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Solvent selection guide for counter-current chromatography

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ABSTRACT

Thirteen two-phases counter-current chromatography solvent systems (five not previously described for high-speed counter-current chromatography) were evaluated for relative polarity by using Reichardt's dye to measure solvachromatic shifts and by using solubility of index compounds. Three groups of systems were classified as lipophilic, polar or intermediate and a solvent selection guide was developed. Several intermediate systems appeared to have similar characteristics based on these evaluations. A method for making alternative solvent systems and determining composition of the two phases without concentration standards was described. Three of the lipophilic systems were tested for the separation of a 180-mg mixture of dimethylphthalate, dioctylphthalate and dodecyl acetate, and the hexane–acetonitrile (1:1) system was found to work best.

INTRODUCTION

Counter-current chromatography (CCC) is a rapidly progressing technique [1–3]. Although many two-phase solvent systems have been reported for CCC separations, there is no organized means for initially selecting one solvent system over others. Lists of CCC solvent systems [1,4] and separations of particular compounds are often arranged from "least" polar to "most" polar upper phase with no accurate measure of the polarity of the phases. Unless the compound to be separated by CCC is very similar to one already separated, there is difficulty in choosing a system.

To provide a framework for the initial selection of a CCC solvent system for a particular separation, we classified two-phase solvent systems by polarity of their upper and lower phases using Reichardt's dye [5]. Reichardt's dye is a resonance-stabilized charge-transfer compound that absorbs light in solution between 400 and 900 nm depending on the polarity of the solvent [5].

A secondary problem in making up two-phase solvent systems for CCC has been the lack of published data on composition of the two phases. Usually the phases of a four-solvent mixture are referred to as the "aqueous" phase or the "polar" phase without regard to true knowledge of their composition. Problems are encountered when the solvent mixture divides into two phases in an 80:20, 90:10, or similar disproportionate volume ratio. Filling the CCC column with one phase and then eluting with the second phase requires similar volumes of each phase and large amounts of solvent mixtures must be prepared to obtain sufficient volume of the smaller volume

phase if they separate disproportionately. Equal volume phases with similar composition to those that separate disproportionately can be devised if the percent composition of each phase is determined by our proposed method.

EXPERIMENTAL[a]

Materials

Solvent systems for study were chosen fromt he literature (systems 3, 4, 5, 7, 8, 10, 11, 12 in Table I) as indicated by Table I citations or developed at our laboratory (systems 1, 2, 6, 9 and 13 in Table I).

The least polar solvent in a system is listed first in each row of Table I. This is the solvent added incrementally to adjust the polarity of the overall system.

Dimethyl phthalate, dioctyl phthalate, dodecyl acetate, ethylene glycol, glycerol, methyl stearate and Reichardt's dye were obtained from Aldrich. Meadowfoam oil, a mixture of vegetable fatty acid triglycerides was supplied by Selim Erhan of our laboratory. Other chemicals are commercially available reagents.

TABLE I

TWO-PHASE SOLVENT SYSTEMS FOR COUNTER-CURRENT CHROMATOGRAPHY

System	Solvent and volume ratio	Ref.
1	Hexane–acetonitrile (1:1)	
2	Hexane–acetonitrile–chloroform (5:5:1)	
3	Hexane–ethanol–water (6:5:1:)	4
4	Hexane–ethyl acetate–acetonitrile–methanol (5:2:5:4)	3
5	Hexane–ethyl acetate–methanol–water (1:1:1:1)	4
6	Chloroform–methanol–water (13:7:2)	
7	Chloroform–methanol–water (1:1:1)	4
8	Chloroform–methanol–water (7:13:8)	4
9	Toluene–acetonitrile–water–ethanol (3:4:3:2)	
10	Chloroform–methanol–0.2 *M* acetic acid (1:1:1)	4
11	Ethyl acetate–ethanol–water (2:1:2)	
12	*n*-Butanol–acetic acid–water (4:1:5)	4
13	*n*-Butanol–ethyl acetate–water (4:1:4)	

Solvachromatic shift

A 1-mg amount of Reichardt's dye was added to 10 ml of one phase (upper or lower) of the two-phase solvent system and shaken. Using the solvent as background, the absorption spectrum of the dye solution was measured from 200 to 900 nm in a 1-cm pathlength quartz cell on a Beckman DU UV spectrophotometer. Samples were diluted to maintain maximum absorbance at or below 1.0 absorption unit. The maximum absorbance between 450 and 900 nm was determined automatically using instrument software and confirmed by inspection of the spectrum. Solvachromatic

[a] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

shifts were similarly obtained for low volatility liquids: dimethyl phthalate, dioctyl phthalate, ethanolamine and ethylene glycol.

Composition of the phases

Composition of the two phases was calculated from peak areas determined on a Hewlett-Packard Model 5790 gas chromatograph using a 15 m × 1.5 mm polydimethylsiloxane-coated capillary column from J & W Scientific. Flame ionization detection, a Hewlett-packard Model 3390A recording integrator and hydrogen carrier gas at 5 ml/min were used. Oven temperature varied depending on the nature of the solvent. When water was present in a system, its concentration was determined by the Karl–Fisher titration method on a Fisher Coulomatic Titrimeter Model 447. Using peak areas of individual solvent peaks for each solvent phase and the total amount of each solvent used, we calculated the concentration of each solvent in each phase. For example, if the total volume of four solvents mixed are A, B, C and D and the resulting volumes of upper and lower phases are V_U and V_L, respectively, then we can designate the volumes we wish to determine A_U, B_U, C_U, D_U, the volumes of solvents A to D in the upper phase, and A_L, B_L, C_L, D_L, the volumes of solvents A to D in the lower phase. A peak area response for each solvent in the upper phase was determined as described above by gas chromatography (GC) to be A_1, B_1, C_1 and D_1. The peak area determination was made three times and an average taken. The same size of injections (0.2 to 2.0 μl) were made for the upper and lower phases and average peak area responses designated A_2, B_2, C_2, D_2 for the lower phase. The fraction $A_1/A_2 \cdot V_U/V_L$ equals the ratio of the volume of solvent A in the upper phase to the volume of solvent A in the lower phase or $A_1/A_2 \cdot V_U/V_L = A_U/A_L$ (i). Since the total volume of solvent A equals the sum of A in the two phases we can write $A_U + A_L = A$ (ii). Substituting (i) in (ii) the volume of A in each phase can be derived. By repeating this calculation for all GC detectable peaks, the compositions of the two phases were determined. Water was determined separately by a Karl–Fisher titration as a volume percentage for each phase. Thus each phase could be made up separately in equal volumes and combined but retain their equilibrium compositions. Compositions of both phases were determined in those cases where the volume of one phase was substantially smaller than the other (systems 4, 6 and 8 in Table I).

Adjusting the solvent system polarity

To determine the effect of added non-polar solvent, either 1, 5 or 10 ml of the least polar solvent in the system was added to a 50:50 mix of upper and lower phase, shaken and the UV absorbance of Reichardt's dye in each phase was measured.

Partition coefficients

Sample (0.1 g) was shaken with 1 ml each of the upper and lower phases at room temperature. A 100-μl aliquot of each phase was dried under a flow of nitrogen at 50°C in an aluminum dish, weighed, and the partition coefficient (k) calculated as the ratio solute concentration in the lower phase to the solute concentration in the upper phase.

Solubility

Solubility of selected compounds, designated index compounds, were deter-

mined at the 2% concentration or lower level by weighing 0.100 g of the compound into a vial, adding 5.00 ml of one of the phases from the solvent mixtures and shaking to dissolve, then allowing it to stand at least 2 h. A 1-ml aliquot was taken to dryness under nitrogen at 50°C, weighed and the amount of the solute dissolved was determined.

CCC separation

Upper phase of a two-phase solvent system was pumped into a 30 m × 1.6 mm I.D. PTFE tube wound into a coil on one side of a Pharmatech revolving CCC apparatus. The solvent mixture was pumped into the end of the teflon tube starting from the center of the coil to the outside of the coil. A brass counterbalance was mounted opposite from the column on the revolving apparatus. After filling, the coil was rotated about its axis as it was simultaneously revolved about the axis between the coil and counterbalance at 750 rpm. The direction of revolution was set to go forward on the apparatus. This direction of revolution and rotation is such that the solvent in the coil is forced back toward the center of the coil, *i.e.*, the inlet. Pump pressure opposes this force and introduces more solvent. Next, lower phase was pumped onto the rotating column at 3.6 ml/min until no further upper phase was displaced at the column outlet. Sample (180 mg) dissolved in 3 ml of upper phase was introduced through a 5-ml sample loop. Lower phase continued to be pumped onto the column. Effluent from the column was sent through a 1 mm pathlength quartz cell in an Isco Model V4 recording UV detector set at 220 nm wavelength and minimum sensitivity. Fractions (14.4 ml) were collected in a Gilson Model GC-100 automatic fraction collector every 4 min for 90 min and then the revolution and pumping were stopped. Nitrogen was used to displace column contents and 14-ml fractions were collected as the column was emptied. The column was washed by pumping 100 ml ethanol through it and blowing it dry with nitrogen. Collected fractions were evaporated in a Haake Buchler Evapotec vortex evaporator at 50°C under vacuum. Identity of the effluent peaks were confirmed by their GC retention time and infrared spectra.

RESULTS AND DISCUSSION

Composition of the phases

A typical calculation of composition is shown for system 4 in Table II. Solvent systems 6 and 8 were similarly analyzed (Table III). Based on the composition in Table II, 2 l of upper and 2 l of lower phase were made and shaken together. Unfortunately, the two phases did not retain their original volumes of 2 l each, which indicates that some of our GC analyses are not accurate enough. Reproducibility of the GC injection and resolution of overlapping peaks are the probable sources of error. Nevertheless the goal of obtaining an alternative for solvent systme 4 was achieved. Instead of 4 l of a solvent system that separates into 441 ml and 3470 ml the alternative system separates into 1640 ml of upper phase and 2334 ml of lower phase but most importantly, solvachromatic shifts of the upper and lower phases of the alternative system 4 are nearly identical with those of the orignal system 4. Absorbance maxima of system 4 upper phase was 561 nm, system 4 lower phase was 533 nm and for alternative 4 upper, 558 nm and alternative 4 lower, 535 nm. We feel that this

TABLE II

PHASE COMPOSITION OF SOLVENT SYSTEM 4

	Solvent			
	Hexane	Ethyl acetate	Aceto-nitrile	Methanol
Volume mixed (ml)	1250	500	1250	1000
Phase volume (ml)				
Upper (U), lower (L), ratio U:L 441, 3470, 0.121				
GC peak area ratio U:L	3.41	1.19	0.0676	0.0726
Solvent volume ratio U:L	0.413	0.145	0.00818	0.00878
Solvent volume to make each phase[a]				
Upper	366	63.2	10.1	8.7
Lower	884	437	1240	991
Composition (%, v/v)				
Upper	81.7	14.1	2.25	1.95
Lower	24.9	12.3	34.9	27.9

[a] Volume of hexane in lower phase calculation: $H_U + H_L = 1250$ where H_U = volume hexane in the upper phase, H_L = volume of hexane in the lower phase. $H_U/H_L = 0.413$; $0.413\ H_L + H_L = 1250$ and $H_L = 1250/1.413 = 884$.

preliminary method can be refined, yet it provides an expedient alternative for the scientist working with two-phase systems that separate into disparate volumes.

Solvent selection guide

The relative polarity of each phase and some solvents, as determined by the solvachromatic shift method are shown in Fig. 1. The midpoint of the absorbance maxima for a solvent system is shown in the boxed-in area. This midpoint was used to arrange most systems from least polar (top of Fig. 1) to most polar (bottom of Fig. 1). Shifts of some solvent systems could not be measured by this method. Acids, salts, or low solubility of the dye prevented shift measurements in these cases. Therefore solvent systems also were classified by the partitioning and solubility of various solutes designated index compounds. Dashed-line boxes in Fig. 1 are systems placed in the chart based on partitioning and solubility of index compounds. Solute (0.1 g) was dissolved in each phase (5.00 ml) separately to determine the percent solubility of index compounds at low levels. Low concentrations were examined so as not to alter the overall polarity of the solvent mixture. If all the solute dissolved, that constituted >20 mg/ml solubility. Meadowfoam oil, methyl stearate and dodecyl acetate were chosen to represent non-polar solutes and dextrose, glycerol, and K_2SO_4 were chosen to represent polar solutes for ranking the various solvent mixtures. The results for

TABLE III

COMPOSITION OF PHASES IN SYSTEMS 6 AND 8

System		Upper %	Lower %
6	Chloroform–methanol–water (13:7:2)	6.7:53.4:39.9	64.3:29.7:6.0
8	Chloroform–methanol–water (7:13:8)	11.7:53.4:34.9	76.7:19.3:4.0

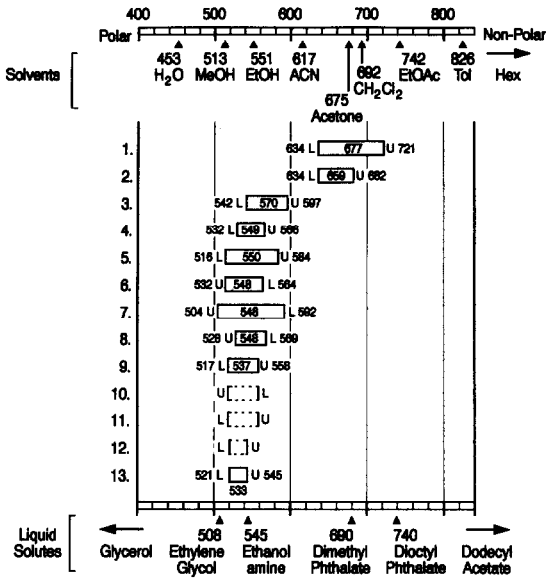

Fig. 1. A solvent selection guide for counter-current chromatography showing the absorbance maxima of Reichardt's dye (1 mg in 10 ml) in each phase of 13 two-phase solvent systems and 15 individual solvents and solutes. Dashed-line boxes were classified by solubility of index compounds, not Reichardt's dye. Midpoint values between upper and lower phase values are given inside the boxed-in spaces. Abbreviations: Hex = hexane; ACN = acetonitrile; MeOH = methanol; EtOH = ethanol; Tol = toluene; EtOAc = ethyl acetate; U = upper phase; L = lower phase. For solvent systems 1–13, see Table I.

these six compounds are shown in Table IV. Relative rankings of polar phases (P_P) from least polar (1) to most polar (13) for all two-phase systems were made using these rules for non-polar solutes (S_N): (1) if methyl stearate (MSt) and meadowfoam oil (MO) were soluble at >19.5 mg/ml phases were ranked by the highest dodecyl acetate (DDA) solubility being least polar; (2) after rule 1, phases in which MSt was soluble at 19.5 mg/ml or greater were ranked by meadowfoam oil solubility; (3) after the above, if MSt solubility < 19 mg/ml and MSt, MO and DDA were $\neq 0$ the phases were ranked least polar by the highest sum of MSt, MO and DDA solubility; (4) after the above, if only MSt solubility = 0 the highest sum of MO plus DDA solubility was ranked most non-polar; (5) after 1 to 4, if MO or DAA solubility = 0 the sum of the other two were used to rank the systems; (6) finally, systems where solubility of MSt, MO and DDA = 0 were considered most polar in the ranking.

Relative rankings of non-polar phases, P_N, was made using the above rules for non-polar solutes, S_N. A similar set of rules using polar solutes (S_P) was used to rank the various phases. The four ranking combinations P_PS_N, P_PS_P, P_NS_N, P_NS_P resulted in Table V for the various phases of systems 9–13. Systems 10, 11 and 12 could not be ranked with Reichardt's dye. Table V results were thus used to place systems 10 and 11 very close to system 9 and system 12 very close to system 13.

There are three groups of systems —the lipohilic (systems 1–3 with 3 the least lipophilic), the polar systems (systems 9–13) and intermediate systems 4–8. Relative placement within each group may depend more on the difference in polarity between

TABLE IV

SOLUBILITY (mg SOLUTE/ml SOLVENT PHASE) OF 13 TWO-PHASE SOLVENT SYSTEMS

Solvent[a] system, phase	Solubility of					
	Methyl stearate	Meadowfoam oil	Dodecyl acetate	Glycerol	Dextrose	K_2SO_4
1U	>20	>20	16.1	0	0	0
L	11	0.32	18.9	17	0	0
2U	>20	>20	18.2	0	0	0
L	>20	3.1	9.9	14.7	0	0
3U	>20	19.1	12.9	1.0	0	1.7
L	13.9	0.84	11	>20	>20	0.24
4U	>20	19.9	13.3	0.30	0	0
L	16.0	12.5	13.0	>20	1.1	0.4
5U	>20	>20	11	1.0	0.18	0
L	0.30	2.2	2.8	>20	>20	1.7
6U	0	0	0	>20	>20	0.64
L	20	18.8	17.3	18.2	6.6	1.0
7U	0	0.54	0.18	>20	>20	2.9
L	19.6	>20	10.7	5.4	0.19	0.34
8U	0	0	0	>20	>20	1.4
L	18.8	19.8	8.9	15.6	2.12	0.32
9U	>20	9.9	>20	13.5	2.0	0
L	0.29	2.2	0	>20	>20	3.7
10U	0.50	0.20	0	>20	>20	2.62
L	>20	19.4	18.6	7.7	0.31	.07
11U	>20	3.5	19.1	12.9	4.0	.09
L	0.6	0.1	0.33	>20	>20	0.47
12U	19.6	3.5	18	>20	6.56	0.47
L	.21	0	0.25	>20	>20	19
13U	>20	2.32	17.4	17.4	8.7	
0.37	L	0.1	0.26	0	>20	>20
19.6						

[a] Make-up of solvent system as in Table I and Fig. 1. U = Upper phase; L = Lower phase.

TABLE V

RANKING OF PHASES IN SYSTEMS 9–13 BASED ON SOLUBILITY OF NON-POLAR SOLUTES (S_N) AND POLAR SOLUTES (S_P) IN UPPER (U) AND LOWER (L) PHASES[a]

Ranking least to most polar	Ranking criteria[a]				System	Average rank
	P_NS_N	P_NS_P	P_PS_N	P_PS_P		
1	10L	9U	11L	11L	9	2.0
2	9U	10L	9L	10U	10	2.0
3	11U	11U	10U	9L	11	2.0
4	12U	13U	12L	12L	12	4.25
5	13U	12U	13L	13L	13	4.75

[a] See discussion for applying solubilities in Table IV to determine relative polarities.

TABLE VI

SHIFT OF UV–VISIBLE ABSORBANCE MAXIMA OF REICHARDT'S DYE IN TWO-PHASE SYSTEMS WHEN NON-POLAR SOLVENT IS ADDED

System	Upper phase shift (nm)			Lower phase shift (nm)		
	+1 ml	+5 ml	+10 ml	+1 ml	+5 ml	+10 ml
3	−19	−12	−13	0	− 3	0
4	+ 5	0	+ 1	−2	− 3	+3
6	+10	+ 6	+ 6	+2	+ 2	+5
7	+ 5	0	+ 1	+1	+ 1	+3
8	+ 7	+ 2	+ 2	0	+ 1	+1
9	+ 1	+ 4	+ 6	+1	− 9	−9
13	0	− 1		−7	−13	

the upper and lower phase than on the average polarity of the system. The average polarity is similar by both the Reichardt's dye test and the index compound test. System 7 for example has a system polarity similar to system 8 but the upper and lower phases of system 7 are further apart in polarity than those of system 8. Partition coefficients of two natural products illustrate this effect. The partition coefficient of kampferol in system 8 is 3.40 and in system 7 is 4.03. The partition coefficient of rutin in system 8 is 2.04 and in system 7 is 2.25. This agrees with Fig. 1 and Table IV in that both are very similar in separation characteristics. However, he values for system 8 are closer to 1.0 than those of system 7 which reflects the closer polarity and more nearly equal partitioning of solute in the two phases.

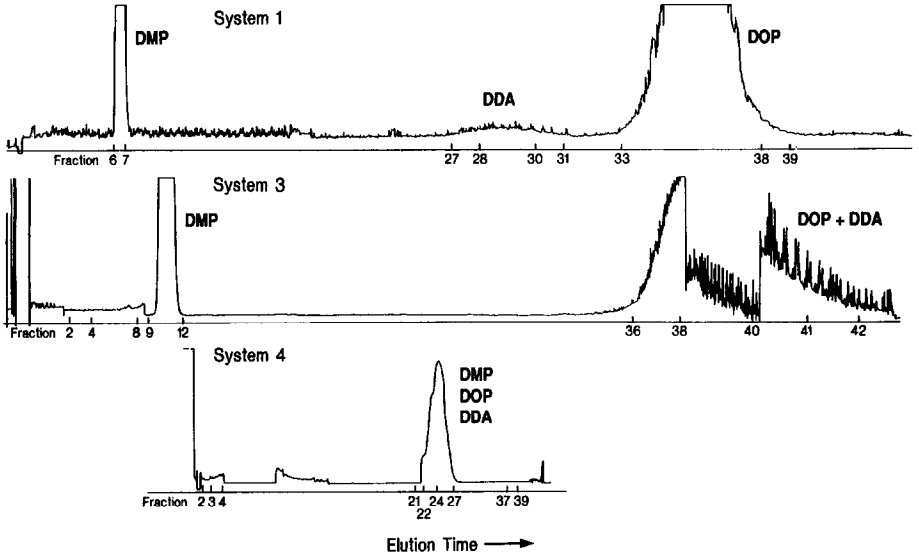

Fig. 2. Chromatograms from CCC Separations of dioctyl phthalate (DOP), dimethyl phthalate (DMP) and dodecyl acetate (DDA). Flow-rate 3.6 ml/min for 90 min, UV 220 nm, mobile phase: lower, 14.4-ml fractions collected. Column contents displaced with nitrogen after fraction 22.

Adjusting the solvent system polarity

Since so many systems have similar partitioning characteristics it seems doubtful that adding 1, 5 or 10 ml of the least polar solvent to a system would have much effect on the polarity of the two phases. Nonetheless, it is a well-known practice for adjusting CCC solvent system polarities. Using Reichardt's dye we tested the effect of adding 1, 5 or 10 ml of the least polar solvent, the first listed for each system in Fig. 1, to 100 ml of each system, 50 ml of each phase (Table VI). In some cases two phases were not maintained, but surprisingly some significant shifts in absorbance maxima did occur. The differences that these shifts make in partition coefficients and separations by CCC need to be investigated further.

CCC separations

The overall usefulness of classifying solvent systems by polarity for CCC separations is clear, but many separations using CCC are necessary to determine whether solvent systems with similar polarities are interchangeable for separations. If we use Fig. 1 and the interpretation above of Table IV data then we would expect that separation of dimethyl phthalate, dioctyl phthalate, and dodecyl acetate might be best accomplished in one of the systems from 1 to 4. Partition coefficients (not reported) and then actual separations as shown in Fig. 2 bore this out. The apparent changes in slope in the chromatogram from system 4 looked like possible separation so the fractions were reevaluated individually in a Hewlett-packard Model 8450A photodiode array UV–visible spectrophotometer linked to a Mod Comp Classic computer. Chromatograms were constructed at several wavelengths and intensities but no separation was evident for system 4 as shown in Fig. 3 for the reconstructed chromatograms at 254 nm. The separation in solvent system 1 is also evident in Fig. 3. We feel that this separation demonstrates the usefulness of the solvent selection guide for selecting a group of solvents. This group should be further examined by partition coefficients to select one best system for a particular separation. Much more research needs to be done on comparing several of the systems for nearly equivalent partition-

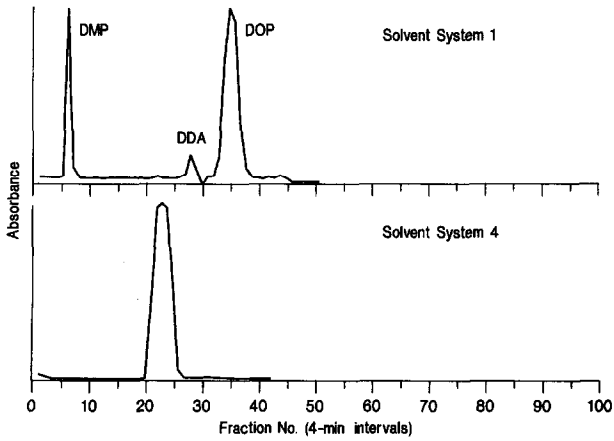

Fig. 3. Reconstructed chromatograms from fractions collected as in Fig. 2 but absorbance was remeasured at 254 nm.

ing ability. We find that many are similar, especially in the group from system 4 to system 8. Each system may have unique properties for a particular type of compound. However, deducing these differences will require that many partition coefficients be evaluated or that another method of evaluating mixed solvent polarity be used.

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