Centrifugal Partition Chromatography. III. Physico-Chemical Properties of Ternary Liquid Systems

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RAPHY. III. PHYSICO-CHEMICAL PROPERTIES
OF TERNARY LIQUID SYSTEMS

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ABSTRACT

Four important physico-chemical solvent parameters that affect centrifugal partition chromatography (CPC) were evaluated. They are (i) the mutual solubility of the solvent(s) in each phase which was analyzed using phase diagrams and tie-lines, the results of which can be used to evaluate the behavior and stability of the biphasic CPC system; (ii) solution density which affects the pressure drop; (iii) interfacial tension which affects droplet formation and (iv) viscosity which affects both pressure drop and chromatographic efficiency. These parameters were studied for three ternary solvent systems. The first one had a very low mutual solubility (water-hexane-octanol). The second one had an intermediate mutual solubility (water-hexane-methanol). The last one had a high mutual solubility (water-pentanol-methanol).

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INTRODUCTION

In two previous papers (1,2), the general features of centrifugal partition chromatography (CPC) (1) as well as the specific and sometimes unusual advantages in selectivity and efficiency (2) were discussed in detail. As a countercurrent chromatography (CCC) technique, CPC uses two immiscible liquids; one is the stationary phase, while the other is the mobile phase. Basically a simplified description of the CPC technique is that of a centrifuge rotor filled by the stationary phase liquid. Then, the mobile phase is pumped through the stationary phase which is held by the centrifugal field in channels inside the rotor. It is possible to treat the interconnecting channel system containing the biphasic solution as a preparative LC, column provided the rotor is spinning at a constant rate.

An important area that is often overlooked in CCC research is the study of the physico-chemical properties of the two liquid phases used in the system. The selection of the solvent system is often empirical. For example, the system chloroform: glacial acetic acid: water (0.1 M HC1),2:2:1, by volume is used for the DNP-amino acid analysis (3-8) because the partition coefficients of those amino acid derivatives are close to unity in this liquid-liquid system. The limitations imposed by the magnitude of the solute partition coefficients is an important criterion in choosing a liquid-liquid system (9). Sometimes prospective solvent systems can be evaluated by thin layer chromatography on silica gel or bonded phase plates (10,11).

In CPC, the criteria used to select a liquid-liquid system are strongly dependent on the goal of the experiment. For example, if the goal of a CPC application is to purify a given compound from impurities, the liquid system will be chosen so that the partition coefficient of the interesting solute is around unity and partition coefficients of the impurities are very different

from unity. If the octanol-water partition coefficient of a solute must be determined, there is no choice, the system must consist of octanol and water. The study of the partition coefficient determinations and preparative sample purification will be topics of subsequent papers.

The aim of the present work is to study the physico-chemical properties of a liquid system relative to CPC. Those properties are (i) the mutual solubility of the two liquid phases, which is related to the CPC "column" life time (1); (ii) the densities of both liquid phases, which affect the driving pressure (1); (iii) the interfacial and surface tension and (iv) the viscosities of both liquid phases, which are related to the solute mass transfer between phases, efficiency, and pressure drop (2).

Three ternary liquid systems are studied: octanol-hexane-water, methanol-hexane-water and pentanol-methanol-water. The approach used to study these three systems could be used with any other liquid-liquid system.

EXPERIMENTAL SECTION

1-Mutual Solubility

The mutual solubility (in mass units) was determined using ternary diagrams. Each point in the ternary diagrams was determined by combining weighed amounts of each of the three liquids. The mixture was shaken and then allowed to thermally equilibrate for 5 min. The mixture was determined to be monophasic or biphasic by visual examination. The composition of each solution was determined by gas chromatography with thermal conductivity detection.

2-Density

Densities of the lower and upper phases were determined by weighing a known volume of each phase. The results given are the mean of three separate determinations.

3-Interfacial and Surface Tension

A Du Nouy Surface tensiometer (Fisher, Model 20) with a platinum ring of 2 cm diameter was used for surface and interfacial tension measurements. Between each measurement, the platinum ring was cleaned by heating to redness with a burner. This device was not able to determine interfacial tension lower than 2 dyne/cm with an acceptable reproducibility and accuracy.

4-Viscosity

The viscosities of phases were determined using a falling ball viscosimeter; the descending time of the ball is viscosity dependent. To obtain an acceptable precision, two different balls, one glass, the other stainless steel, were used depending on the phase viscosity.

The solvents were from Burdick and Jackson (Muskegan, MI) or Fisher Scientific (Pittsburg, PA), and were used as received. Their physicochemical properties are listed in Table I. Before each experiment, the two phases of the liquid system under investigation were allowed to equilibrate for at least 20 min in an ultrasonic bath at 20°C.

RESULTS AND DISCUSSION

1-Solubility

Three different liquid-liquid systems were chosen, based on their mutual solubility behavior. The first system had a very low mutual solubility between the aqueous and the organic phase. This solvent system consisted of octanol-hexane-water. Octanol and hexane are fully miscible and both of them have a low water solubility. The system with an intermediate solubility was: methanol-hexane-water. Methanol is fully miscible with water and it can dissolve large amounts of hexane. The third system methanol-pentanol-water has a high mutual solubility. Methanol is freely soluble in both

Table I. Physico-chemical parameters of pure liquids at 20°C.

Compound	m.w. g/mol	b.p. °C	density g/cm³	viscosity cP	surf. tension dyne/cm
Octanol	130	194.5	0.827	7.20	27.5
Pentanol	88	137	0.814	4.10	26.9
Methanol	32	65	0.791	0.60	22.6
Hexane	86	69	0.660	0.33	18.4
Water	18	100	0.999	1.001	73.0

Data from Weast, R. C., Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 65th Ed. (1985).

pentanol and water. Furthermore, methanol enhances greatly the water solubility in pentanol.

The ternary mass diagrams of the three liquid-liquid systems are shown in Figures 1-3. The black areas correspond to the monophase mixtures which cannot be used in CPC. The area containing the tie-lines delineates the biphasic region that can be used in CPC. The tie-lines allow one to calculate the quantity and composition of the two phases obtained when three liquids are mixed. To do this, the lever rule must be used (12). Consider, for example, the solution represented by point B in Figure 2 which corresponds to a mixture of hexane (60 g), methanol (20 g) and water (20 g). It is possible to calculate the quantity and composition of the two phases obtained with the Point B mixture as follows. According to the lever rule:

distance AB x mass of A = distance BC x mass of C   (1)

and

mass of A + mass of C = mass of B   (2)

Figure 1: Ternary mass diagram for the hexane-ocatanol-water system. The white area containing tie-lines represents the solvent compositions at which 2 phases exist (biphasic). The dark area indicates a monophasic (homogeneous) solution.

The compositions of Points A and C are: methanol 49%-water 51% w/w (traces of hexane) and hexane 99%-methanol 0.9%-water 0.1% w/w, respectively. The length AB is equal to 0.61xAC. The length BC is equal to 0.39xAC.

From Eqs. 1 and 2, one obtains:

$$\frac{0.61xAC}{0.39xAC} = \frac{mass\ of\ C}{mass\ of\ A} = \frac{100 - mass\ of\ A}{mass\ of\ A} = 1.56 \qquad (3)$$
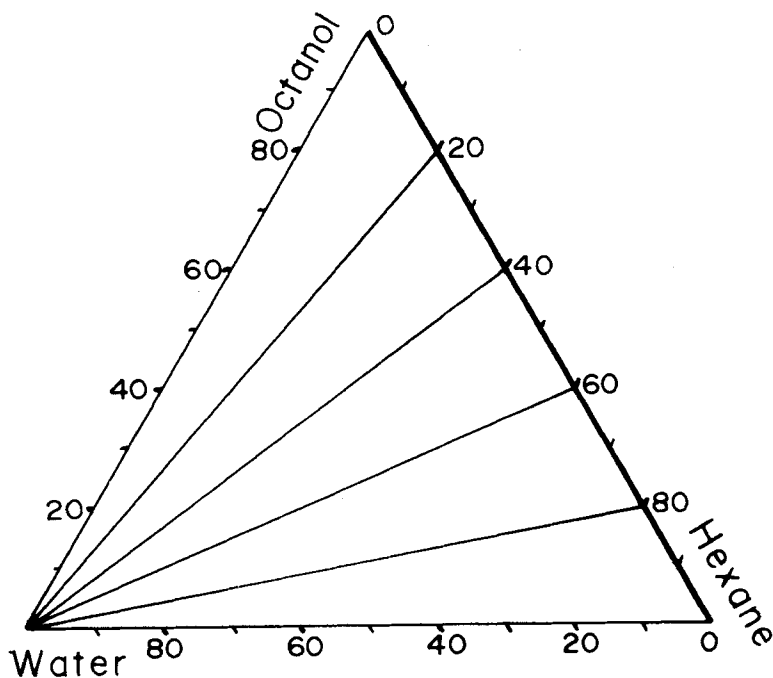
Figure 2: Ternary mass diagram for the hexane-methanol-water system. The white area containing tie-lines represents the solvent compositions at which 2 phases exist (biphasic). The dark area indicates a monophasic (homogeneous) solution. See the Results and Discussion section for an explanation of points A, B and C.

from which the mass of A is calculated to be 39 g and the mass of C, 61 g. The 100 g mixture, corresponding to Point B, separates into two phases: 39 g of aqueous phase with the A composition and 61 g of organic phase with the C composition.

In CPC, the useful quantity is the volume of each phase. The solution density must be used to convert the mass to a volume. Table II lists several different phase volumes obtained after mixing different amounts of various
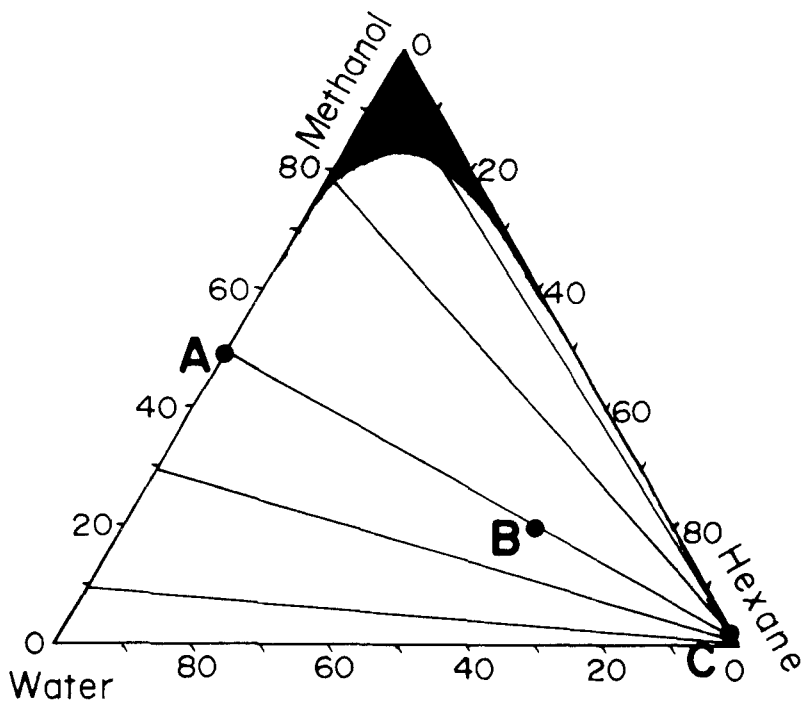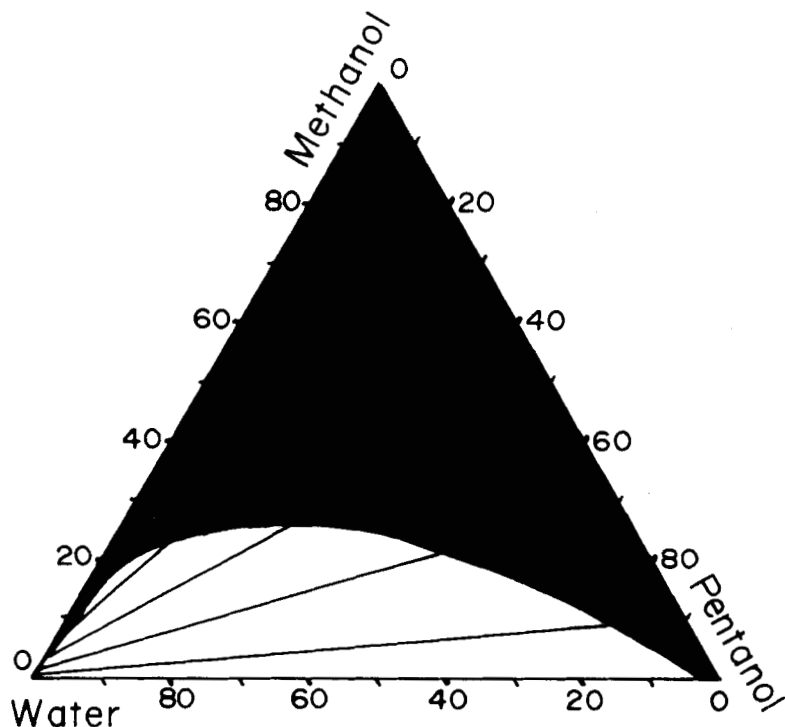
Figure 3. Ternary mass diagram for the pentanol-methanol-water system. The white area containing tie-lines represents the solvent compositions at which 2 phases exist (biphasic). The dark area indicates a monophasic (homogeneous) solution.

solvents. As expected, by looking at the mass diagram of Figure 2, the mixture of 25 mL of hexane with 25 mL of methanol gives only 8 mL of hexane upper phase and 42 mL of methanol rich lower phase.

2-Density

In the first paper of this series (1), we demonstrated that the pressure drop (P) in CPC could be described using:

$$P = n \left[\Delta\rho \, \omega^2 \, R \, h + \mu \gamma \, F \right] \qquad (4)$$

Table II. Volume characteristics and density of selected mixtures

Mixture (mL)			Aqueous Lower Phase		Organic Upper Phase	
hexane	methanol	water	volume	density	volume	density
25.0	25.0	0.0	42.0	0.720	8.0	0.666
25.0	24.75	0.25	35.9	0.726	14.1	0.664
25.0	23.75	1.25	28.4	0.773	21.6	0.664
25.0	22.5	2.5	26.3	0.796	23.7	0.653
25.0	20.0	5.0	25.2	0.828	24.8	0.652
pentanol	methanol	water	volume	density	volume	density
25.0	0.0	25.0	23.7	1.000	26.3	0.831
25.0	1.25	23.75	22.7	0.978	27.3	0.834
25.0	2.5	22.5	21.8	0.967	28.2	0.846
25.0	5.0	20.0	20.4	0.960	29.6	0.850
25.0	10.0	15.0	12.5	0.950	37.5	0.865

Temperature 20°C; if 25 mL of water was shaken with any 25 mL octanol-hexane mixture, both aqueous and organic phases had about the same volume (25 mL).

in which n is the total number of channels (2400), $\Delta\rho$ is the density difference between the mobile and the stationary phase (g/cm^3), ω is the spin rate (rd/s), R is the rotor radius (cm), h is the channel height (cm), μ is the mobile phase viscosity (cP), γ is a geometrical characteristic of the channel and duct system (cm^{-3}), and F is the flow rate (mL/min). Eq. 4 shows that the pressure drop

PURE   PURE
OCTANOL   HEXANE
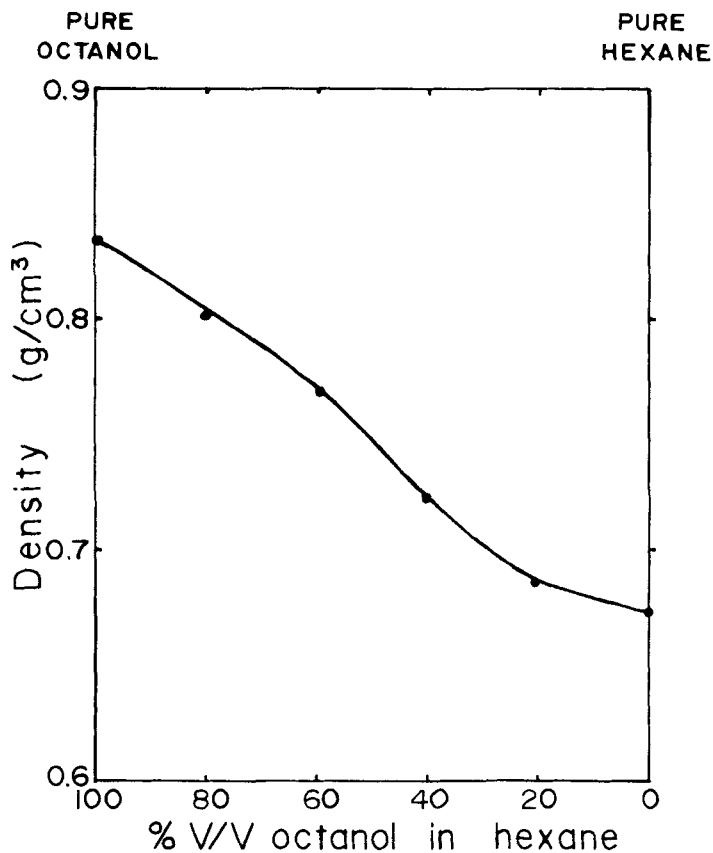
Figure 4: Change in the organic phase density of the hexane-octanol-water
          system versus the hexane percentage.

increases linearly with the density difference between the stationary and the
mobile phase.

Table II lists the density of each phase for the two solvent systems that
contain methanol. Figure 4 shows the density variation of the organic phase
versus the octanol-hexane percentage ratio. For the three systems studied,
the density measurements correspond to the tie-lines shown on Figures 1-3.

For the octanol-hexane-water system, the density of the organic phase evolves continuously from the pure octanol density to the pure hexane density. The aqueous phase density is always very close to unity because its composition does not change appreciably from that of pure water. With the methanol-hexane-water system, the organic phase density is close to the pure hexane density (0.66 g/cm^3) and the aqueous phase density increases as the water content increases (Table II). In the case of the pentanol-methanol-water system, the density variations of both aqueous and organic phases seems relatively small. The aqueous phase compositions show small variations (tie-lines of Figure 3), but the organic phase compositions change appreciably. The water and methanol contents increase and the pentanol content decreases. The density increases due to water are almost compensated by the density decreases due to methanol.

3-Interfacial and Surface Tension

In CPC, the interfacial tension plays a role in the droplet formation mechanism: the lower the interfacial tension, the easier the droplet formation and the higher the efficiency. Unfortunately, a low interfacial tension also increases the likelihood of emulsion formation. This "flooding" point, described in a previous paper (2), would occur at a low flow rate. Table III lists the surface and interfacial tensions for several compositions of the three liquid systems already described.

The interfacial tension roughly corresponds to the difference between the surface tension of the two liquid phases. The surface tension of each phase depends on its respective composition as determined by the tie-lines (Figs. 1-3). For example, the surface tension of the aqueous phase of the system hexane-octanol-water is almost constant (~35 dyne/cm), corresponding to an octanol-saturated aqueous solution. The surface tension

of the organic phase of the system hexane-methanol-water increases as the methanol content decreases.

The efficiency obtained with the hexane-methanol-water 50-49.5-0.5 v/v systems was higher than any of the other liquid-liquid systems studied. This mixture has the lowest interfacial tension, but the viscosity must also be considered.

4-Viscosity

In CPC, viscosity is a very important parameter affecting the efficiency. The diffusion coefficients of solutes are inversely proportional to their liquid viscosities (Einstein equation). A high viscosity will result in a poor mass transfer of solutes between phases, and a poor efficiency. Viscosity is also a parameter of the dynamic term of the pressure drop equation (Eq. 4).

Table III lists the viscosities of the saturated phases obtained with the liquid systems represented in Figures 1-3. The most important viscosity change occurs in the organic phase of the octanol-hexane-water system. When the hexane content of the organic phase increases from 20% v/v to 40% v/v, the viscosity of the octanol-hexane phase drops from 6.1 cP to 1.7 cP. The viscosity of the organic phase used by Terada et al. (hexane-octanol, 80-20, v/v) (13) in CPC, was only 0.65 cP. From a practical point of view, it is possible to work with the CPC apparatus and a mobile phase of pure octanol i.e., with a viscosity as high as 7.1 cP (14). However, a low viscosity makes the CPC system much easier to handle.

The viscosities of the other phases correspond to the liquid compositions indicated by the tie-lines in Figures 1-3. For example, the viscosity of the organic phase of the hexane-methanol-water system is close to the pure hexane viscosity (Tables I and III). The viscosity of the aqueous

Table III. Viscosity and surface tension measurements

mixture (v/v)			aqueous phase		organic phase		
hexane	octanol	water	viscosity[a]	surface tension[b]	viscosity[a]	surface tension[b]	inter-facial tension[b]
mL	mL	mL	cP	dynes/cm	cP	dyne/cm.	
0.0	25.0	25.0	0.8	33	7.1	30	4.8
5.0	20.0	25.0	1.1	30	6.1	26	4.1
10.0	15.0	25.0	1.0	35	1.7	23	5.2
15.0	10.0	25.0	1.0	40	0.8	24	6.5
20.0	5.0	25.0	0.9	32	0.65	21	8.0
25.0	0.0	25.0	1.0	78	0.34	20	45.0
hexane	methanol	water	viscosity	surf. t.	viscosity	surf. t.	interf.
25.0	25.0	0.0	0.6	15	0.42	16	<2
25.0	24.75	0.25	0.9	19	0.47	18	<2
25.0	20.0	5.0	1.2	25	0.47	18.5	6.4
25.0	15.0	10.0	1.4	32	0.48	20	12
25.0	10.0	15.0	1.1	38	0.48	20	17
25.0	5.0	20.0	1.1	45	0.49	20	23
pentanol	methanol	water	viscosity	surf. t.	viscosity	surf. t.	interf.
25.0	0.0	25.0	1.1	32	2.1	27	3.5
25.0	5.0	20.0	0.9	28	1.4	26	<2

[a]viscosity precision: 0.2 cP (steel ball); 0.04 cP (glass ball).
[b]surface tension accuracy 6%. It was not possible to measure accurately a surface or interfacial tension lower than 2 dyne/cm.

phase of the same system gives a maximum for the methanol 40%-water 60% v/v composition (1.4 cP). This viscosity maximum for methanol-water mixtures is well-know to individuals who do reversed phase HPLC, as the column back pressure increases significantly with increasing viscosity.

It is clear that the use of ternary solvent systems in CCC and CPC allows one to modulate the phase polarity and therefore the partition coefficients of solutes between the phases. The ability to change the mobile phase "strength" or the stationary phase polarity makes any CPC separation tunable. It is possible, as in classical preparative LC, to optimize the selectivity, the peak resolution, the duration of the experiment and the solvent volume used.

An important point, not considered in this work, was the temperature effect. A temperature increase enhances the mutual liquid solubility and decreases the densities and, especially, the viscosities of most liquids. These effects will be the topic of a subsequent study.

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