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Development of a phase system for intermediate polarity compounds in centrifugal partition chromatography

STEVEN J. GLUCK* and MICHAEL P. WINGEIER
Analytical Sciences Department, Building 1897, Dow Chemical, Midland, MI 48667 (USA)

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ABSTRACT

A new phase system and an approach to developing phase systems relying on composition, physical data and an empirical polarity index have been proposed for general use in centrifugal partition chromatography. The system developed possesses a comparably low viscosity necessary for improved efficiency at a composition where compounds of intermediate polarities have a high solubility in both the upper and lower layers. This system should be useful for traditional isocratic use and in normal-phase ascending gradient separations, because the composition and polarity changes of the lower phase were small relative to the changes in the composition and polarity of the upper phase.

INTRODUCTION

Centrifugal partition chromatography (CPC) is an alternative to preparatory and process-scale liquid chromatography (LC) for the isolation of impurities or purification of a crude material. Compared to LC, CPC has a relatively higher capacity [1,2] attributable to the large volume of stationary phase available to partitioning solutes. Common solvents are used as both the mobile and stationary phases in contrast to LC. Thus, CPC potentially has an economic advantage relative to LC because of a much lower solvent consumption at comparable mass throughputs and the absence of an expensive chromatographic packing. The absence of the silica or otherwise potentially adsorbtive or catalytic surface commonly used in column-packing materials means that sensitive samples have less of a chance to be altered. In contrast to LC, the CPC stationary phase may be switched to become the mobile phase; at the same time the mobile phase becomes the stationary phase (dual-mode operation). Thus, sample recoveries are always 100% and no material is irreversibly sorbed.

Alternatively, LC has the advantage of having a relatively simple method development scheme, because of the limited number of choices for a stationary phase. With a chemically bonded stationary phase or a bare solid surface, LC method development requires little consideration for the effects of the mobile phase on the stationary phase compared to CPC or other counter-current chromatographic techniques. Indeed, the "art" has been reduced to a technology of using expert systems

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for analytical separation development and, more recently, extensive modeling has occurred even in the areas of preparatory separations. In common practice, most LC preparative separations are optimized by investigating the separations on different manufacturers variations of the same sorbent material rapidly on a small scale.

In CPC, phase development is a required and more complex task. Virtually any solvents may be incorporated into a phase system, and most phases developed in the literature have not been chosen out of a systematic optimization scheme. Phase development for CPC seems to have focused along the empirical needs of a crude material to be isolated or purified rather than the development of a phase system which should be applicable to many materials. As an example, the chloroform–methanol–water system has been reported with different mixing ratios by different authors. However, most of these mixing ratios were shown by Foucault and Nakanishi [3] to reside close to the same tie-line of the ternary solvent diagram indicating that all of the authors were using similar upper and lower phase compositions. Another example includes the use of up to five different solvents in the phase system [4]. Perhaps an efficient and well understood ternary system could achieve or at least be a starting place for these types of challenges with less method development time.

Identification of a small set of generally useful phases to fit many applications continues to be a major challenge for optimizing CPC separations. The physical properties of an ideal solvent system for CPC are those which allow the maximum separation efficiency and the highest system throughput. In LC, and usually in CPC, this is a trade-off which must be optimized due to the limiting rate of mass transfer of a solute from the stationary phase to the mobile phase. In CPC, as phase viscosity is lowered, efficiency improves. This phenomenon is dramatic in situations of viscosities of less than about 0.5 cP where an "inverse Van Deemter"-type relationship is observed [5]. In these special cases, separation efficiency reaches a minimum and then increases with flow-rate. This phenomenon is believed to be due from improved mass transfer caused by an increase in mixing and an increase in the interfacial surface area between the two phases. Attempts to increase efficiency with higher flow-rates of the commonly used phase systems usually result in exceeding the apparatus pressure limits of 850 p.s.i.g. before any increase in efficiency is observed. However, over the useful flow-rates, the lower viscosity phase systems appear to have improved efficiencies.

The elution volume of the solute is dependent on the volume of the stationary phase, the volume of the mobile phase and the partition coefficient of the solute between those phases. The phase volumes are experimentally controlled by the flow-rate and angular velocity of the rotor. Experimental control of the partition coefficient is significantly dependent on the polarity of each phase and the polarity and polarizability of the solute. For development and investigative purposes, the use of a solvochromatic parameter may be used to judge the relative phase polarity of mixed solvent systems. This is a rapid, simple and precise parameter to measure compared to actually determining the partition coefficients of a series of solutes for development purposes. Reichart and Dimroth's dye (hereafter referred to as Reichart's dye) number 30 has been documented to relate the relative solvent strengths of aqueous mixtures of acetonitrile–tetrahydrofuran–methanol [6]. Hence, the use of this dye can guide the development of mixed-phase system polarity.

Useful characterization of the solvent system for CPC should include the phase diagram with the densities, viscosities and the polarities of the upper and lower layers formed by the various mixing ratios of the solvents. The separation of hydrocarbon-type materials is done well on a hexane–methanol–1% water system which has been partially characterized in refs. 3, 7 and 8. Many other phases have been used for different applications of intermediate polarity compounds. One of the more common phase systems is the 1-butanol–water system. It is the goal of this paper to present a scheme for characterization of CPC phases and to present a generally useful phase for separating solutes of intermediate polarity.

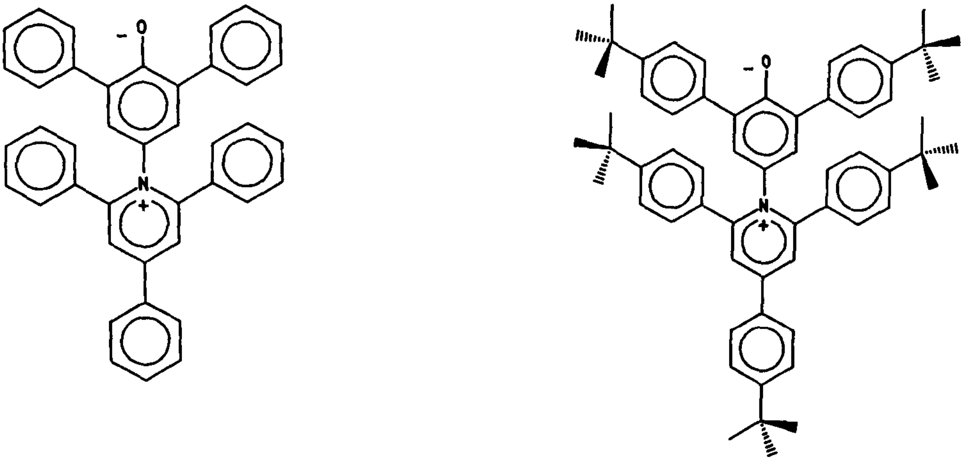
EXPERIMENTAL

Viscosity measurement

Kinematic viscosities were measured at 22°C using Cannon-Fenske routine viscometers (Fisher Scientific). Solution densities (22°C) were taken from the mass of 5.00 ml dispensed from a volumetric pipet. Viscosities in centipoise were the density times the kinematic viscosity.

Measurement of solvent polarity

A small amount, 0.01–0.2 mg, of Reichardt's dye, I (Aldrich catalogue number 27,244-2), was dissolved in 5 ml of the test solution (Fig. 1). The solution was placed in a 1-cm-pathlength cuvette and the absorbance was scanned from 800 to 400 nm using a 2-nm slit width and a 0.5-nm sampling interval at the fast slew setting of a Shimadzu UV-3101 PC spectrophotometer. Reichardt's dye has too low of a solubility to be used in non-polar solvents and thus the penta *tert.*-butyl substituted analogue, II (Aldrich catalog number 27,305-8), was used in these cases (Fig. 1). From the absorbance



I

II

Fig. 1. Structures of Reichardt's dye (I) and the penta *tert.*-butyl substituted analogue (II).

maximum, λ_{max}, in nm of the solvatochromatic dyes, the energy of transition, E_t, was calculated:

$$E_t(\text{kcal/mol}) = 28590/\lambda_{max}$$

The E_t of II, E_t', can be converted to the E_t of I, commonly known as $E_t(30)$, by a simple linear transformation [9]:

$$E_t(30) = \frac{E_t' - 3.434}{0.9143}$$

To convert the E_t to a dimensionless unit, Reichardt and Harbusch-Gornert [9,10] have recommended the normalized E_tN. The normalized E_tN uses the polarity extremes of water and tetramethylsilane (TMS) as reference solvents:

$$E_tN = \frac{E_t(\text{test solvent}) - E_t(\text{TMS})}{E_t(\text{water}) - E_t(\text{TMS})} = \frac{E_t(\text{test solvent}) - 30.7}{32.4}$$

Therefore, non-polar liquids have an E_tN close to 0 and polar liquids have an E_tN close to 1.

Partition coefficient measurement

Partition coefficients, K, were determined by adding 1 μl of a 50% mixture of ethyl acetate and 1-butanol to 3 ml each of upper and lower phases. The phases were shaken vigourously and allowed to separate twice, thus allowing equilibrium to be established. On-column capillary gas chromatographic (GC) analysis with flame ionization detection was performed for the peak-area determinations of the test solutes. The peak area of the solute in the upper layer divided by the peak area of the solute in the lower layer was taken to be the partition coefficient.

Phase composition determinations

Phase composition was determined by split-injection capillary GC analysis with external standard calibration. The response factors were determined to shift with the composition of the samples and thus the composition of each sample was first estimated and then multiple standards were prepared with a composition similar to that of each sample. Water composition was determined by percentage difference. The tie-lines connecting the upper and lower layers on the methyl *tert.*-butyl ether, acetonitrile and water phase diagram intersected within ± 1% of the bulk composition ratio and thus served as an internal check on the determination accuracy.

CPC apparatus

Example separations were performed on a Sanki Model LLN CPC system manufactured by Sanki Engineering. Both twelve-cartridge (4800 50-μl stages) and six-cartridge rotors were used. All separations were at 22°C under the conditions listed.

TABLE I

PHYSICAL AND CHEMICAL DATA REPRESENTATIVE OF SOME PHASES USED IN CPC

The viscosities and densities are at 22°C.

Phase[a]	Volume		Viscosity (cP)		E_tN		Density (g/ml)	
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower
1% Water in MeOH–hexane (1:1)	2	3	0.34	0.37	0.62	0.50	0.665	0.763
Hexane–ACN	1	3	0.35	0.59	0.23	0.45	0.668	0.745
THF–ACN–Hexane (1:2:2)	1	4	0.37	0.39	0.38	0.40	0.709	0.764
EtOAc–EtOH–water (5:2:4)	5	6	0.40	1.59	0.40	0.58	0.759	0.950
Hexane–EtOAc–DMF–water (3:3:4:1)	4	1	0.49	1.48	0.45	0.58	0.816	0.946
CCl$_4$–MeOH–water (10:9:1)	1	2	0.67	1.22	0.69	0.80	0.814	0.933
Hexane–EtOAc–DMF–water (15:21:18:2)	5	1	1.31	0.58	0.45	0.59	0.814	0.946
CHC$_3$–MeOH–nPrOH–water (45:60:10:40)	3	1	1.10	0.97	0.69	0.57	1.10	1.51
BuOH–nPrOH–water (4:1:5)	4	3	1.84	0.95	0.70	0.62	0.960	1.19
BuOH–HOAc–water (4:1:5)	1	1	3.02	1.56	—[b]	—[b]	0.848	0.979
BuOH–water (1:1)	1	1	3.06	1.38	0.65	0.89	0.840	0.984
14% Aquaphase PPT–5% PEG 8000 in water	1	1	6.87	42	—[c]	—[c]	1.03	1.05
MtBE–isooctane–MeOH–water (10:2:8:6)	2	5	0.92	1.17	0.61	0.74	0.746	0.872

[a] MeOH = Methanol; ACN = acetonitrile; THF = tetrahydrofuran; EtOAc = ethyl acetate; EtOH = ethanol; DMF = dimethylformamide; CCl$_4$ = tetrachloromethane; CHCl$_3$ = chloroform; nPrOH = *n*-propanol; BuOH = butanol; HOAc = acetic acid; aqueous PPT from Perstorp Biolytica; MtBE = methyl *tert.*-butyl ether.

[b] Not applicable to acidic solutions.

[c] The absorbance background in these solutions was too high to be able to determine the maximum for Reichardt's dye.

RESULTS AND DISCUSSION

Some fundamental data for a set of CPC phases are presented in Table I for perspective. These data include the relative volumes of the upper and lower layers, the viscosities of each layer, the densities of each layer and the E_tN values for each layer. The hexane–methanol–1% water phase is the most efficient phase ever reported [8]. Typical chromatograms for this phase are shown in Fig. 2a and b which indicate excellent efficiencies. These chromatograms are only presented as a goal for future separation to be obtained in other phases. The low viscosity of this phase system was an important criterion for the high efficiency. The disadvantage of this phase system is the low solubility many materials of moderate polarity exhibit in the hexane phase thus limiting its use for isolation and purification.

In contrast, the butanol–water system has found wide use for isolating intermediate polarity compounds such as peptides and natural products in other liquid–liquid chromatographic apparate and CPC [11]. Compared to this system, a methyl *tert.*-butyl ether–acetonitrile–water (30:30:40) phase system exhibits improved chromatographic efficiency. Fig. 3 shows the relationship between peak width at half height *versus* elution time for a series of test solutes run on both of these systems under similar experimental conditions (5 ml/min ascending flow-rate, 300-rpm, 120-ml six-cartridge rotor). In the butanol–water system, the peak width increased more rapidly than in the methyl *tert.*-butyl ether-acetonitrile–water (30:30:40) phase

TABLE II

PHYSICAL AND CHEMICAL DATA FOR THE METHYL *tert.*-BUTYL ETHER–ACETONITRILE–WATER PHASE SYSTEM

The viscosities and densities are at 22°C. MtBE = methyl *tert.*-butyl ether; ACN = acetonitrile; EtOAc = ethyl acetate; BuOH = butanol.

MtBE–water–ACN composition (v/v/v)	Weight% ACN		Weight% MtBE		E_tN		Density (g/ml)		Viscosity (cP)		K		α
	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	EtOAc	BuOH	
25:25:0	0.0	0.0	95.2	4.0	0.284	0.942					8.52	3.95	2.16
25:25:5	8.5	6.8	85.1	4.0	0.487	0.895					8.80	4.66	1.89
25:25:10	18.1	11.5	70.3	4.4	0.569	0.861	0.76	0.97	0.472	1.2	8.00	4.30	1.86
25:25:15	25.7	14.2	58.1	4.7	0.617	0.839					6.92	4.20	1.65
25:25:20	30.7	16.9	47.1	5.2	0.642	0.824	0.79	0.96	0.549	1.18	6.36	4.08	1.56
25:25:25	38.0	18.4	42.2	5.3	0.663	0.817					5.51	3.65	1.51
25:25:30	41.0	20.1	37.4	5.7	0.681	0.807					4.78	3.24	1.48
25:25:35	43.7	22.0	32.1	5.9	0.690	0.800	0.8	0.94	0.612	1.18	4.18	2.86	1.46
25:25:40	45.6	23.2	28.6	6.2	0.702	0.789	0.81	0.94	0.639	1.15	3.58	2.58	1.39
25:25:45	45.2	24.3	24.2	6.6	0.711	0.783	0.84	0.95	0.755	1.14	3.04	2.23	1.36

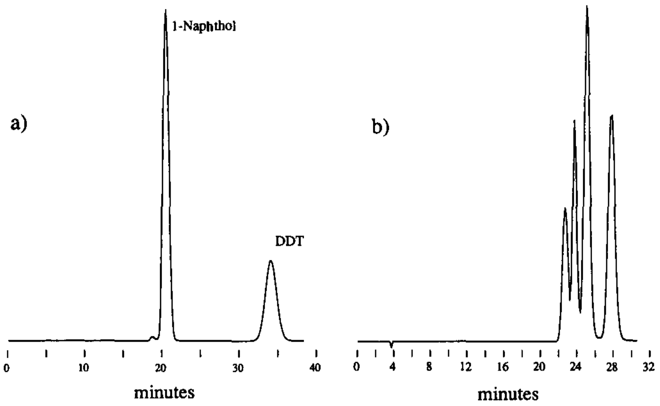


Fig. 2. Example separations of a test mixture using the highest-efficiency CPC phase system known to date. The phases were made from 1% water in methanol equilibrated with an equal volume of hexane. The mobile phase volume (V_m) was 92–94 ml and the total system volume (V_t) was 215 ml (twelve cartridges). (a) 1-Naphthol and DDT. (b) Alltech reversed-phase mixture D consisting of uracil–phenol–benzaldehyde–N,N-diethyl-*m*-toluamide–toluene–ethyl benzene. Component identities have not been determined in this chromatogram.

system. Therefore, the latter phase system would provide better resolution for similarly retained compounds than the butanol–water system. However, neither of these phase systems approach the optimum potential efficiency of the apparatus as shown in Fig. 2a and b.

Phase diagram

The composition of the various mixtures of the methyl *tert.*-butyl ether–acetonitrile–water phase system are given in Table II along with the E_tN polarity values, the densities and the viscosities. The viscosities of this system are lower than others which have been used for moderate-polarity solutes and thus this system should provide superior efficiencies. The volume percent approximation of the weight percent

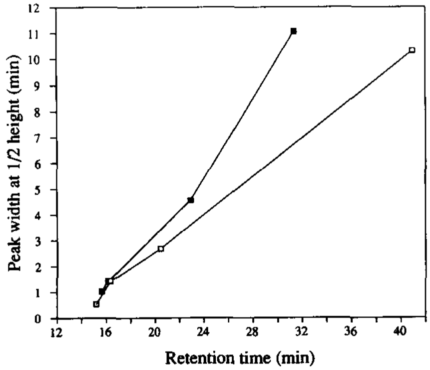


Fig. 3. Relationship between peak width at half height *versus* retention time comparing 1-butanol–water (■) to the methyl *tert.*-butyl ether–acetonitrite–water (30:30:40) phase system, both applicable to the separation of intermediate polarity compounds.

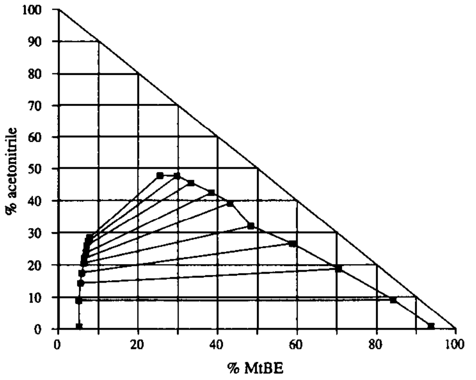


Fig. 4. Approximation of the volume percent phase diagram (calculated from the weight percent phase diagram) of the methyl *tert.*-butyl ether (MtBE)–acetonitrile–water system.

phase diagram is shown in Fig. 4. The composition of the upper and lower layers of ten different solvent mixing ratios were determined by GC. The points on the left represent the composition of the upper layers. The lines between the points are the tie-lines where any solvent mixing ratio will separate into the same upper and lower layer compositions. The binodal is the line representing the boundary between a biphasic and homogeneous phase system. Inspection of the phase diagram indicates that this system could be useful in normal-phase gradient elution. Successful gradient elution in CPC requires one of the phases to have a large change in composition along the binodal of the phase diagram while the other phase has a small change [3].

Phase polarity

The E_tN values show the phases converging in polarity as the tie-lines approach the Plait point (the Plait point is the solvent composition on the binodal where the

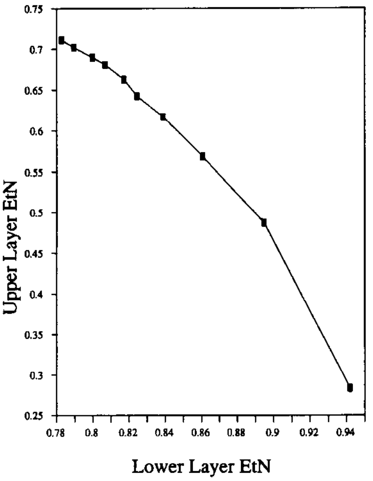


Fig. 5. E_tN polarity index of the upper layer *versus* the lower layer of the methyl *tert.*-butyl ether–acetonitrile–water system.

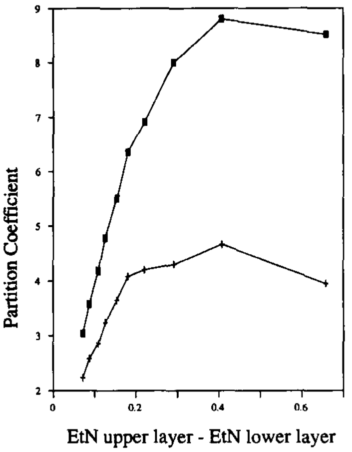


Fig. 6. Partition coefficients of ethyl acetate (■) and 1-butanol (+) *versus* the difference in the polarity index, E_tN, of the upper and lower layers.

tie-lines converge). Therefore, as the polarity of the upper phase increases, the polarity of the lower phase decreases. This relationship is plotted in Fig. 5. The polarity change in the lower phase is small relative to that of the upper phase which is another factor making the use of a normal-phase, ascending-mode gradient attractive.

A pair of solutes exhibiting good solubility in all compositions of this ternary system, ethyl acetate and 1-butanol, was chosen to illustrate the relationship between phase polarity and partitioning. As the differences in the E_tN values of each phase increase, the partition coefficients of both solutes increase (Fig. 6). In addition, the α values (the ratio of the partition coefficients) correlate in a near linear fashion to the differences in the E_tN values (Fig. 7).

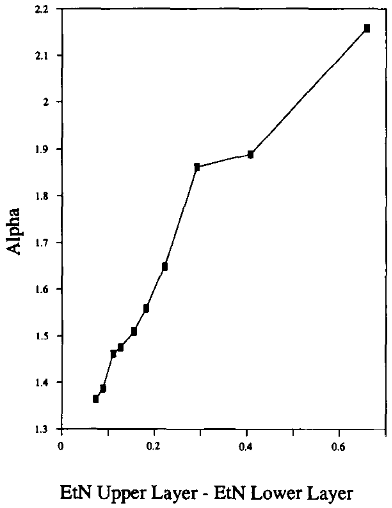


Fig. 7. α values of ethyl acetate and 1-butanol *versus* the difference in the polarity index, E_tN, of the upper and lower layers.

CONCLUSIONS

A new phase system has been developed for general use in CPC. It possesses relatively low viscosities at intermediate ranges of polarities. It should be applicable to the isolation or purification of intermediate-polarity substances. The phase diagram was established for understanding the composition of the phases prepared with different solvent ratios. This phase system should be useful in traditional isocratic separations and also in normal-phase, ascending-gradient separations because the composition and polarity of the lower phase changes little with respect to changes in the composition and polarity of the upper phase.

The relationship between the E_tN, K and α values reveals what may prove to be a rapid means to screen new, potential phases and their usefulness in CPC. The measurement of E_tN is simple, rapid and can be carried out in many laboratories. Classifying CPC phases by their E_tN provides a semi-quantitative measure of phase polarity, a critical selectivity parameter. However, it should be cautiously recognized that E_tN values lack the ability to measure other important selectivity parameters and cannot be determined in acidic media. But even with a recognized quantitative limitation of E_tN values as a means to predict partitioning, we have demonstrated their utility for phase development.

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