intense centrifugal force field leads to a significant retention of one phase (called the stationary phase) even at large flow rates of the other phase (the mobile phase).

Basically, in one of these two systems (centrifugal droplet CCC, CDCCC), the column is made of numerous small channels connected in series, in which the flow of the mobile phase is parallel to the direction of the centrifugal force. In this system, the direction of the flow in the channels depends on the density of the mobile phase relative to that of the stationary phase (outward flow when the mobile phase has a lower density).

In the other system (high-speed CCC, HSCCC), the flow and field vectors are not parallel. The column is made of a Teflon tube helically coiled in one or several layers wound on a core sylinder (with radius *r*) rotating with an angular speed Ω around its own axis. This assembly (column and cylinder) is itself rotating with the same angular velocity in the same direction around the main axis of the system. *R* is the distance between the two parallel axes. The ends of the column can be labeled "head" or "tail", depending on both the type of helix (right-handed or left-handed) and the direction of rotation. It is found experimentally that in order to more or less retain the stationary phase in the column, the lighter phase has to be pumped either from the tail to the head or from the head to the tail, depending on the nature of the two-phase solvent system and on the β ratio, where $\beta = r/R$.[2] A classification of solvent systems, established by Ito according to their hydrodynamic behaviors in the HSCCC device,[3] is reported in Table 1.

The first group encompasses solvent systems for which the lighter phase has to flow toward the head of the column to ensure some retention of the heavier phase. Because the polarities of the two phases (one of which being water) are significantly different for all the investigated systems in this group, Ito labeled these solvent systems as "hydrophobic" systems. A second group of so-called "hydrophilic" solvent systems, for which the nonaqueous phase is quite polar, exhibits an opposite behavior (flow of the lighter phase toward the tail). A third group of "intermediate" systems exhibits a more complex behavior as they behave like hydrophobic systems for $\beta > 0.3$ and like hydrophilic systems for $\beta < 0.3$.

The detailed hydrodynamic behavior of two-phase solvent systems in CCC devices is extremely complex and is at the present stage very poorly understood. Yet it would be desirable to have good models of this flow behavior based on intrinsic physicochemical properties of solvent systems in order to optimize the separation and to select the operating conditions of the CCC apparatus, especially for the HSCCC device. This paper is a preliminary attempt toward this goal.

It is well-known that the physics of biphasic liquid systems in a gravitational field is controlled by the capillary wavelength. One therefore expects that this parameter plays a key role in

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(2) Sandlin, J. L.; Ito, Y. *J. Liq. Chromatogr.* **1985**, *8*, 2153–2171.
(3) Ito, Y. *J. Chromatogr.* **1984**, *301*, 377–386.

Table 1. Values of Interfacial Tension, Densities, Viscosities, and Experimental Settling Times for the Main Solvent Systems Used by Ito[a]

solvent systems (v/v)	surface tension δpure (dyn/cm)		interfacial tension γ (dyn/cm)				density (g/cm³) ρ1			ρ2			dynamic viscosity (cP) η1			η2			settling times (s) T		T'	
	δ1	δ2	δ1-δ2	lit.	I/C	lab	pure	IC	lab	pure	I/C	lab	pure	I/C	lab	pure	I/C	lab	I/C	lab	I/C	lab
Hydrophobic[b]																						
hexane/water (1:1)	18.4[d]	72.8[c]	54.4	51.1[d]	52	45.8	0.66	0.66	–	1.00	1.00	–	0.33	0.41	–	1.00	0.95	–	<1	1	8	10
ethyl acetate/water (1:1)	23.9[d]	72.8[c]	48.9	6.8[d]	31	6.6	0.90	0.92	–	1.00	0.99	–	0.46	0.47	–	1.00	0.89	–	15.5	23	21	25
chloroform/water (1:1)	27.1[d]	72.8[c]	45.7	31.6[d]	42	–	1.48	1.50	–	1.00	1.00	–	0.58	0.57	–	1.00	0.95	–	3.5	4.5	5.5	5.5
Intermediate[b]																						
hexane/methanol (1:1)	18.4[d]	22.5[c]	4.1	<2[f]	4	1.2	0.66	0.67	–	0.79	0.74	–	0.33	0.50	–	0.60	0.68	–	5.5	12.5	6	14
ethyl acetate/acetic acid/water (4:1:4)	–			–	16	3.5	–	0.94		–	1.01		–	0.76		–	0.81		15	13.5	16	14
chloroform/acetic acid/water (2:2:1)	–			–	12	–		1.35			1.12			0.77			1.16		29	34.5	27.5	34
n-butanol/water (1:1)	24.6[d]	72.8[c]	48.2	1.8[d]	3	1.9	0.81	0.85		1.00	0.99		2.95	1.72		1.00	1.06		18	19	14	13
n-butanol/0.1 M NaCl (1:1)	24.6[d]	72.9	48.3		4		0.81	0.85		1.00	0.99		2.95	1.66		1.01	1.04		16		14.5	
n-butanol/1 M NaCl (1:1)	24.6[d]	74.4[c]	49.8		5		0.81	0.84		1.04	1.04		2.95	1.75		1.10	1.04		23.5		21.5	
Hydrophilic[b]																						
n-butanol/acetic acid/water (4:1:5)	–			–	<1			0.90			0.95			1.63			1.40		38.5	33	37.5	30
n-butanol/acetic acid/0.1 M NaCl (4:1:5)	–			–	<1			0.89			1.01			1.68			1.25		32		30.5	
n-butanol/acetic acid/1 M NaCl (4:1:5)	–			–	1			0.88			1.05			1.69			1.26		26.5		24.5	
sec-butanol/water (1:1)	23.5	72.8[c]	49.3		<1		0.81	0.87		1.00	0.97		4.21	2.70		1.00	1.67		57	45	58	40
sec-butanol/0.1 M NaCl (1:1)	23.5	72.9	49.4		<1		0.81	0.86		1.00	0.98		4.21	1.96		1.01	1.26		46.5		49.5	
sec-butanol/1 M NaCl (1:1)	23.5	74.4	50.9		3		0.81	0.84		1.04	1.03		4.21	1.91		1.10	1.29		34		33.5	
New[b]																						
DMSO/heptane (1:1)	43.5[e]	20.4[d]	23.1	–		10.8	1.10[e]		1.08	0.66		0.65	2.24		2.16	0.41		0.40		3.5		3
DMF/heptane (1:1)	36.8	20.4[d]	16.4	–		3.1	0.95		0.89	0.66		0.66	0.92		0.80	0.41		0.41		5		2.5
toluene/water (1:1)	28.5[c]	72.8[c]	44.3	36.0		31.8	0.87		0.86	1.00		1.00	0.59		0.53	1.00		0.89		6		11
o-xylene/water (1:1)	30.3[c]	72.8[c]	42.5	36.1		28.4	0.88		0.88	1.00		1.00	0.62		0.74	–		0.89		6.5		20
methanol/acetic acid/heptane (1:1:1)	–		–			2.1			0.88			0.69			0.92			0.41		4.5		5
chloroform/ethyl acetate/water/methanol (2:2:2:3)	–		–			1.3	–		1.09	–		0.93	–		0.68	–		1.66		13.5		13.5

[a] Values obtained from ref 9 unless otherwise noted. lit, literature values; lab, our laboratory; I/C, Ito and Conway. The errors of the measurements carried out in our laboratory are as follows viscosity, ±0.01 cP; density, ±0.01 g/cm³; interfacial tension, ±0.1 dyn/cm. [b] Ito's classification. [c] Reference 10. [d] Reference 11. [e] Reference 12. [f] Reference 13.

the flow behavior in CCC. The capillary wavelength and derived parameters—the characteristic settling velocities—are calculated for various solvent systems used for countercurrent chromatography.

The classification methods based on these parameters are compared to those based on interfacial tension and on experimental settling time and to Ito's classification. In addition, six new solvent systems, not previously used, are studied to compare the four initial classification methods with the experimental observations. The advantages of each method are finally discussed.

THEORETICAL BACKGROUND

Capillary Wavelength. The dynamics of the macroscopic behavior of the interface of two immiscible liquids is governed by the evolution of waves. In the case of liquids extending far away from the interface, these waves are the capillary waves where the interfacial tension tries to prevent curvature of the interface at small curvature radii. Gravity can produce instability if the upper fluid is heavier. The linear relation between the temporal frequency and the wavelength of the waves is given from hydrodynamics by the following law, called the dispersion law:[4]

$$\langle \rho \rangle \omega_r^2 = \gamma\left(\frac{\Delta \rho g}{\gamma}k + k^3\right) \tag{1}$$

where ω_r is the real part of the temporal frequency, ω, of spatial undulations with the form

$$\Phi \approx \exp\{i(\omega t - kx)\} \tag{2}$$

where Φ is the amplitude of the undulations and x the distance along one direction of the interface from some reference point. The wavenumber $k = 2\pi/\lambda$ is related to the wavelength λ of the perturbation, g is the gravity acceleration, γ is the interfacial tension, and ρ_1 and ρ_2 are the densities of the lower and upper fluids, respectively, with $\langle \rho \rangle = (\rho_1 + \rho_2)/2$ and $\Delta \rho = \rho_1 - \rho_2$. From this law we can build with dimensional analysis a typical length to scale and renormalize almost all the problems related to capillary waves. Indeed a natural spatial frequency is obtained with $k_{cap} = (|\Delta \rho| g/\gamma)^{1/2}$. The associated wavelength is the capillary wavelength:

$$\lambda_{cap} = \frac{2\pi}{k_{cap}} = 2\pi\left(\frac{\gamma}{|\Delta \rho| g}\right)^{1/2} \tag{3}$$

This length is typically of the order of 1 cm for classical fluids in normal gravity. An equivalent definition is often used defining the capillary length:

$$L_{cap} = 1/k_{cap} \tag{4}$$

The dispersion relation for capillary waves in (1) is only valid when dissipation is excluded, as in very thick layers of fluid. In the case where one has a very thin layer of a fluid much more viscous than the other, dissipation is important, the relation (1) is no longer correct, and the dispersion relation is then[5]

$$\omega_i = -\frac{h^3 k \gamma}{3\eta}\left[\frac{\Delta \rho g}{\gamma}k + k^3\right] \tag{5}$$

with ω_i is the imaginary part of the temporal frequency, h the

thickness of the thin layer, and η the viscosity of this thin layer of viscous fluid. For relation 5 we recover the same spatial scale with λ_{cap}. The characteristic time, τ, for growth and formation of viscous small drops in layers of depth h is obtained from eq 5, when an instability exists ($\Delta \rho < 0$) and the maximum growth rate is at $k = k_{cap}/2^{1/2}$, so that

$$\tau = 12\eta\gamma/h^3\Delta \rho^2 g^2 \tag{6}$$

We are interested in the process of breaking of drops involved in the dynamics of phase migration. Many models explain the size and the characteristic times of the drops. For high and intermediate values of the Reynolds numbers, the physical main mechanism of disintegration is the pressure difference between the dynamic pressure induced by velocity fluctuations and the capillary pressure.[5] In the case of low Reynolds numbers ($Re \approx 1$), breaking of drops comes from surface instabilities and amplified oscillations of the capillary waves in the interface between the two phases.[6] The characteristic size of drops and bubbles is commonly determined by the dynamics of these capillary waves. So it seems reasonable to try to identify the occurrence of capillary instabilities and its signature with the capillary wavelength λ_{cap}.

The capillary wavelength λ_{cap} is a function of the value of the gravity acceleration. In systems submitted to rotation, centrifugal forces can modify this value and in consequence the value of this scale.

Characteristic Settling Velocity. In order to characterize the hydrodynamic behavior of immiscible liquids in CCC, Ito and Conway[7] have classified solvent systems according to the time required for the two phases to settle in a unit gravitational field after more or less vigorous shaking. If one assumes that this time corresponds to the time t_{sed}, required for a droplet of diameter d of liquid 2 to settle across a height H of a continuous phase 1, one has

$$t_{sed} = H/v_{sed} \tag{7}$$

where V_{sed} is the sedimentation velocity of the droplet, which is expressed in terms of the droplet friction coefficient f and of the force F_g exerted by the gravitational field with acceleration g on the droplet, as

$$v_{sed} = F_g/f \tag{8}$$

The force F_g is equal to

$$F_g = (\pi/6)d^3\Delta \rho g \tag{9}$$

and the friction coefficient is given by the Rybczynski–Hadamard equation[8] taking into account the liquid motion inside the droplet as

$$f = 3\pi d\eta_1\left(\frac{3\eta_2 + 2\eta_1}{3\eta_2 + 3\eta_1}\right) \tag{10}$$

where η_1 and η_2 are the dynamic viscosities of liquids 1 and 2 in equilibrium with each other. When η_2 is much larger than η_1, as is the case for a solid particle, one retrieves the classical Stokes equation ($f = 3\pi d\eta_1$), while, when $\eta_2 \ll \eta_1$

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(5) Reference 4: Chapter VIII.
(6) Clift, R.; Grace, J. R.; Weber, M. E. *Bubbles, Drops and Particles*; Academic Press: New York, 1978; Chapter 12.
(7) Ito, Y.; Conway, W. D. *J. Chromatogr.* **1984**, *301*, 405–414.
(8) Hadamard, J. C. *R. Acad. Sci.* **1911**, 1735–1738.

(case of a bubble rising in a liquid), one gets $f = 2\pi d\eta_1$. Combining eqs 8–10, one gets

$$v_{sed} = \frac{d^2 \Delta\rho g}{18\eta_1\left(\dfrac{3\eta_2 + 2\eta_1}{3\eta_2 + 3\eta_1}\right)} \qquad (11)$$

The dynamics of the sedimentation process can be described by assuming that the size of the droplet is of the order of magnitude of the capillary wavelength λ_{cap}. Writing $d = A\lambda_{cap}$, with A of the order of 1, one gets with eq 3

$$v_{sed} = \frac{2\pi^2 A^2}{9} \frac{\gamma}{\eta_1\left(\dfrac{3\eta_2 + 2\eta_1}{3\eta_2 + 3\eta_1}\right)} \qquad (12)$$

This equation shows that the sedimentation velocity does not depend on the intensity of the gravitational field and on the density difference between the droplet and the continuous phase. This arises from the fact that a change in the field intensity or in the density difference induces a change in the size of the droplets in such a way that the sedimentation velocity is constant. Equation 12 allows one to define two characteristic settling velocities. The first one, V_{low}, involves the fall of a droplet of the heavier phase (low) in the continuous lighter one (up). The second, V_{up}, deals with the rise of a droplet of the lighter phase into the heavier one:

$$V_{low} = \frac{\gamma}{\eta_{up}\dfrac{2 + 3(\eta_{low}/\eta_{up})}{3 + 3(\eta_{low}/\eta_{up})}} \qquad (13)$$

and

$$V_{up} = \frac{\gamma}{\eta_{low}\dfrac{2 + 3(\eta_{up}/\eta_{low})}{3 + 3(\eta_{up}/\eta_{low})}} \qquad (14)$$

γ is the interfacial tension of the two liquid phases, η_{up} is the dynamic viscosity of the lighter phase, and η_{low} the viscosity of the heavier phase.

The characteristic velocities are proportional to the reciprocal of the time of settling (or equivalently, the time of rising, depending on the sign of the density difference) across some given height H. It is interesting to note that, as V_{up} and V_{low} do not depend on g, these velocities do not depend on the selected angular velocity of rotation. This is because, as mentioned above, the field intensity influences in the same way the size of the capillary wavelength (or more precisely, its square) and the sedimentation velocity of a particle of a given size.

EXPERIMENTAL SECTION

Apparatus. In order to compare new solvent systems with those classified by Ito, we used a high-speed countercurrent chromatograph similar to the type J instrument used by Ito (see description of this system in ref 1). It consists of two Gilson Model 303 pumps (Villiers-le-Bel, France) for pumping the organic and the aqueous phases. The pumps were connected to a Model CPHV 2000 HSCCC system (derived from a Pharmatech 2000 apparatus; SFCC, Neuilly-Plaisance, France). It is equipped with three identical columns, symmetrically arranged around the orbital axis of rotation. They

are made of 1.60-mm-i.d. PTFE wound around their axis. They are mounted in series to give a total capacity of 112 mL. Because each column is made of several layers of coiled tube, β ranged from 0.32 to 0.70.

Interfacial tension measurements were achieved on a Wilhelmy balance (MGW Lauda Tensiometer, Lauda-Königshofen, Germany). All measurements were performed at 21 ± 1 °C.

A graduated cylinder of 10-cm height and 1-cm internal diameter was used for the measurements of settling times.

Solvents. All organic solvents were of HPLC grade. Dimethyl sulfoxide (DMSO), dimethylformamide (DMF), methanol, acetic acid, and n-heptane were purchased from Prolabo (Paris, France); all other organic solvents were from Rathburn (Chromoptic, Montpellier, France). Water was supplied by a MilliQ apparatus (Millipore, St. Quentin-en-Yvelines, France).

Measurement of Interfacial Tension. All glass vessels were washed and immersed in sulfochromic mixture for 6 h, washed with distilled water, and dried at room temperature for overnight. Solvent mixtures were allowed to stand for 6 h to ensure complete equilibrium of the two-phase systems at room temperature. Prior to measurements, the stirring piece of the Wilhelmy balance was carefully washed using a sulfochromic mixture. The measurements were achieved in the ascending mode, the stirring piece going from the lower phase to the upper one. They were repeated several times in order to obtain good precision.

Measurement of Settling Times. The settling time is the time required for a two-phase solvent mixture to be completely separated into two layers in a unit gravitational field. We used the same method described by Ito and Conway for their measurements.[7] The two-phase solvent system was first equilibrated in a separatory funnel at room temperature so that it was completely separated into two layers. A 2-mL aliquot of each phase was introduced into the graduated cylinder, which was then sealed with a glass stopper. Two different experiments were then done. The first consisted of gently inverting the cylinder five times and measuring the time T necessary for the solvent system to settle. The second involved five vigorous shakings of the cylinder and allowed the measurement of time T'. The measurements were repeated several times to compute mean values for T and T'.

Measurement of Viscosity. Viscosities were measured using a capillary viscometer. The kinematic viscosity ν (in centistokes) was computed using the formula

$$\nu = K(t - u) \qquad (15)$$

where K is the constant of the capillary tube ($K = 0.005$ cm^2/s^2) and t the observed time (in seconds). u is a correction value, given in tables (in seconds). The absolute viscosity η (in centipoise) is then obtained from the equation

$$\nu = \eta/\rho \qquad (16)$$

where ρ is the density of the phase (in grams per cubic meter). All measurements were performed at 21 ± 1 °C. As the relative variation of the viscosity with the temperature is typically 1%/°C, the precision of the temperature, ± 1 °C, is sufficient to ensure a high enough accuracy of the viscosity measurements for the classification purpose.

Determination of the Hydrodynamic Behavior of Solvent Systems. In order to classify new solvent systems among the three groups listed by Ito (described above), experiments were performed with the HSCCC device. The coil was first entirely filled with one phase. The apparatus was then rotated while the other phase was pumped into the column. The effluent from the outlet of the column was collected. The elution was continued until the total elution volume exceeded the column internal volume. The four possible elution modes (pumping either the lighter or the heavier phase, either from the head or the tail of the column) were implemented. The configuration for which the largest amount of stationary phase was retained was selected as the basis of classification of the studied solvent system within one of the three groups.

RESULTS AND DISCUSSION

Calculation of the Parameters. *Obtaining Physical Data.* The capillary wavelength involves two physical parameters which are the interfacial tension γ of the two immiscible phases and their density difference $\Delta\rho$. The characteristic settling velocity requires knowledge of the interfacial tension of the solvent system and the viscosity of each phase. Table 1 displays the values obtained from the literature[9-13] at a temperature of 20 °C, by Ito and Conway[7] and by our laboratory.

Three approximations of the interfacial tension can be made. The first value is given by the difference of the surface tensions δ of pure phases (that is, their interfacial tensions with air), shown in Table 1 for γ. The second way to calculate the interfacial tension is to carry out the same approximation as the first one but with the surface tension of each phase saturated with the other. This is the direct application of Antonoff's law stating that "The interfacial tension between two liquids, mutually saturated with each other, is equal or very approximately equal to the difference between the surface tensions of the two phases".[14] This method was chosen by Ito and Conway,[7] and the results (γ) are given in Table 1. We performed experiments based on the direct measurement of the interfacial tension of the two-phase system and the results are shown in Table 1. We also show values obtained from the literature[9-13] when available. The δ_1 values are rough calculations but are useful when compared to the δ_2 values. The bigger the difference between δ_1 and δ_2, the more miscible the phases. Apart from the hexane/water system, the values determined by Ito and Conway are not in good agreement with the literature γ values. Our experimental determinations closely agree with the data reported in the literature for all the investigated solvent systems. Consequently, further calculations of λ_{cap} and V use literature results when available. Otherwise, our laboratory values are applied, and when neither literature or laboratory values are available, Ito and Conway measurements are used.

We also show the densities of each phase in Table 1. In this case, as the values of the references are for pure phases and Ito and Conway measurements are for one phase saturated

with the other, there are some minor differences. Also viscosities come from references for pure solvents while their measurements involve equilibrated phases. The differences between the values show that the viscosity is more dependent than the density on the composition of each liquid phase. The last columns show the settling times measured by Ito and Conway and also our measurements obtained following the procedure they used and previously described.

The values of interfacial tensions, densities, viscosities and settling times displayed in bold-face type in Table 1 are those used for the following calculations of capillary wavelength and characteristic settling velocities and for the classifications based on interfacial tension and experimental settling times.

Calculation of the Capillary Wavelength. Equation 3 involves the gravity acceleration g. In HSCCC devices, the column is submitted to combined rotations around two different axes and consequently g must be replaced by the effective gravitational field g^*. The latter actually involves two fields. The first one is the constant gravitational field of the Earth, equal to $g = 981$ cm/s^2 and always directed toward the bottom. The second one is the centrifugal force field, continuously varying in intensity and direction. As in this study one is concerned with the ability of the capillary wavelength, and of derived characteristic settling velocities, to classify solvents systems, the absolute value of λ_{cap} is of minor importance. Consequently, the centrifugal force field is averaged by $R\Omega^2$, which allows comparison of various solvent systems using the relative numerical values of λ_{cap}. Studies involving the variation of g^* on the absolute value of λ_{cap} are currently under investigation in our laboratory. We introduce the x parameter, defined as the ratio of the centrifugal and gravitational effects:

$$x = R\Omega^2/g \qquad (17)$$

When x is small ($x \ll 1$), the Earth's gravitational field is the most important, $g^* \cong g$. In contrast, if x is large ($x \gg 1$), the centrifugal force field is the main one and we can approximate g^* with $R\Omega^2$. The x parameter is also known as the relative centrifugal force (RCF).[15] For the HSCCC device using the usual rotational speed, x is large ($x \cong 100$) so that the capillary wavelength is written as

$$\lambda_{cap} = 2\pi\left(\frac{\gamma}{g^*|\Delta\rho|}\right)^{1/2} = 2\pi\left(\frac{\gamma}{(xg)|\Delta\rho|}\right)^{1/2} \qquad (18)$$

The capillary wavelength of each solvent system can thus be calculated for the high-intensity force fields involved in HSCCC devices. All the computed λ_{cap} values at $x = 100$ are shown in Table 2.

The values show that the capillary wavelength for hydrophobic solvent systems is larger than that for the intermediate solvent systems and the latter is larger, for most cases, than that of hydrophilic systems. Small values of λ_{cap} lead indeed to smaller droplets of one phase in one another and thus increase the stability of the created emulsion. In other words, hydrophilic solvent systems easily lead to stable emulsions. Their stationary phase is consequently less retained in the column of the HSCCC device than the one of hydrophobic solvent systems. This phenomenon is well-known in CCC,[2]

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solvent systems (v/v)		λ_{cap} (cm)^a	charact settling veloc (m/s)	
			V_{low}	V_{up}
hexane/water	(1:1)	0.246	138.56	70.12
ethyl acetate/water	(1:1)	0.198	16.35	9.77
chloroform/water	(1:1)	0.159	42.02	63.36
hexane/methanol	(1:1)	0.083	2.79	2.18
ethyl acetate/acetic acid/water	(4:1:4)	0.142	5.49	5.22
chloroform/acetic acid/water	(2:2:1)	0.145	12.94	17.97
n-butanol/water	(1:1)	0.072	1.32	1.94
n-butanol/0.1 M NaCl	(1:1)	0.107	3.03	4.41
n-butanol/1 M NaCl	(1:1)	0.100	3.61	5.49
n-butanol/acetic acid/water	(4:1:5)	<0.090	>0.75	>0.84
n-butanol/acetic acid/ 0.1 M NaCl	(4:1:5)	<0.058	>0.74	>0.93
n-butanol/acetic acid/1 M NaCl	(4:1:5)	0.049	0.47	0.69
sec-butanol/water	(1:1)	<0.063	>0.47	>0.69
sec-butanol/0.1 M NaCl	(1:1)	<0.058	>0.64	>0.91
sec-butanol/1 M NaCl	(1:1)	0.080	1.96	2.69
DMSO/heptane	(1:1)	0.101	28.48	6.96
DMF/heptane	(1:1)	0.074	8.52	4.97
toluene/water	(1:1)	0.322	77.58	51.13
o-xylene/water	(1:1)	0.348	57.48	49.59
heptane/acetic acid/methanol	(1:1:1)	0.067	5.71	2.97
chloroform/ethyl acetate/water/ methanol	(2:2:2:3)	0.057	1.03	2.12

$^a x = 100.$

Figure 1. Ranges of interfacial tension for the hydrophilic, intermediate, and hydrophobic solvent system groups and six new solvent systems.

as the retention of stationary phase is higher for hydrophobic systems than that of intermediate systems, which is in turn higher than that of hydrophilic systems.

Calculation of Characteristic Settling Velocities. Equations 13 and 14 are used with the values of interfacial tension and viscosities given in Table 1. The values of V_{up} and V_{low} are all shown in Table 2.

The characteristic settling velocities for hydrophobic solvent systems are larger than those for the intermediate solvent systems, which are, in most cases, themselves larger than those for the hydrophilic systems. The stability of an emulsion created in a hydrophilic system is thus expected to be increased compared to that of a hydrophobic system. This is directly related to the lower retentions of stationary phase observed with hydrophilic solvent systems.

Exceptions are the chloroform/acetic acid/water system for which V_{up} is larger than the lowest V_{up} value of hydrophobic systems and the sec-butanol/1 M NaCl system for which both V_{up} and V_{low} exceed the corresponding lowest values of intermediate systems.

Classification and Prediction Using Physical Data. The understanding of the hydrodynamic phenomena involved in CCC columns is of major importance to rationalize the choice of solvent systems. Nevertheless, the pragmatic chromatographer is interested in knowing the direction in which the mobile phase has to flow to provide the highest retention of stationary phase for the solvent system he chose. This requires classification of the solvent system between the three groups previously defined prior to its use in a CCC device. Four different methods are described in this paper to classify the solvent systems used in CCC from their physical and chemical properties. A fifth one, based on the density difference, could have been used. However, the values given in Table 1 show that hydrophilic and intermediate solvent systems have very
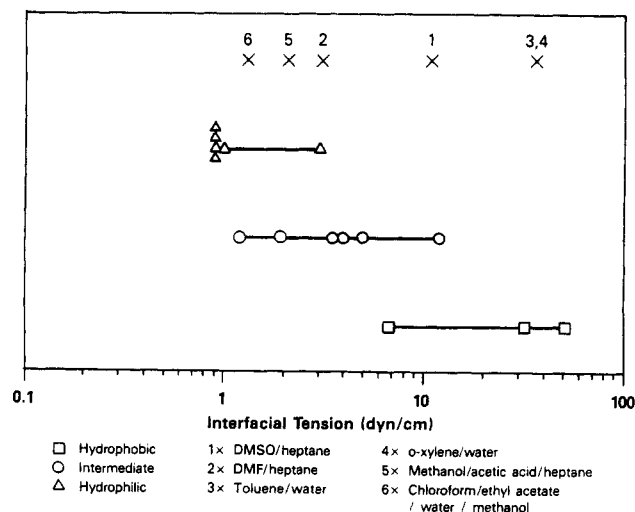
similar density differences, preventing that method from being considered as a classification method. The values of the interfacial tension, the experimental settling time, the capillary wavelength, and the characteristic settling velocity are plotted for all the solvent systems studied by Ito and Conway.[7] We determined that each physical parameter was able to classify the solvent systems and that each classification was correlated with that described by Ito. For each parameter, ranges were determined, related to the three groups of solvent systems previously defined. Six "new" solvent systems were studied. For each of them, the interfacial tension, the experimental settling time, the capillary wavelength, and the characteristic settling velocity were obtained to classify the solvent system using the ranges previously defined. As the groups they belong to are known from their hydrodynamic behaviors experimentally observed on the HSCCC apparatus, it was possible to compare the validity of each classification method.

Use of Interfacial Tension γ. Figure 1 shows the interfacial tension ranges for each group of solvent systems, on a logarithmic scale. In this figure, as well as in Figures 2–4, four different elevations have been arbitrarily assigned to the three groups and to the six new systems. For each group, one part of the range overlaps another. However, it is possible to give ranges of interfacial tension where no overlap occurs for the 15 investigated solvent systems:
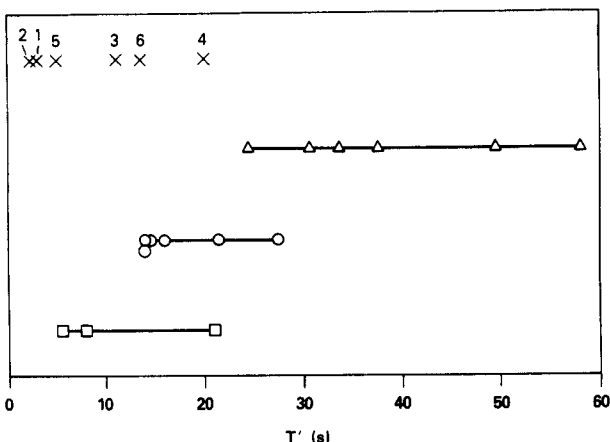
$\gamma \leq 1.2$	hydrophilic
$1.2 \leq \gamma \leq 3$	*hydrophilic or intermediate*
$3 \leq \gamma \leq 6.8$	intermediate
$6.8 \leq \gamma \leq 12$	*intermediate or hydrophobic*
$12 \leq \gamma$	hydrophobic

with γ expressed in dynes per centimeter. These range overlaps demonstrate that the classification based on γ is not reliable.

Use of Experimental Settling Times T and T′. Parts A and B of Figure 2 show the experimental settling time ranges for each group of solvent systems. Again, for each one, one part of the range overlaps another. This demonstrates that the classifications based on the times T or T′ (defined in the Experimental Section) are not reliable. From the behaviors of the 15 solvent systems, one can find some ranges which are
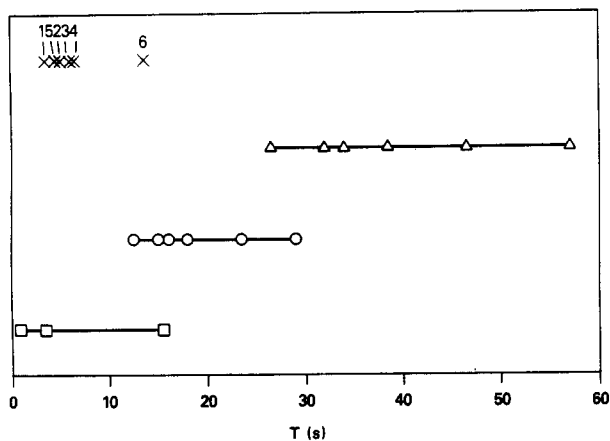
Figure 2. Ranges of experimental settling times T (A, top) and T' (B, bottom) for the hydrophilic, intermediate, and hydrophobic solvent system groups and six new solvent systems.

not ambiguous:

$29 \le T$	hydrophilic
$26.5 \le T \le 29$	*hydrophilic or intermediate*
$15.5 \le T \le 26.5$	intermediate
$12.5 \le T \le 15.5$	*intermediate or hydrophobic*
$T \le 12.5$	hydrophobic

and

$27.5 \le T'$	hydrophilic
$24.5 \le T' \le 27.5$	*hydrophilic or intermediate*
$21 \le T' \le 24.5$	intermediate
$14 \le T' \le 21$	*intermediate or hydrophobic*
$T' \le 14$	hydrophobic

where T and T' are in seconds. These two classification methods were previously proposed by Ito and Conway.[16]

Use of Capillary Wavelength λ_{cap}. As the classification method is based on the relative values of λ_{cap}, its results are independent on the x value. Formula 18 is therefore used with $x = 100$, as this value corresponds to the average centrifugal force field obtained in HSCCC devices. Figure
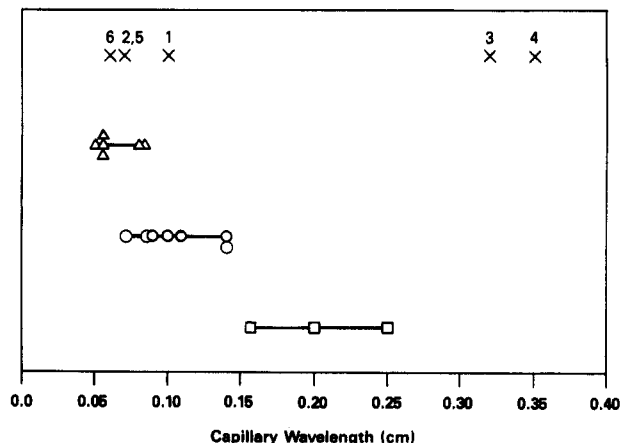
(16) Reference 1: p 369.

Figure 3. Ranges of capillary wavelength for the hydrophilic, intermediate, and hydrophobic solvent system groups and six new solvent systems.

3 displays the capillary wavelength ranges for the hydrophobic, intermediate, and hydrophilic systems. The hydrophilic and intermediate groups share a small range. This classification method is consequently more reliable than the previous ones as only one small range overlap occurs. It may allow one to determine the behavior of not previously studied solvent systems in an HSCCC column. The ranges are

$\lambda_{cap} \le 0.072$	hydrophilic
$0.072 \le \lambda_{cap} \le 0.090$	*hydrophilic or intermediate*
$0.090 \le \lambda_{cap} \le 0.145$	intermediate
$0.159 \le \lambda_{cap}$	hydrophobic

where λ_{cap} is in centimeters.

Use of Characteristic Settling Velocities V_{low} *and* V_{up}. The fourth way to classify the solvent systems among the three groups defined by Ito is to use the characteristic settling velocities. The ranges for the three solvent systems are gathered in Figure 4A,B, on a logarithmic scale. They demonstrate that the use of the characteristic settling velocity V_{low} is not a completely reliable classification method as the hydrophilic and intermediate groups share a small part of their ranges:

$V_{low} \le 1.32$	hydrophilic
$1.32 \le V_{low} \le 1.96$	*hydrophilic or intermediate*
$1.96 \le V_{low} \le 12.94$	intermediate
$16.35 \le V_{low}$	hydrophobic

However, V_{up} leads to a worse classification as the intermediate group overlaps the ranges of the hydrophilic and hydrophobic groups:

$V_{up} \le 1.94$	hydrophilic
$1.94 \le V_{up} \le 2.69$	*hydrophilic or intermediate*
$2.69 \le V_{up} \le 9.77$	intermediate
$9.77 \le V_{up} \le 17.97$	*intermediate or hydrophobic*
$17.97 \le V_{up}$	hydrophobic

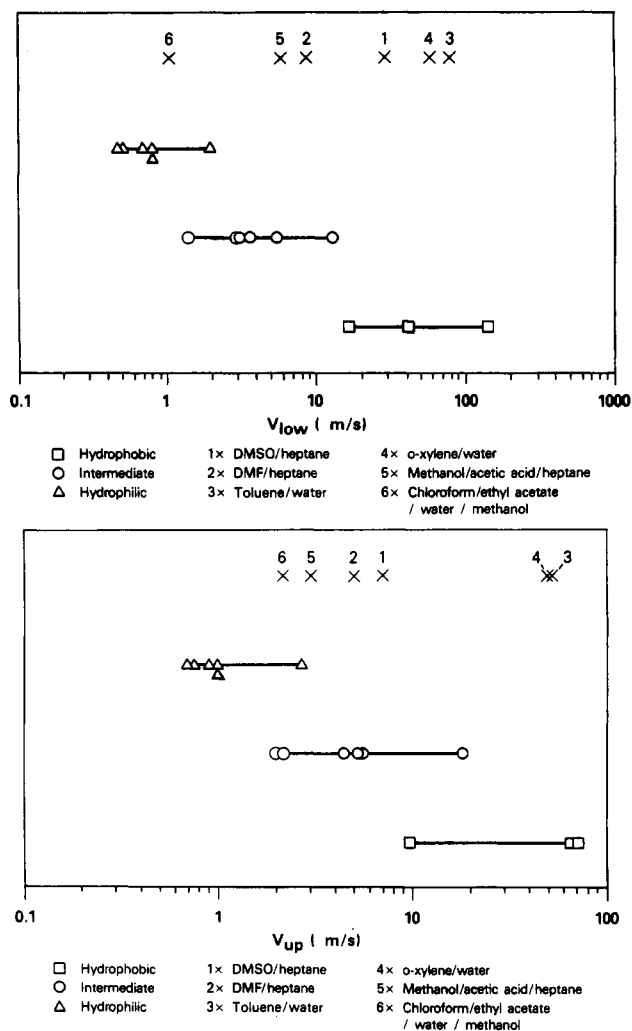where V_{low} and V_{up} are in meters per second.

Figure 4. Ranges of characteristic settling velocities for the hydrophilic, intermediate, and hydrophobic solvent system groups and six new solvent systems: (A, top) droplets of heavier phase falling in the lighter phase; (B, bottom) droplets of lighter phase going up in the heavier phase.

organic phase/water systems are very similar to those investigated by Ito and Conway. Two other solvent systems are completely organic: DMSO/heptane and DMF/heptane. The λ_{cap} and V_{low} and V_{up} parameters give the right prediction for both systems. The lower λ_{cap} value for the system containing DMF probably reflects the higher capability of this system to form an emulsion compared to conventional systems. In practice, the DMF/heptane system is less convenient for HSCCC than the DMSO/heptane system:[17] its retention of stationary phase is limited to 50%, which is lower than the average value (80%) for hydrophobic solvent systems. A problem occurs with the three-component solvent system heptane/acetic acid/methanol. The characteristic settling velocities give the right behavior whereas the capillary wavelength leads to the wrong prediction. The higher reliability of V_{low} and V_{up} compared to that of λ_{cap} may be explained from the physical definitions of these parameters given in the theoretical introduction. λ_{cap} reflects the dynamics of formation of droplets at the interface of the two liquid phases. V characterizes the dynamics of the migration of droplets of size λ_{cap}. Consequently, the characteristic settling velocity seems to be a better parameter than the capillary wavelength and thus better describes the hydrodynamic phenomena involved inside the CCC columns. The last new solvent system prepared from four solvents, chloroform/ethyl acetate/water/methanol, leads to wrong predictions for the classification methods based on λ_{cap} and V_{low}. One reason could lie in the difficulties met for measuring the interfacial tension, as the aqueous phase of this system is the upper one, contrary to the five other original solvent systems. An underestimation of γ lowers both λ_{cap} and V and leads one to consider the solvent system as more hydrophilic than it actually is.

CONCLUSION

Solvent systems were classified by four methods based only on their physicochemical properties. The use of interfacial

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Application to New Solvent Systems.

The above results show that λ_{cap} and V_{low} and V_{up} allow, to some extent, a reliable classification of the solvent systems studied by Ito. The values of γ, $\Delta\rho$, and η given in Table 1 for six original solvent systems allowed calculation of their corresponding λ_{cap} and V_{up} and V_{low} parameters, as displayed in Table 2. It was then possible to use the various ranges previously described to classify each new solvent system within the three groups defined by Ito. All the results are gathered in Table 3. The HSCCC device used for the determination of the hydrodynamic behavior of each solvent system had β values larger than 0.3 (which is the case for most commercial HSCCC devices). It is consequently impossible to differentiate intermediate and hydrophobic solvent systems, as indicated in the last column of Table 3. Since all the six new solvent systems behave like so-called hydrophobic or intermediate systems, wrong conclusions are drawn from the physical parameters investigated when a hydrophilic behavior (system 3 in Table 3) is predicted.

Among the six new solvent systems, two of them are made of an organic phase (toluene or o-xylene) added to water. All the classification methods predict the right behavior for these two systems, which is that of hydrophobic systems. These

(17) Bully, M. DEA de Chimie Analytique, Université Claude Bernard, Lyon I, 1990.
(18) Rolet, M.-C. Thèse de Chimie Analytique, Université Paris VI, 1993.

tension is most straightforward, but the important overlaps between the three solvent system groups may lead to ambiguous conclusions. The classification based on experimental settling times also involves overlaps. Furthermore, the determination of these times is largely experimental and hardly reproducible (see the significant differences experimentally found between Ito and Conway determinations and our values in Table 1). Therefore, even if this method gives the right predictions for the six original solvent systems, it cannot be used as a reliable classification. The capillary wavelength method separates two of the three ranges, but the results for new solvent systems do not correlate well with the experimental observations. The characteristic settling velocities V_{up} and V_{low} again involve overlap of ranges, but the results obtained correlate better with experiments than those deduced from the use of capillary wavelength. The V_{up} parameter allows the right prediction of classification for the six new solvents. The reason probably lies in the fact that the capillary wavelength is an indicator of the size of the droplets which may be formed in HSCCC while the characteristic settling velocities reflect the dynamic behavior of the droplets in a gravitational or centrifugal field.

However, the capillary wavelength or the characteristic settling velocity V_{low} does not predict the hydrophobic-like behavior of the chloroform/ethyl acetate/water/methanol system. This illustrates the complexity of the hydrodynamic phenomena occurring in the HSCCC column. Indeed, the methods are based on the determination of the mobilities of the droplets in the field but do not take into account the characteristic time of formation of these droplets. The latter is assumed to be small compared to the characteristic settling times in the cross section of the column. The characteristic time for droplet formation may be significantly different in the case of a system with a fairly low interfacial tension.

It is clear that the hydrodynamic phenomena occurring in HSCCC, where the field is varying along the path of a given fluid particle, cannot be explained by the simple model described above. Nevertheless, the globally satisfying predictability of the classification method based on parameters derived from the capillary wavelength provides good confidence that the basic physical approach underlying this method describes the essential features controlling the hydrodynamic behavior of a two-phase solvent system in HSCCC.

In the present study, we have explored the concept of capillary wavelength for the purpose of classifying solvent systems in HSCCC. One can expect that models based on this concept will allow the description of other dynamic aspects of CCC (such as retention of stationary phase, band-broadening phenomena, etc.) both in HSCCC and in CDCCC. Work in this direction is in progress.[18]