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Solvatochromic studies in polyethylene glycol–salt aqueous biphasic systems

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Abstract

The polarities of the co-existing phases of a polyethylene glycol (PEG)-2000–K_3PO_4 aqueous biphasic system (ABS) have been examined using Reichardt's carboxylated pyridinium-*N*-phenoxybetaine dye as a probe. Using this probe, the polarities of these phases have been compared to those of conventional solvent extraction systems and micellar systems using values obtained from the literature. In general, these extraction systems are comparable in polarity to rather polar solvents. Data on the free energy of transfer of solvents suggests that this may be due to the failure of the probe to account for the real polarity of the salt-rich phase compared to the polymer-rich phase. Examination of the monophasic region of these systems suggests that the reason for this is that the probe is partitioned to a discreet solvent domain dominated by PEG, even though phase separation of the solution is not observed. The use of linear free energy relationships for the characterization of ABS is briefly discussed.

Keywords: Aqueous two-phase systems; Solvatochromic studies; Linear free energy relationships; Poly(ethylene glycol)

1. Introduction

Aqueous solutions of polymers have excited some recent interest for their ability to solubilize otherwise poorly soluble species without the involvement of organic solvents [1]. Such systems include *inter alia*, cloud point extraction (CPE) [2], micellar extraction (ME) [3], thermoseparating polymer systems (TPS) [4] composed of PEO–PPO (polyethylene and polypropylene oxides) and other co-polymer solutions, aqueous biphasic systems (ABS) [5], and dendrimers [6]. This ability to enhance the solubility of, or to capture, hydrophobic substances appears to be manifest both in free solution and when the polymer is bound to a physical support as for example in aqueous biphasic extraction chromatography (ABEC) [7].

It has been suggested that these wholly aqueous solvent extraction systems (WASE) represent environmentally benign alternatives to the conventional use of organic solvents in liquid–liquid extraction processes [1]. It is, therefore, of interest to be able to make comparisons of the observed solubilization and distribution properties of solutes between the different micellar and biphasic systems, and between these systems and the bulk solvent phases of traditional solvent extraction. Through illumination of the molecular forces involved in these systems a ''tool box'' of polymeric extractants may be made available to aid in the replacement of a solvent based process with one based on solubilization using aqueous polymeric solutions.

Numerous methods are available by which such

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comparisons may be made and there is only space to mention a few here. These include solvatochromic methods, which in their simplest form measure the effect of the solvent on some molecular property, and are generally interpreted in terms of solvent polarity [8]. For instance the Py scale of Dong and Winnick [9] has been used to produce an empirical ranking of solvent polarities, based on the ratio of the first and third vibronic bands of the emission spectrum of the fluorescent probe, pyrene. This scale has been used to examine the solvent-like environment of Triton X-114 micelles [10] which seemed to be equivalent to the bulk phase of the solvent 1,2-dichloroethane (1.46) at 5°C. Increase in temperature to 25°C close to the critical point leads to an apparent decrease in polarity equivalent to that of 1,5-pentanediol (1.36).

Similarly, Reichardt's $E_T(30)$ scale of solvent polarity is based on the molar transition energy of the intramolecular charge transfer band of a betaine dye [11], carboxylated pyridinium-*N*-phenoxy-betaine. Polymer solutions pertinent to the characterization of polymer–polymer ABS have been studied using this method [12] and the polarity of various micellar systems [13] has been reported.

Another comparative approach to the study of the solvent-like properties of these polymeric systems may be sought in the direct comparison of the partition of solutes. The relationship between Log*P* in 1-octanol–water and distribution in ABS [14–17] or micellar [18–21] and cloud point systems [22,23] has often been pointed out, which is really only a restatement of the ''Collander equation'' [24]:

$$\mathrm{Log}P_1 = a + b\,\mathrm{Log}P_2 \quad (1)$$

where the subscripts refer to partition coefficients in different solvent–water systems and the remaining terms are constants of proportionality. Strictly, such relationships only apply to chemically related solutes, and where many solutes are considered, the two systems will show many such relationships for each solute type [24]. In addition, the solute–solvent interactions may be quite different for different solvent systems where the solvent may be a hydrogen bond acid or base (or both), or neutral and may contain varying amounts of water at equilibrium [24,25].

Similar comparisons between extraction systems have been made for ABS on the basis of the free energy of transfer of a methylene group [$\Delta G_{tr}(CH_2)$] [26,27], since this is directly related to the distribution coefficient through:

$$\Delta G_{tr} = -RT \ln K \quad (2)$$

Similar limitations noted for the ''Collander equation'' may be thought applicable. It is interesting to reflect that some ''hydrophobicity scales'' are derived in a similar way [28,29].

A more chemically illuminating way of comparing solvent properties may be thought to be available through the development of linear solvent free-energy relationships (LSERs or LFERs) as proposed by Kamlet and Taft [30,31] and more recently Abraham [32,33]. The most recent of these are no longer solvatochromic in nature, but are based on chemical equilibria and are thus Gibbs energy related. The LSER of Abraham takes the form:

$$\mathrm{Log}\,SP = c + rR_2 + s\pi_2^{\mathrm{H}} + a\Sigma\alpha_2^{\mathrm{H}} + b\Sigma\beta_2^{\mathrm{H}} + vV_x \quad (3)$$

where c is a constant, R_2 is the solute excess molar refraction, π_2^{H} is the dipolarity/polarizability obtained from partition measurements, and $\Sigma\alpha_2^{\mathrm{H}}$ and $\Sigma\beta_2^{\mathrm{H}}$ are the effective hydrogen-bond acidity and basicity respectively. V_x is the McGowan volume [32–35].

In this paper we report on some of our recent work on partitioning in ABS in which we have examined the polarity of the phases of PEG–salt ABS using the betaine dye of Reichardt. We also briefly report on other work on the solvent-like properties of these systems using LFERs which is currently in progress in our laboratories.

2. Experimental

The chemicals, K_3PO_4, $(NH_4)_2SO_4$, thymol blue, and poly(ethylene glycol) (PEG, average MW= 2000), were obtained from Aldrich (Milwaukee, WI, USA) and were of reagent-grade. Reichardt's carboxylated betaine dye was a kind gift of Professor Dr. Christian Reichardt (Department of Chemistry, University of Marburg). All water was purified using

a Barnsted commercial deionization system (Dubuque, IA, USA).

Solvatochromic studies were performed using both thymol blue and Reichardt's betaine dye as the solvatochromic reporting molecules. Initial studies have concentrated on the PEG-2000–K_3PO_4 ABS which was made up on a weight/weight percent basis. Measurements of the solvatochromic shifts in monophasic PEG–salt mixtures (below the binodal curve) were made at 20°C. Measurements on the separated phases of biphasic systems were performed 2°C below this equilibrium temperature to counteract heating in the spectrophotometer and prevent clouding and redistribution of the added dye due to the onset of phase separation.

Monophasic systems were made up to give a total weight of 5 g and 10 μl of a 2 mg/ml solution of thymol blue was then added. In the case of Reichardt's betaine dye, a concentration of 0.5 mg/ml was used giving a final concentration of ca. 5×10^{-5} *M* (10 μl added to a 5-g solution containing polymer and/or salt). The solutions were mixed thoroughly and the absorbance of an aliquot of the solution scanned using a Cary 3C UV–Visible scanning spectrophotometer (Varian Optical Spectroscopy, Mulgrave, Victoria, Australia) set to sample at 0.25-nm intervals with a signal-to-noise ratio of 1000.

Peak height was determined by the method of Kamlet and Taft [30] in which the wavelengths either side of the absolute peak maximum having 90% of the peak maximum absorbance (KT_{90}) are averaged. In some cases with the betaine dye, this criterion was changed to 95% peak maximum to cope with situations where the solvatochromic peak could not be detected otherwise because of the broadness of the peak. There were occasions when the betaine solvatochromic peak maximum could not be distinguished by either method (see below).

For symmetrical peaks with noise-free data the absolute peak maximum and the KT_{90} method should be equivalent. In reality, and in the case of the data presented here, the absolute peak maximum is subject to greater variability, but the Kamlet and Taft parameter gives a peak maximum shifted to consistently lower wavelengths due to peak asymmetry. The molar transition energy (E_T, kcal mol^{-1}) of the peak of maximum absorption is calculated [12] as:

$$E_T = hcN_A\nu_{max} \quad (4)$$

where ν_{max} is the wave number of the absorption maximum, *h* is Planck's constant, *c* is the speed of light in a vacuum, and N_A is Avagadro's number.

The determination of the preliminary LSER relating the partition coefficient of a limited number of organic solutes using Abraham's published solute descriptors [34,35] for the PEG-2000–$(NH_4)_2SO_4$ ABS was determined by multiple linear regression. Partition coefficients, expressed as log *D* [Eq. (5)], for generating this data were based on the distribution of radioactive tracers [16,17]. Carbon-14 labeled organic radiotracers were purchased from Sigma (St. Louis, MO, USA) and upon receipt, diluted with water to an activity of approximately 0.06–0.08 μCi/μl for use as the 'spike' in the partitioning experiment. For standard liquid scintillation analyses, Ultima Gold Scintillation Cocktail (Packard Instrument, Downers Grove, IL, USA) and a Packard Tri-Carb 1900 TR Liquid Scintillation Analyzer (Packard Instrument) were used. Precise details of this technique have been published elsewhere [7].

Tracer quantities (1–4 μCi) of the radionuclide of interest were added and the system centrifuged (2 min, 2000 *g*), then vortex-mixed for 2 min. The phases were disengaged by centrifugation. Equal aliquots of each phase were then removed for standard liquid scintillation analysis. Since equal aliquots of each phase were analyzed, and the activity of the tracers is directly proportional to their concentration, the distribution ratios were determined as in Eq. (5). All measurements were carried out at least in duplicate.

$$D = \frac{\text{Activity in counts per min in the upper PEG-rich phase}}{\text{Activity in counts per min in the lower salt-rich phase}} \quad (5)$$

3. Results

3.1. Response of solvatochromic dyes to salt concentration

Fig. 1 shows the absorption spectra of Reichardt's betaine dye in pure potassium phosphate (K_3PO_4) solutions. There is an obvious difference between the peak maximum of the solvatochromic absorption

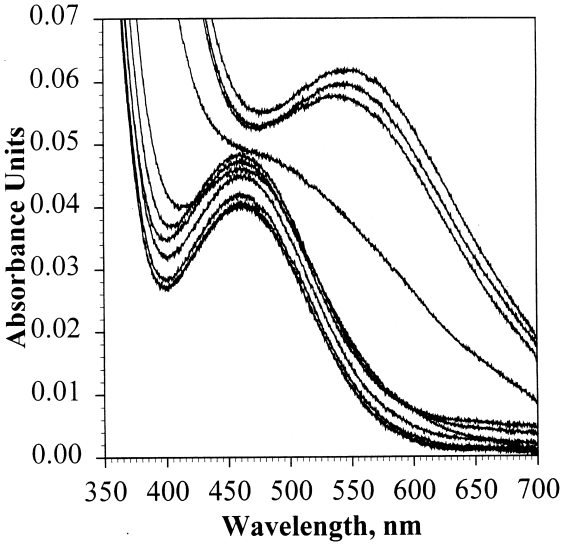

Fig. 1. The principle solvatochromic absorption band of Reichardt's betaine dye shows small bathochromic shifts in adsorption in the region of 460 nm at low phosphate concentrations (0–16%, w/w) and in the region of 550 nm at higher concentrations (20–36%, w/w). The region in between is marked by a diffuse adsorption spectrum where determination of the peak maximum is difficult. Details of the molar transition energies obtained from these spectra are shown in Fig. 2.

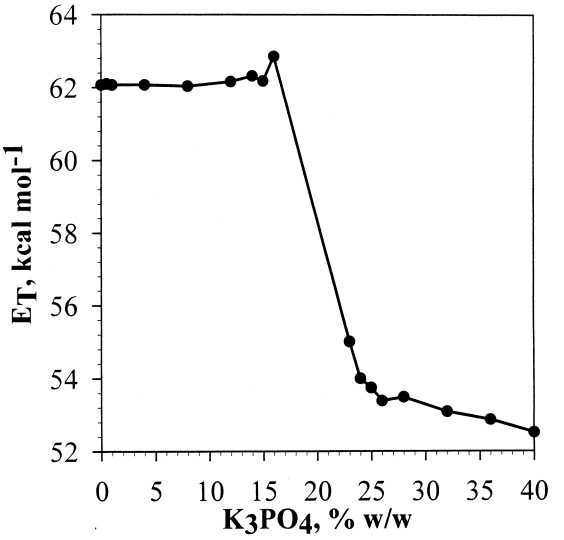

Fig. 2. Molar transition energies of the betaine dye derived according to Eq. (4) for the data shown in Fig. 1 showing the apparent salting-out transition caused by high concentrations of K_3PO_4.

band of this dye when in low to moderate concentrations of phosphate compared to high concentrations of phosphate where it becomes shifted to much shorter wavelengths. This is almost certainly due to aggregation and self association of the dye due to the high salt concentration. Beyond 16% (w/w) K_3PO_4 it is not possible to determine a peak maximum for the solvatochromic absorption peak using the simple methods employed. This becomes possible again at 23% (w/w) K_3PO_4.

Fig. 2 shows the molar transition energy associated with the solvatochromic peak as calculated using Eq. (4). The molar transition energy is largely unaffected by the increase in phosphate concentration until about 16% (w/w) potassium phosphate. This marks the beginning of a large change in the molar transition energy associated with the increasing self association of the dye which is apparent above 23% (w/w) potassium phosphate. The region in between these concentrations of K_3PO_4 is marked by an almost complete absence of data points due to the difficulty of obtaining the peak of maximal absorption.

Similar spectra obtained for thymol blue (data not shown) in increasing concentrations of K_3PO_4 in deionized water showed a peak maximum at about 595 nm which is reasonably close to that reported by Zaslavsky et al. [12] of 597 nm. The adsorption spectrum of this dye was largely unaffected by the increasing concentration of K_3PO_4 until the concentration reached about 30% (w/w). At this concentration the adsorption band changed shape, broadened and decreased in magnitude. Again changes to the absorption spectrum and the increase in E_T above 30% (w/w) phosphate are probably due to increasing self association and aggregation of the dye in the high concentrations of salt present.

From these results we conclude, that provided the dyes do not begin to self associate [above 30% (w/w) K_3PO_4 in the case of thymol blue or 16% (w/w) in the case of the betaine dye], the changing polarity of these aqueous salt solutions will not induce any change in the molar transition energy of these dyes. The dyes do not report on the polarity of salt containing solutions, but such solutions will be reported as having essentially the same polarity as

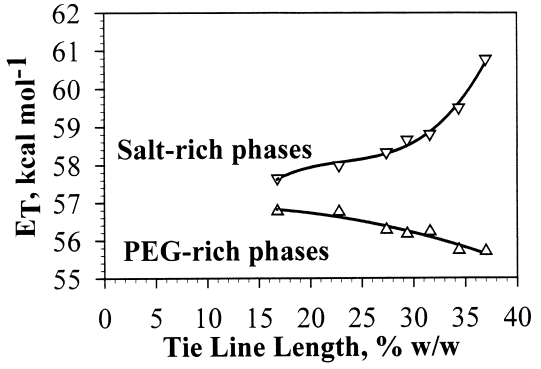

Fig. 3. Molar transition energies of the betaine dye for the coexisting phases of a PEG-2000–K_3PO_4 ABS as a function of the tie line length of the ABS.

pure water. The dyes respond differently to salt concentration through differences in their salting-out constant (K_s).

3.2. Response of solvatochromic dyes to ABS phase composition

Fig. 3 shows the molar transition energy associated with the solvatochromic peak of Reichardt's betaine dye added to the separated top and bottom phases of a PEG-2000–K_3PO_4 ABS. The dye was not partitioned in the systems but added directly to the separated phases which were examined spectroscopically at a temperature about 2°C below the critical temperature to prevent clouding. The compositions and tie line length relationships of these systems are given in Table 1. The betaine dye was superior to thymol blue in distinguishing the apparent polarity difference between the two phases because of its greater spectral shift. At the shortest tie line lengths examined, there was hardly any difference in the molar transition energy of thymol blue in the PEG-rich and salt-rich phases (data not shown).

There are a number of interesting features of the relationship between the molar transition energy of this dye and the apparent polarity of the environment on which it is reporting which are worth indicating. The polarity of the *top phase* as measured by the molar transition of this betaine molecular probe varies by only a relatively small amount in moving from systems lying relatively close to the critical point to systems of quite extended tie line length. In terms of the molar transition energy this variation covers little more than 1 kcal mol^{-1}. By contrast, in moving from the critical point to systems of extended tie line length, the polarity of the *lower, salt-rich phase*, as indicated by the probe, increases much more (just over 4 kcal mol^{-1}). In fact, it seems reasonable to suppose, the real increase in polarity of this phase could be much greater than this. Firstly, the probe is only responsive to the environment of the PEG, of which there is less and less present in the lower phase as the tie line length increases. Secondly, the response of the probe in solutions containing only salt is identical to that of water and thus may not reflect the true relative polarity of the salt-rich phase.

The molar transition energy of the betaine dye may also be expressed relative to the concentration of PEG in the co-existing phases of these ABS. This is shown in Fig. 4. It is apparent that there is a very rapid decrease in polarity of the probe associated with a relatively small increase in PEG content in separated lower, salt-rich, phases. This is probably

Table 1
Composition of the PEG-2000–K_3PO_4 biphasic systems whose solvatochromic behaviors are shown in Figs. 3 and 4

System	PEG (%, w/w)	PO_4^{3-} (%, w/w)	V_R	PEG_{TOP} (%, w/w)	$PO_{4\ TOP}^{3-}$ (%, w/w)	PEG_{BOT} (%, w/w)	$PO_{4\ BOT}^{3-}$ (%, w/w)	TLL
1	11.5	9.0	1.23	17.4	6.8	5.2	11.6	16.8
2	12.8	9.05	0.94	23.2	4.9	2.5	13.2	22.8
3	14.0	9.1	0.84	26.4	4.1	1.5	14.1	27.4
4	14.5	9.4	0.79	28.0	3.9	0.8	15.0	29.4
5	15.3	9.6	0.77	29.6	3.8	0.5	15.6	31.6
6	16.2	9.9	0.74	32.0	3.4	0.2	16.4	34.4
7	17.3	10.0	0.70	34.4	3.2	0.1	17.0	37.0

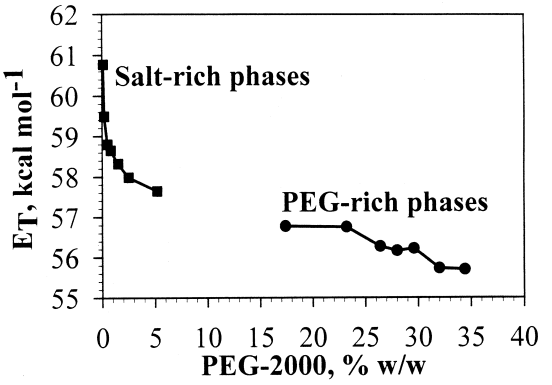

Fig. 4. Molar transition energies of the betaine dye for the coexisting phases of a PEG-2000–K_3PO_4 ABS as a function of the PEG concentration in the phase.

due to the selectivity of the response of the probe and its preferential hydration in PEG-ordered aqueous solution. Thus the probe does not really measure the true relative polarity of the separated PEG-rich and salt-rich phases, but appears to be much more closely related to its preference for hydrating in the PEG environment and therefore to its *partition coefficient* in these systems. For the PEG-rich phases, the increase in E_T is much less rapid. ABS top phases, are marked by an increasing rejection of salt and a consequent increase in PEG concentration, but this appears to make little difference to the apparent polarity of the phase as reported by the dipolar dye. It is also apparent that there is a unique value of E_T for each phase of the system and that the spectral response of this probe could be used to determine the phase diagram. However, even the very large bathochromic response of this probe might not allow the distinction of phases much closer to the critical point than have been examined here. Certainly thymol blue reported that the lowest tie line lengths examined here were of essentially the same polarity due to its much smaller bathochromic spectral shift (data not shown).

It is also interesting to note that close to the critical point, both phases are really rather close to their minimum polarity as reported by this dye for top phases at extended tie line length and really rather a long way from the polarity of water. As tie line length increases top (polymer-rich) phase polarity decreases little but that of the lower (salt-rich) phase rapidly returns to a polarity close to that of water. As a result of this it seems possible to conceive of a situation in which some polar solutes could show an increase in overall solubility in the system away from the critical point [36].

3.3. Solvatochromism in the monophase region

Fig. 5 shows the spectral data for the betaine dye in a 0.5% (w/w) K_3PO_4 solution to which increasing concentrations of PEG-2000 have been added. The bathochromic shift of the solvatochromic absorption band with increasing concentration of added PEG is apparent from the decrease in the molar transition energy [E_T, from Eq. (4)]. The plot is, in effect, examining the change in polarity of a series of solutions, of increasing PEG concentration, lying outside the PEG-2000–K_3PO_4 phase diagram and lying very close to the PEG concentration axis. Only at the very highest concentrations of added PEG does the composition of these solutions begin to approximate that of a typical biphase and there the approximation is to a PEG-rich phase of quite extended tie line length. The reported molar transition energy of somewhat less than 56 kcal mol^{-1} for the solution containing 38% (w/w) PEG-2000 is consistent with

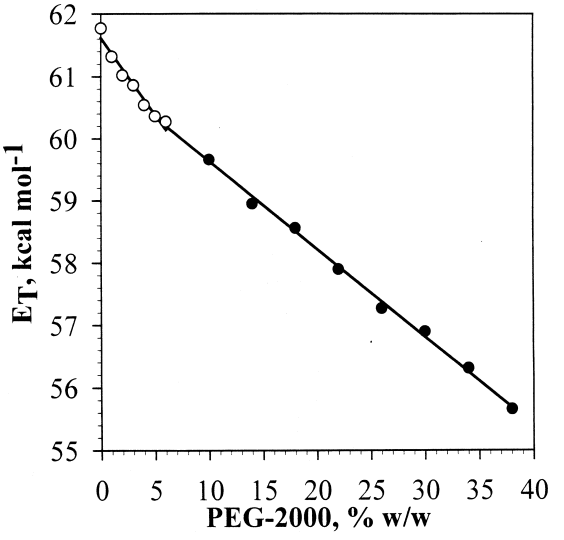

Fig. 5. Change in molar transition energy of Reichardt's betaine dye in response to increasing concentrations of PEG-2000 in a solution of 0.5%, (w/w) K_3PO_4.

this idea since this is close to values found for the separated PEG-rich phases shown in Figs. 3 and 4.

Perhaps of more interest is the apparent disjunction in the essentially linear relationship between the molar transition energy of the betaine dye and the concentration of PEG in the solution. The E_T *vs.* PEG relationship appears to consist of two linear segments of differing slope and intercept. Similar results, consistent with ours, were obtained by Zaslavsky [12] in various polymer solutions in the presence of 10 m*M* universal buffer. In his universal buffer solution this disjunction was observed, using the Reichardt's dye, to occur at approximately 13% (w/w) PEG for PEG molecular weights above 600. For molecular weights below this, the transition in the slope of the relationship between E_T and PEG concentration was observed at much higher concentrations which increased with decreasing molecular weight for shorter oxyalkylene chains (reaching over 40 wt% for diethylene glycol). It was assumed [12] that this marked a transition to a solution regime, above this concentration, in which all the water was specifically under the influence of long range ordering forces by the PEG molecules [12], but still below the concentration (52%, w/w) above which no water seems to be unbound according to DSC and NMR results [12,36].

It may be worth emphasizing that this behavior, of a dipolar species in the presence of PEG, is very different from the situation in which polymer molecules form an anisotropic phase with a high degree of order. An example of this is given in Fig. 6 which shows the effect of increasing concentrations of the non-ionic surfactant Triton X-100 in 0.5% (w/w) K_3PO_4 on the molar transition energy of the betaine dye. Because, even at 1% (w/w), Triton X-100 is above its critical micelle concentration, there is only a single discrete shift in the molar transition energy, compared to that of the surfactant-free salt solution, whatever the concentration of Triton added. This seems to imply that any changes in micelle size or shape do not affect the absorption spectrum of this dye. It is interesting to note that the molar transition energy of the solvatochromic peak of the betaine dye in Triton X-100 solutions is similar to the highest values found in the PEG-2000 solutions. This may reflect the association of the betaine dye with the outer, hydrophilic surface of the triton micelles and not the hydrophobic core. The outer surface of the micelle consists of relatively hydrophilic oxyalkylene chains of about ten monomer units in length, approximately equivalent to a PEG molecule of MW=400, and the core is dominated by octylphenyl head groups.

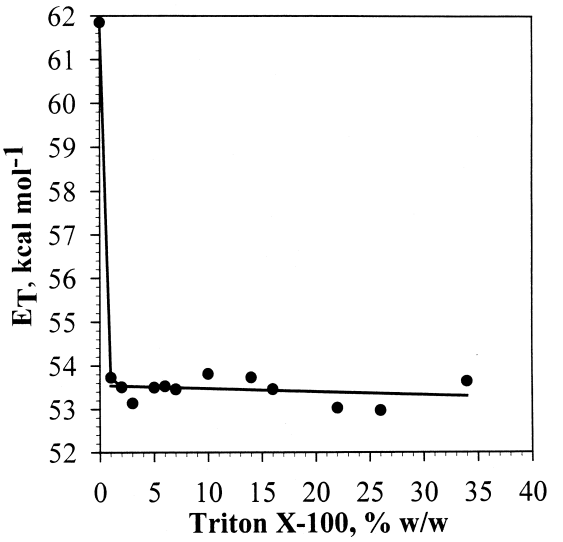

Fig. 6. Change in molar transition energy of Reichardt's betaine dye in response to increasing concentrations of Triton X-100 in a solution of 0.5% (w/w) K_3PO_4.

Returning to the discontinuity in the polymer concentration vs. molar transition energy relationship, Fig. 7 shows that the polymer concentration at which this transition occurs is dependent on the salt concentration. The relationship between the molar transition energy of the betaine dye and the concentration of PEG-2000 is shown at increasing concentrations of added K_3PO_4. It is clear that the characteristic concentration of PEG at the point of the discontinuity is dependent on the salt concentration. The transition in slopes is found at lower concentrations of PEG as the concentration of phosphate increases as shown in the figure. In addition, the steepness of the initial and final slopes increases as the phosphate concentration increases until finally it is difficult to distinguish the difference between the slopes at all. These transitions occur, at a lower salt concentration for the betaine dye than for thymol blue in this system (data not shown). These observations seem to make it harder to accept Zaslavsky's

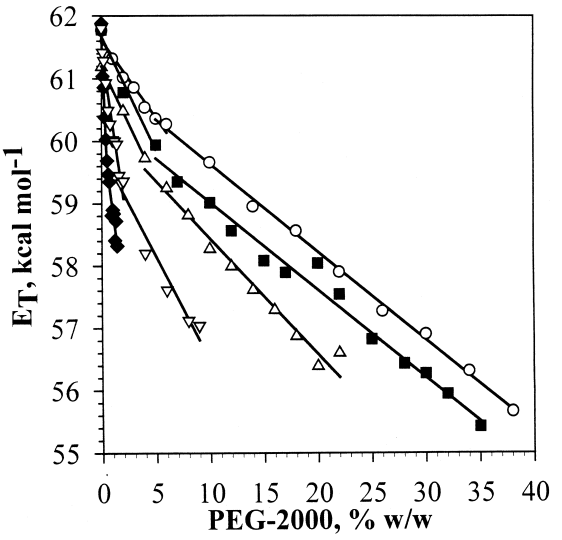

Fig. 7. Change in molar transition energy of Reichardt's betaine dye in response to increasing concentrations of PEG-2000 in solutions containing increasing levels of K_3PO_4 (○ 0.5%, w/w; ■, 2.5%, w/w; △, 5%, w/w; ▽, 10%, w/w; ♦, 14%, w/w).

hypothesis [12] that this transition represents the region in which all the water falls under the long range ordering of PEG. If his interpretation is correct, it is hard to understand why it is dependent on salt concentration and even harder to understand why it is dependent on the nature of the dipolar molecular probe.

Superficially, the effects illustrated in Figs. 5 and 7 resemble the effects of dielectric enrichment [37] by mixed solvents of differing dielectric polarities (i.e., the preferential solvation of the dipolar solute by one of a mixture of solvents). However, since the dye is freely transportable within the liquid medium these effects may represent the partition of the solute to different solvent sites or domains [37]. It may be obvious that the spectra collected are time averaged spectra representing contributions from a mixture of different dye populations. In the absence of specific deconvolutions of these spectra the decrease in E_T may still be thought of as representing the increased partition of the molecular population to less polar regions of the solvent. In principle, the domains which the dipolar molecules occupy could lie in the solvent sphere of the salt, in the bulk water, or in the solvent sphere of the polymer or indeed in any intermediate position. This location probably depends on the polarity of the dye and its partition coefficient hence the difference between the two dyes. This location, and the partition coefficient of the solute, should certainly be a function of the salt concentration and the polarity of the species as, for instance, in classical ''salting-out'' relationships [38,39]. Perhaps the transition (in slopes) may be related to the partition of the dipolar dye to PEG domains (PEG-structured water) as represented at first by a regime characteristic of salt-rich phases containing PEG monomers and a later regime characteristic of PEG-rich phases containing more confluent PEG populations (all of which lie outside the phase diagram). Whatever the truth may be, and a study which included deconvolution of the spectra of the molecular populations involved would be valuable, it is interesting that each salt regime shown in Figs. 5 and 7 tends toward a more or less maximal and similar decrease in solution polarity.

Zaslavsky [12] has reasoned that if the solvatochromic dyes reported only on the bulk water (i.e., were only present in that part of the solution away from the hydration shell of the PEG molecules) no solvatochromic shift would be observed. Equally, if the dyes were solvated close to the hydration shell of the phosphate solution, again no solvatochromic shift would be observed (see Fig. 2). Similarly, if the probe were located exclusively in the hydration shell of the PEG, a single bathochromic shift would be observed (resembling Fig. 6) which would be independent of concentration. What is *actually* observed is a relationship between the concentration of PEG and the solvatochromic shift and from this Zaslavsky argues that the probe reports on the boundary between the bulk solvent and the PEG hydration shell. This is consistent with our observations since the rate of decrease in apparent polarity changes at a critical PEG concentration, which is salt concentration dependent, perhaps marking a transition between different solution regimes characteristic of salt-rich phases and PEG-rich phases, respectively.

It is remarkable that the probe never reports on the phosphate hydration sphere as evidenced by the consistently large solvatochromic shifts observed even in high concentrations of phosphate (see Figs. 4 and 5). The probe is not reporting the bulk dielectric polarity of the medium, but only that surrounding the

PEG molecules as they progressively come into closer and closer contact. This seems to imply, in other words, that these dyes are already displaying a form of partition between distinct regions of the solution even before phase separation has taken place. In turn this implies that the phenomenon of aqueous phase separation represents a progressive sorting of these solvent domains between two co-existing phases of divergent composition as a result of the reduced population of free water molecules available to provide for complete miscibility.

3.4. Solvatochromic solvent comparisons

The measured polarity of the coexisting phases of a polymer–salt ABS using Reichardt's carboxylated pyridinium-*N*-phenoxybetaine [11] can be used to compare the solvent characteristics of this system to those of more conventional solvent extraction systems. Table 2 shows the polarity obtained, according to Reichardt's $E_T(30)$ scale of solvent polarity [8], for a number of conventional solvents as well as for some thermoseparating polymer and micellar systems obtained by ourselves and others [1,13,40–43]. The values obtained using the carboxylated betaine dye differ slightly from those obtained using the non-carboxylated dye, however, only the carboxylated form is soluble in aqueous solutions containing salts. Discrepancies between these two scales may largely be accounted for by the difference in the response of the betaine dye in protic and aprotic solvents when hydrogen bonding interactions may be imposed on polarity and polarizability effects [8]. Table 2 shows $E_T(30)$ values for the conventional solvents and water, and E_T values obtained with the carboxylated betaine for various polymeric systems.

It is clear from comparison of the solvent polarity of the aqueous polymeric media to the conventional solvent systems, that these represent solvents of a generally higher polarity than most of the solvents in common use for the development of solvent extraction strategies. The polymer solution environments, represented in Table 2, are clearly not as polar as water but none, on the basis of E_T, are as weakly polar as, for example, 1-octanol. Table 2 indicates a range of E_T values for the Pluronics,

Table 2
Extracting solvent systems compared by polarity and $\Delta G_{tr}(CH_2)$

Solvent system[a]	$E_T(30)$ or E_T	Ref.	$-\Delta G_{tr}(CH_2)$ (kcal mol^{-1})	Ref.
n-Hexane	31.0	[8]	1.02	[27]
n-Octane	31.1	[8]	0.77	[27]
p-Xylene	33.1	[8]	0.64	[27]
Benzene	34.3	[8]	0.84	[27]
Chloroform	39.1	[8]	0.85	[27]
MIBK	39.4	[8]	0.72	[27]
MEK	41.3	[8]	0.43	[27]
1-Octanol	48.3	[8]	0.73	[27]
1-Butanol	50.2	[8]	0.54	[27]
$C_{12}EO_8$	52.8	[13]		
Brij35	52.8	[13]		
Triton X-100	53.0	[13]		
Pluronics	51–55	[42]		
CTAB–DTAB	53–54	[13]		
PEG–PO_4^{3-} PEG-rich phase	55.7	[17]	0–0.85	[17]
SDS	57.5	[13]		
Ficoll-400–Dextran-70	60.5/60	[12]	0.016	[27]
PEG-6000–Dextran-500	58.5/60.5	[12]	0.025	[27]
Water	63.1	[8]		

[a] MIBK = Methylisobutyl ketone (4-methyl-2-pentanone); MEK = methylethyl ketone (2-butanone); $C_{12}EO_8$ = poly(oxyethylene)-[8]-lauryl ether; Brij35 = poly(oxyethylene)-[23]-lauryl ether; Triton X-100 = *t*-octylphenoxypolyethoxyethlanol; Pluronics = trade name BASF block copolymers of poly(ethylene oxide) and poly(propylene oxide); CTAB = cetyltrimethylammonium bromide; DTAB = dodecyltrimethylammonium bromide; SDS = sodium lauryl sulfate.

since the polarity of the environment about which the probe reports is dependent on the relative proportions of the EO and PO groups present which varies widely for this group of polymers [1]. The lower values shown may reflect solubilization in the proximity of the PO groups of the micellar core. For some of these thermoseparating polymers the polarity of the probe environment is as low as that of the octyl or nonylphenyl ethers or the polyoxyalkylene monoethers. For lower proportions of PO groups the probe environment approaches the polarity of the PEG-rich phase of a PEG-2000–K_3PO_4 ABS.

For the Ficoll–PEG and PEG–Dextran systems, we have estimated the polarity of each phase from published data [12] and this is shown in Table 2. For these polymer–polymer two-phase systems, the polarity of the phases is only slightly reduced over that of water. Data such as is shown in Table 2 allows simple comparisons of solvents to be made, however, it must be remembered that scales based upon the response of probes to the polarity of the molecular environment do not fully describe the range of solubilizing features of particular solvents [8]. In addition, the betaine probe is unable to distinguish the polarity of a salt-containing phase from the polarity of water.

Table 2 also shows the free energy of transfer of a methylene group for these conventional and polymeric solvent systems. The free energy of transfer in the polymeric systems may be very small, particularly for the polymer–polymer ABS. In other words, such systems may be exquisitely sensitive to minor differences in surface properties of partitioned solutes. On the other hand the $\Delta G_{tr}(CH_2)$ for PEG–salt two-phase systems seems to be tunable over a wide range so that the free energy of transfer may approach that of a system like benzene–water. Also implied is that the observed partition is very much dependent on the solubility in the aqueous phase as has been noted by others [13]. In PEG–salt ABS, the aqueous phase is characterized by the presence of high concentrations of salt which will strongly influence solubility in this phase. In large part, this explains the apparent discrepancy between the modest polarity reported by the molecular probe and the relatively large free energy of transfer of a methylene group characteristic of these systems.

3.5. Solvent description by linear solvent free-energy relationships

A more intimate description of the solvent properties of different extraction systems is made available through the development of LSERs, the most recent of which are those due to Abraham [32–35] highlighted in the introduction. The contribution of various physico–chemical aspects of the properties of solute and solvent is encapsulated in a simple multiple linear regression. Solutes are characterized by descriptors [Eq. (3)] whose derivation and relationship to other LSER systems is discussed in Ref. [32–35]. These descriptors are the McGowan volume (V_x) which encapsulates the energy for cavity formation in the phases and is thus related to hydrophobicity, and the molar refractivity (R_2) which may encapsulate the effect of London dispersion forces but also contains contributions from polarizability which is specifically encapsulated (along with dipolarity) in the π_2^H term. The remaining terms reflect the solute hydrogen bond acidity ($\Sigma\alpha_2^H$) and hydrogen bond basicity ($\Sigma\beta_2^H$), whilst c is a simple constant.

LSERs as a general approach to solvent characterization are only rarely used in an engineering context but may be quite powerful [44,45]. On the other hand, they are very widely applied to QSAR problems. In this context it is interesting to note the increasing tendency to question the traditional $\text{Log}P_{o/w}$ approach to the estimation of solute hydrophobicity [25,46–48] and to suggest that the modeling of the interaction of solutes with biological membranes requires a more sophisticated approach. It has been suggested [25] that this problem requires a solvent–water partitioning set consisting of an amphiprotic solvent such as 1-octanol, an inert solvent such as an alkane, and a hydrogen bond donor such as chloroform with propylene glycol dipelargonate (PGDP) as the hydrogen bond acceptor. It has been pointed [48] out that PGDP has numerous deficiencies, such as marked UV adsorption and high viscosity, and di-*n*-butyl ether has been suggested as a replacement. Other authors have suggested [49] that ABS themselves represent the preferred method for the rational quantitation of the hydrophobicity of chemical compounds. This is

based on the idea that hydrophobicity is a measure of the intensity of molecular interactions with water [49], thus, it follows that quantitative partitioning in aqueous two-phase systems (in which the compositions of the phases are about 90% water) represents the preferred method for its evaluation. Additionally, current trends in agrochemistry, pharmacology, etc., suggest that peptides, proteins, and generally larger and more complex molecular species such as polymer–drug conjugates and liposomes (which must be analyzed in their native aqueous conformations) are totally unsuited to the application of traditional methods. This represents a highly interesting and potentially fruitful area for research.

Table 3 shows the Abraham solvent parameters for the above solvent–water systems compared to some WASE systems [1,13,24,41–44]. For the most part, these are micellar systems, but data for a PEG-2000–$(NH_4)_2SO_4$ phase system is also given (shown as PEG–salt ABS). This is from our own data and it must be emphasized is highly preliminary being based on only 11, mostly aromatic, solutes [16,17] which do not, in any sense, represent a well balanced solute set.

The main features of interest may be indicated. For solvent–water systems, the volume (V_x) parameter is always large and positive reflecting the energetic penalty attached to cavity formation in the hydrogen bonded aqueous phase. Where the solvent contains water at equilibrium (1-octanol) this parameter is reduced. It is interesting to reflect that the micellar systems also show a smaller volume parameter. This parameter seems exceptionally low in the ABS systems to the point where one might question its veracity. However, the observation might also be thought to parallel the observations made on polarity and free energy of transfer made earlier for micellar and solvent partitioning systems. The *r* parameter is always small and positive since there is little tendency for water to interact by London dispersion forces, but it is interesting to reflect that this parameter is rather higher for the two systems containing PEG (Brij35 = poly(oxyethylene)-[23]-lauryl ether). The dipolarity/polarizability parameter(s) is almost always negative due to the high dipolarity of water, the exception being the PGDP system. The *a* parameter reflects the solvent hydrogen bond basicity (solute hydrogen bond acidity), and for the 1-octanol–water system there is almost no difference between the phases, a factor which also appears to be true for the ABS. Hexane and chloroform do not favor the partition of hydrogen bond acceptors. This feature seems enhanced in some of the micellar systems. The *b* parameter is a measure of solvent acidity (solute hydrogen bond basicity), and for many of the systems this is highly negative, but for SDS, DTAB, and PEG the partition of hydrogen bond acceptors is relatively enhanced especially in the case of the ABS.

We are continuing our investigations of this important area of solvent characterization through the use of LSERs which we believe will be of importance in the development of ABS as the method of choice for the determination of solute and

Table 3
Abraham's [32–35] solvent descriptors[a] for various partitioning systems

Solvent[b]	c	$r(R_2)$	$s(\pi_2^H)$	$a(\Sigma\alpha 2^H)$	$b(\Sigma\beta_2^H)$	$v(V_x)$	Ref.
Hexane	0.36	0.58	−1.72	−3.60	−4.76	4.34	[26]
1-Octanol	0.09	0.56	−1.05	0.03	−3.46	3.81	[26]
$CHCl_3$	0.13	0.12	−0.37	−3.39	−3.47	4.52	[26]
$n-Bu_2O$	0.18	0.82	−1.50	−0.83	−5.09	4.69	[48]
PGDP	0.13	0.37	0.62	−1.02	−4.91	4.19	[48]
SDS	−0.62	0.32	−0.57	−0.08	−1.48	3.25	[50]
DTAB	−0.87	0.57	−0.40	0.28	−1.82	2.98	[50]
CTAB	−0.76	0.76	−0.32	1.02	−3.78	3.57	[50]
Brij35	−1.39	1.63	−0.37	1.62	−3.83	3.65	[50]
PEG–salt ABS	−0.36	1.64	−0.48	−0.04	−1.28	1.10	This study

[a] Terms are defined in Eq. (3).
[b] PGDP = Propylene glycol dipelargonate; SDS, DTAB, CTAB and Brij35 are defined in Table 2.

solvent properties. The above LSER for the PEG–salt ABS can only be considered highly preliminary, but is given simply to illustrate the kind of information which may be forthcoming from this type of analysis. The importance of ABS for the characterization of solute physico–chemical properties is currently greatly underestimated. Traditionally, partition in a variety of solvent–water systems has been used for the characterization of solutes for use in studies of, for example, bioavailability, drug action, and toxicity. The increasing molecular complexity of modern pharmacological and agrochemical products renders this approach increasingly obsolete. These issues will form the basis of another publication.

4. Conclusions

We have examined the polarity of the coexisting phases of a polymer salt–ABS using Reichardt's carboxylated pyridinium-*N*-phenoxy betaine. The $E_T(30)$ values obtained using this dye have been compared to values obtained for various micellar systems, for some PEO–PPO copolymer solutions and for the phases of a polymer–salt ABS (PEG-2000–K_3PO_4).

It appears that the solvent polarity of the presently used aqueous polymeric media do represent solvents of a generally higher polarity than most of the solvents in common use for the development of solvent extraction strategies. Few, if any, seem to represent an environment as weakly polar as for example that represented by 1-octanol. One reason for this is that such probes of solvent polarity do not fully describe the range of solubilizing features of particular solvents. Another is that the betaine probe seems unable to distinguish the polarity of the equilibrium phase employed in actual extraction experiments to the same degree that it does for the extracting phase. In, for example, a 1-octanol–water partitioning experiment, the polarity of the phase in equilibrium with the 1-octanol extractant would, likely, not depart significantly from that of pure water. However, in practice the polarity of the equilibrium phase of a PEG–salt ABS departs significantly from that of pure water being composed of relatively high concentrations of inorganic salts. Nevertheless, studies of solvent polarity are able to focus attention on the similarities and differences between different solvent systems and may be of great assistance in the selection of solvents or in their replacement by aqueous polymeric systems.

The solvatochromic dyes also appear to indicate that there is a segregation of solutes into different solvent domains below the critical point in ABS. If this is the case, then the process of ABS biphase formation at the critical point and above may be thought of as the increasing segregation of these different domains in to the different co-existing phases as the free unbound water necessary for their mutual miscibility is depleted.

Some preliminary data on the application of LSERs to the description of the solvent properties of ABS has also been presented from which we have begun to argue that ABS may represent the most appropriate systems for solute characterization for QSAR studies at this time. Further work in this important area is forthcoming.

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