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## EXPERIMENTAL OBSERVATIONS OF THE HYDRODYNAMIC BEHAVIOR OF SOLVENT SYSTEMS IN HIGH-SPEED COUNTER-CURRENT CHROMATOGRAPHY

### III. EFFECTS OF PHYSICAL PROPERTIES OF THE SOLVENT SYSTEMS AND OPERATING TEMPERATURE ON THE DISTRIBUTION OF TWO-PHASE SOLVENT SYSTEMS

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#### SUMMARY

Statistical studies were made to correlate the hydrodynamic behavior of two-phase solvent system in counter-current chromatography (CCC) to their physical properties including interfacial tension, viscosity, and the difference in density of the two phases. Settling time measured under unit gravity provided a reliable numerical index for the hydrodynamic behavior of the solvent systems in a centrifugal force field. Viscosity and settling time were strongly correlated (correlation coefficient,  $r = +0.88$ ) while interfacial tension ( $r = -0.65$ ) and phase density difference ( $r = -0.45$ ) showed moderate and weak correlation, respectively. Studies of the effect of temperature on settling time as well as a preliminary apparatus operated at higher temperature show that raising the temperature will improve the performance of high-speed CCC.

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#### INTRODUCTION

Studies of the hydrodynamics of high-speed counter-current chromatographic (CCC) systems described in Part II (see ref. 2; for Part I, see ref. 1) have demonstrated that the two-phase solvent systems display characteristic behavior related to the hydrophobicity of the non-aqueous phases. The hydrophobic solvent systems exhibit a strong hydrodynamic trend in the coiled column where the two solvent phases are unilaterally distributed in such a way that the upper phase is always directed toward the head and the lower phase toward the tail. The hydrophilic solvent systems show the opposite hydrodynamic tendency, with the upper phase being directed toward

the tail and the lower phase toward the head. In the solvent systems with moderate hydrophobicity intermediate between the above two extremes, the hydrodynamic tendency is sensitive to the centrifugal conditions. In a system having a column of small helical diameter with a large radius of revolution, the hydrodynamics of the intermediate solvent group approaches that of the hydrophilic solvent group while with reversed conditions (large helical diameter and small radius of revolution) the hydrodynamics becomes quite similar to that of the hydrophobic solvent group.

In the present studies, efforts were made to correlate the hydrodynamics of the two-phase solvent systems with three major physical properties: interfacial tension, viscosity, and the difference in density between the two phases. Measurement of the settling time of the two-phase solvent systems under unit gravity gave a parameter which provided a reliable numerical index which correlated with the hydrodynamics observed for the solvent in a centrifugal force field.

## EXPERIMENTAL

### *Two-phase solvent systems*

The present studies were performed using the same set of fifteen two-phase solvent systems (Table I) employed in previous studies (see Experimental in Part II; ref. 2).

### *Measurement of physical properties of the two-phase solvent system*

Major physical properties of the solvent systems including density, viscosity, and interfacial tension were measured using conventional methods as described below. The densities of the upper and the lower phases in each solvent system were measured using a set of hydrometers (Fisher Scientific, Pittsburgh, PA, U.S.A.) and the density difference between the two phases was calculated. The viscosity of each solvent phase was measured using a falling-ball type viscometer (VWR Scientific, Baltimore, MD, U.S.A.). A modified Wilhelmy balance was used to determine the surface tension of each solvent phase against air, using water (72 dyne/cm) and hexane (30 dyne/cm) for standardization of the instrument for aqueous and organic phases respectively. The interfacial tension was calculated as the difference in surface tension between the upper and the lower phases of each solvent pair.

All measurements above were performed at room temperature of  $22 \pm 1^\circ\text{C}$ .

### *Measurement of settling time of the two-phase solvent system*

In order to correlate the hydrodynamic phase distribution in the rotating coiled column to the physical properties of the solvent system, simple tests were performed to measure the settling time of the two solvent phases, *i.e.*, the time required for the solvent mixture to be completely separated into two layers in a unit gravitational field. Each two-phase solvent system was first equilibrated in a separatory funnel at room temperature and separated into two phases. A 2-ml aliquot of each phase, total volume 4 ml, was delivered to a 5-ml graduated cylinder which was then sealed with a glass stopper. The solvents in the cylinder were gently mixed by inverting the cylinder five times and the cylinder was immediately placed on a flat table in an upright position and the time required for the two phases to settle was measured. The measurement was repeated several times to obtain a mean value. The data were found

to be fairly reproducible and the variations were usually within a few seconds. After the above measurements were completed, a second set of tests was performed in a similar manner except that the cylinder was vigorously shaken five times to see the tendency for emulsification. The test was again repeated several times to obtain a mean value for each solvent system.

For the butanol solvent systems, additional measurements were obtained at raised temperatures ranging from the room temperature up to 80°C. The settling times were similarly measured after the two-phase solvent system was thoroughly equilibrated at the desired temperature which was maintained by immersing the container in a water bath.

## RESULTS AND DISCUSSION

### *Three physical properties of the two-phase solvent system*

As described earlier, conventional two-phase solvent systems may be classified into three categories according to their stationary phase retention profile and hydrodynamic behavior in a rotating coiled column. The hydrophobic solvent group, characterized by high hydrophobicity of the non-aqueous phase, has a strong hydrodynamic tendency for the upper phase, either the non-aqueous or the aqueous phase, to unilaterally distribute on the head side of the column. The hydrophilic solvent group, characterized by high hydrophilicity of the non-aqueous phase, exhibits the reverse hydrodynamic behavior with the lower phase being distributed on the head side. The rest of the solvent systems with moderately hydrophobic non-aqueous phases belong to an intermediate solvent group and display transitional behavior where either the upper or the lower phase can occupy the head side of the column depending on the centrifugal conditions as expressed by the parameter  $\beta$  (see Fig. 2 in Part II; ref. 2).

In order to correlate the above hydrodynamics with the physical nature of the solvent systems, three major physical properties, *i.e.*, interfacial tension, viscosity, and density were measured for each solvent system (Table I).

Among three physical properties measured for the solvent system, interfacial tension ( $\Delta\gamma$ ) shows extremely high correlation with both the hydrophobicity and the hydrodynamic behavior of the solvent system in that the value is highest for the hydrophobic solvent group and lowest for the hydrophilic solvent group. The viscosity of each phase ( $\eta_U$  or  $\eta_L$ ) or the mean viscosity of both phases ( $\bar{\eta}$ ) is also very well correlated with the solvent group, being lowest in the hydrophobic solvent systems and highest in the hydrophilic solvent systems. The density difference between the upper and the lower phases ( $\Delta\rho$ ) appears to be less well correlated with the solvent groups. Although the value is generally high in the hydrophobic solvent group and low in the hydrophilic solvent group, a discrepancy is presented by ethyl acetate-water in the hydrophobic solvent group which shows the second lowest value among all solvent systems. Thus all three physical properties examined have some significant relationship to the stationary phase retention profile and the hydrodynamic behavior of the solvent systems in high-speed CCC.

### *Settling time for the two-phase solvent systems*

The time required for the two-phase solvent system to settle in a unit gravi-

TABLE I

## PHYSICAL PROPERTIES OF THE TWO-PHASE SOLVENT SYSTEM

$\eta_U/\eta_L$  = Upper phase/lower phase;  $\bar{\eta}$  = mean viscosity of the two phases;  $\rho_U/\rho_L$  = upper phase/lower phase;  $\Delta\rho$  = density difference between the two phases;  $T$  = settling time after gentle mixing;  $T'$  = settling time after vigorous shaking.

Solvent group	Two-phase solvent system (volume ratio)	Interfacial tension (dyne/cm) $\Delta\gamma$	Viscosity (c.p.)		Density (g/cm <sup>3</sup> )		Settling time (sec)	
			$\eta_U/\eta_L$	$\bar{\eta}$	$\rho_U/\rho_L$	$\Delta\rho$	$T$	$T'$
Hydrophobic	Hexane-water	52	0.41/0.95	0.68	0.66/1.00	0.34	< 1	8
	Ethyl acetate-water	31	0.47/0.89	0.68	0.92/0.99	0.07	15.5	21
	Chloroform-water	42	0.95/0.57	0.76	1.00/1.50	0.50	3.5	5.5
Intermediate	Hexane-methanol	4	0.50/0.68	0.59	0.67/0.74	0.07	5.5	6
	Ethyl acetate-acetic acid-water (4:1:4, v/v/v)	16	0.76/0.81	0.79	0.94/1.01	0.07	15	16
	Chloroform-acetic acid-water (2:2:1, v/v/v)	12	1.16/0.77	0.97	1.12/1.35	0.24	29	27.5
	<i>n</i> -Butanol-water	3	1.72/1.06	1.40	0.85/0.99	0.14	18	14
	<i>n</i> -Butanol-0.1 <i>M</i> NaCl (1:1, v/v)	4	1.66/1.04	1.35	0.85/0.99	0.15	16	14.5
	<i>n</i> -Butanol-1 <i>M</i> NaCl (1:1, v/v)	5	1.75/1.04	1.40	0.84/1.04	0.20	23.5	21.5
Hydrophilic	<i>n</i> -Butanol-acetic acid-water (4:1:5, v/v/v)	< 1	1.63/1.40	1.52	0.90/0.95	0.05	38.5	37.5
	<i>n</i> -Butanol-acetic acid-0.1 <i>M</i> NaCl (4:1:5, v/v/v)	< 1	1.68/1.25	1.47	0.89/1.01	0.11	32	30.5
	<i>n</i> -Butanol-acetic acid-1 <i>M</i> NaCl (4:1:5, v/v/v)	1	1.69/1.26	1.48	0.88/1.05	0.16	26.5	24.5
	<i>sec.</i> -Butanol-water	< 1	2.7 /1.67	2.19	0.87/0.97	0.10	57	58
	<i>sec.</i> -Butanol-0.1 <i>M</i> NaCl (1:1, v/v)	< 1	1.96/1.26	1.61	0.86/0.98	0.12	46.5	49.5
	<i>sec.</i> -Butanol-1 <i>M</i> NaCl (1:1, v/v)	3	1.91/1.29	1.60	0.84/1.03	0.19	34	33.5

tational field provides a reliable numerical index to classify the hydrodynamic behavior of the solvent systems in a centrifugal force field.

Results of a simple experiment conducted to measure the settling time of the two-phase solvent system in a 5-ml capacity graduated cylinder, are shown in Table I where  $T$  indicates the settling time after gentle mixing by inverting the cylinder five times and  $T'$ , after vigorous shaking of the cylinder five times. The results clearly show a close correlation between the settling times in a unit gravitational field and the hydrodynamic behavior of the solvent phases observed in a centrifugal field. The settling times of the hydrophobic solvent systems are shortest ranging from 5 to 16 sec while those of the hydrophilic solvent systems are longest ranging from 38 to 59 sec. The intermediate solvent systems gave moderate values ranging from 15 to 29 sec with the exception of the non-aqueous system hexane-methanol which had a short settling time of 5.5 sec. The difference between  $T$  and  $T'$  for each solvent system was found to be quite small in both the intermediate and hydrophilic solvent systems. However, in the hydrophobic solvent group  $T'$  becomes much greater than  $T$  especially in hexane-water.

Because of the parallel trend observed with the hydrophobicity of the solvent group, the settling time may serve as a useful parameter for correlating the physical properties of the solvent system with the hydrodynamic behavior of the solvent systems in a centrifugal force field.

The values for  $\Delta\gamma$ ,  $\Delta\rho$  and  $\bar{\eta}$  (mean viscosity of the upper and the lower phases) in Table I are respectively plotted against the settling time ( $T$ ) of the solvent system in Fig. 1 where the open circle represents the hydrophobic solvent systems; the solid circle, the hydrophilic solvent systems; and the semisolid circle, the intermediate solvent systems. The correlation coefficient ( $r$ ) for each plot of physical property with settling time ( $T$ ) was computed for the set of fifteen solvent systems. The density difference ( $\Delta\rho$ ) gave a weak negative correlation ( $r = -0.45$ ) (Fig. 1A) and the interfacial tension ( $\Delta\gamma$ ), a moderate negative correlation ( $r = -0.65$ ) (Fig. 1B). A strong positive correlation ( $r = +0.88$ ) was observed between the settling time and the mean viscosity ( $\bar{\eta}$ ) as illustrated in Fig. 1C. When the correlation coefficient between the viscosity and the settling time is computed individually for each phase, the aqueous phase (including the lower phase of hexane-methanol) gave the highest value of 0.89 and the non-aqueous phase, a lower value of 0.83 (Fig. 2).

The above findings suggest that among various physical properties of the solvent system viscosity may play a major role in retention of the stationary phase. The less viscous the solvent system, the higher the level of retention to be expected. Although the interfacial tension and density difference between the two phases appear to be less important factors than the viscosity, they would exert a strong influence on the settling times near the plait point of the solvent as described later in detail.

#### *Effects of the temperature on the settling time*

As described earlier in Part II (ref. 2), hydrophilic solvent systems such as *n*-butanol-acetic acid-water (4:1:5, v/v/v) and *sec.*-butanol-water exhibit unique hydrodynamic behavior: the upper phase is distributed toward the tail and the lower phase toward the head. Therefore the reversed elution mode is required to obtain retention of the stationary phase in the helical column. These solvent systems are characterized by high viscosity and low interfacial tension associated with longest

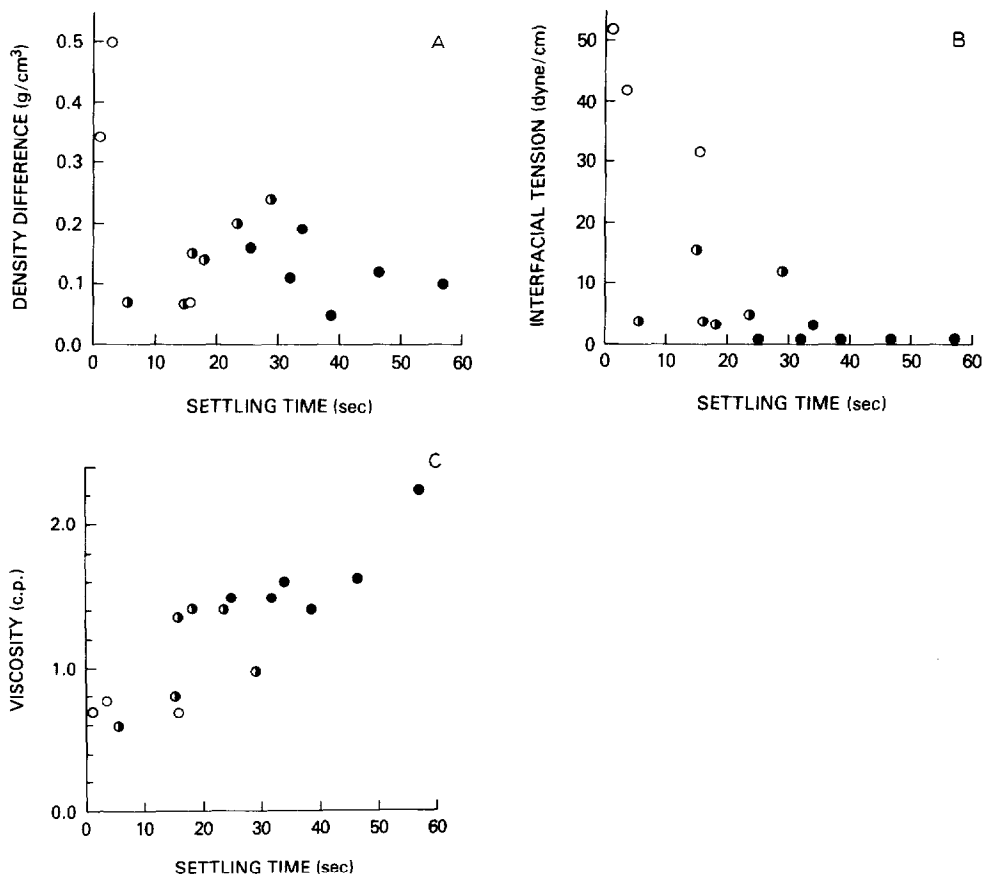


Fig. 1. Correlation between the settling time and three major physical properties of the two-phase solvent system. Open circle indicates the hydrophobic solvent system; semisolid circle, the intermediate solvent system; and solid circle, the hydrophilic solvent system. (A) Density difference of the two phases vs. settling time ( $r = -0.45$ ). (B) Interfacial tension vs. settling time ( $r = -0.65$ ). (C) Mean viscosity vs. settling time ( $r = +0.88$ ).

settling times among all solvent systems examined. The highly significant correlation found between viscosity and settling times of various solvent systems strongly suggests that the above unique hydrodynamic behavior of the hydrophilic solvent systems may be attributed to their high viscosity and that reduction of the viscosity by raising the temperature of these solvent systems would reduce their settling times to the range observed for other solvent systems, thus converting their reversed hydrodynamic mode to the normal mode. This reversal of the hydrodynamic behavior of the butanol solvent systems will provide an obvious advantage in performing high-speed CCC in that all conventional solvent could then be eluted with the normal mode with high flow-rates of the mobile phase.

In order to demonstrate the feasibility of this reversal of hydrodynamics, settling times of the butanol solvent systems were measured at various temperatures ranging from room temperature to around 80°C. Fig. 3A shows effects of temperature

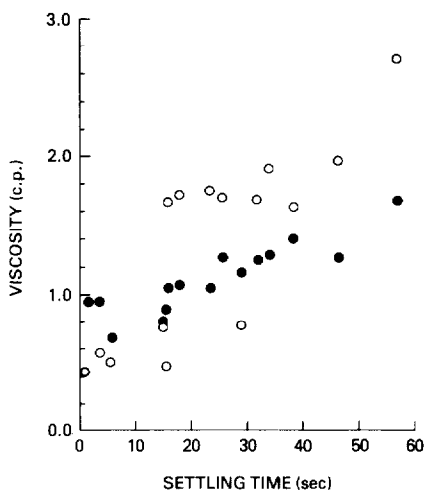


Fig. 2. Correlation between the settling time and viscosity of each solvent phase. Open circle: viscosity of non-aqueous phase vs. settling time ( $r = +0.83$ ). Solid circle: viscosity of aqueous phase including the lower phase of hexane-methanol vs. settling time ( $r = +0.89$ ).

on the settling times of *n*-butanol-water and *sec.*-butanol-water. The settling times of the *sec.*-butanol-water system sharply decline to less than 20 sec on increasing the temperature to 50°C. The settling time of the less viscous *n*-butanol-water system exhibits a similar trend, dropping to less than 10 sec at 50°C. As judged from the settling times, it is expected that at a temperature of 50°C these solvent systems would produce phase distribution diagrams similar to those of the intermediate and hydrophobic solvent systems which allow high retention levels of the stationary phase with the normal elution mode. It is interesting to note that the curves of the settling time vs. temperature for these binary butanol systems (Fig. 3A) are quite similar to that of the viscosity vs. temperature plot for *n*-butanol (Fig. 3B), which is consistent with the high positive correlation between the settling time and viscosity described earlier.

Fig. 4 shows the effects of temperature on the settling times of the ternary

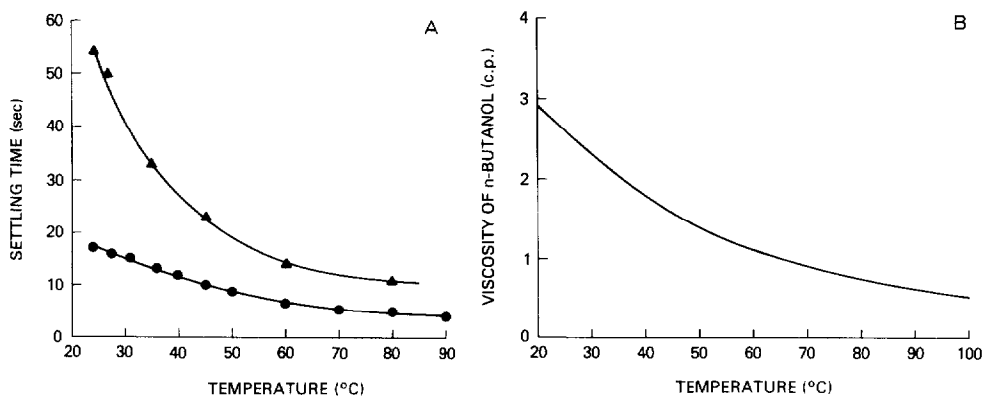


Fig. 3. (A) Effects of temperature on the settling time of binary butanol systems. ●, *n*-butanol-water; ▲, *sec.*-butanol-water. (B) Effects of temperature on the viscosity of *n*-butanol.

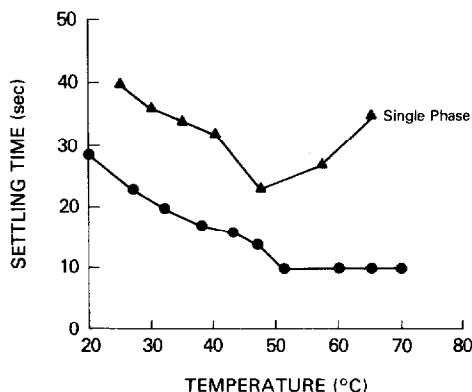


Fig. 4. Effects of temperature on the settling time of ternary *n*-butanol systems. ●, *n*-butanol-acetic acid-water (9:1:10); ▲, *n*-butanol-acetic acid-water (4:1:5).

butanol systems. In *n*-butanol-acetic acid-water (4:1:5, v/v/v) the settling times decline with an increase in temperature to 50°C reaching a minimum of 23 sec. Increase in the temperature up to 65°C further results in increase of the settling times and the system forms a single phase. The increase of the settling times near the plait point is apparently caused by the extremely low interfacial tension and the small difference in density between the two phases. This anomaly can be corrected by reducing the amount of acetic acid in the solvent system as shown in the settling time curve obtained for *n*-butanol-acetic acid-water (9:1:10, v/v/v) which declines to about 10 sec at 50°C.

Alternatively, the above problem may be solved by the addition of a salt in the aqueous solution. Fig. 5 illustrates effects of salt concentration on the settling time vs. temperature curve in *n*-butanol-acetic acid-aqueous solution (4:1:5, v/v/v). At 0.1 *M* sodium chloride concentration the solvent system still retains the anomalous zone although it has shifted about 15° toward the right on the temperature axis. Increase of the sodium chloride concentration to 1 *M* normalizes the settling time curve which declines to 15 sec at 50°C.

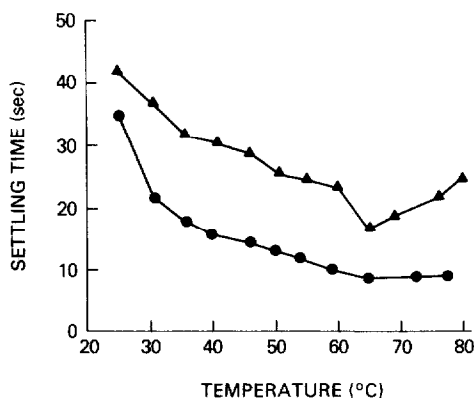


Fig. 5. Effects of temperature on the settling time of quadruple *n*-butanol systems containing NaCl. ●, *n*-butanol-acetic acid-1 *M* NaCl (4:1:5); ▲, *n*-butanol-acetic acid-0.1 *M* NaCl (4:1:5).



The above findings clearly indicate that at higher temperature the hydrophilic solvent systems may display the normal hydrodynamic behavior observed in the hydrophobic and intermediate solvent systems, provided that the phase composition is kept away from the vicinity of the plait point. Raising the temperature slightly above room temperature may also reduce the settling time of the intermediate solvent systems and therefore would improve the retention of stationary phase and increase partition efficiency during the separation.

Performing CCC at elevated temperature will provide a number of beneficial effects including (1) conversion of the hydrodynamic behavior of the hydrophilic solvent systems to the normal mode, (2) improved retention of the stationary phase resulting in higher peak resolution, (3) higher partition efficiency due to reduction of mass transfer resistance, (4) increased sample loading capacity due to higher solubility, (5) prevention of altered hydrodynamics at the column inlet when a highly concentrated sample is introduced with resultant loss of the stationary phase as described in the following section.

These expectations have been confirmed recently using a high-speed CCC apparatus equipped with a temperature control system which has been constructed in our laboratory.

#### *Practical application of the settling time in high-speed CCC*

The close correlation found between the settling time and the stationary phase retention profile and hydrodynamic behavior of the solvent systems provides useful guidance in developing applications for CCC. From the data obtained with simple settling tests, the elution mode and other operational conditions to attain a satisfactory retention level of the stationary phase may be predetermined<sup>3</sup>. In a standard multilayer coiled column ( $\beta = 0.5-0.8$ , 10 cm revolutionary radius)<sup>4</sup>, a solvent system with a settling time less than 30 sec would display normal hydrodynamic behavior which distributes the upper phase toward the head and the lower phase toward the tail. Accordingly, the elution should be performed by pumping the upper phase from tail to head (broken curve in the phase distribution diagram, Fig. 3 of Part II, ref. 2) or the lower phase from head to tail (solid curve in the phase distribution diagram, Fig. 3 of Part II, ref. 2). The flow-rate of the mobile phase may also be judged from the settling times. For a 1.6 mm I.D. column, flow-rates over 240 ml/h may be applicable if the settling time is less than 10 sec, and a reduced flow-rate should be used for solvent systems with longer settling times. When the settling time of the solvent system considerably exceeds 30 sec, it is most likely that the hydrodynamic behavior of the two phases is reversed, the upper phase now being distributed toward the tail and the lower phase toward the head. Accordingly, the upper phase should be pumped from the head toward the tail (solid curve) and the lower phase pumped from the tail toward the head (broken curve) at the reduced flow rates; typically 60 ml/h or less for the 1.6 mm I.D. column. For this type of solvent system, the best stationary phase retention is attained with a small  $\beta$  value around 0.25 (see the phase-distribution diagrams in Fig. 3 in Part II, ref. 2).

In preparative-scale separations, the effect of the sample concentration on the settling time might alter the hydrodynamic behavior of the solvent system in the inlet portion of the column where the sample is introduced. Generally speaking, the settling time of two-phase solvent systems increases with the solute concentration. For

example, a high solute concentration in an intermediate solvent system (with a settling time of less than 30 sec) could increase the settling time substantially over 30 sec. The resulting change in the hydrodynamic behavior of the solvent phases in the column inlet when the sample is introduced may reverse the motion of the upper phase, producing detrimental effects on the retention regardless of the choice of the elution mode. In most cases, this problem can be eliminated by diluting the sample solution before injection. An alternative and superior solution to this problem may be to raise the temperature of the solvent system as described in the foregoing section. When the above precautions are followed, high-speed CCC may be universally applied using conventional solvent systems and normal mode elution with minimum carry-over of the stationary phase.

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