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Partitioning of small organic molecules in aqueous biphasic systems

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Abstract

Aqueous biphasic systems (ABS) are suitable for the separation of small organic molecules in industrial and environmental applications and thus, it is important to correlate partitioning behavior of model organic solutes with their structure in order to develop predictive models. The partitioning behavior of five, uncharged, substituted benzenes (benzene, toluene, chlorobenzene, 1,4-dichlorobenzene and 1,2,4-trichlorobenzene) were studied in ABS prepared from stock solutions of 40% (w/w) PEG-2000 and increasing concentrations of four water-structuring salts (K_3PO_4 , K_2CO_3 , $(NH_4)_2SO_4$ and NaOH). For a given solute and a defined concentration of salt, the partition coefficients increase as the ΔG_{hyd} value of the salt anion becomes more negative (e.g., $D_{benzene}$ increases in the order $OH^- < SO_4^{2-} < CO_3^{2-} < PO_4^{3-}$). In a given salt, the distribution ratios increase in the order benzene < toluene < chlorobenzene < 1,4-dichlorobenzene < 1,2,4-trichlorobenzene. The partitioning behavior of the solutes in PEG–salt ABS was found to be strongly correlated with their partitioning coefficients in 1-octanol–water biphasic systems. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Aqueous biphasic systems; Partitioning; Benzenes

1. Introduction

Separations processes are ubiquitous in the chemical industry and yet receive little attention in terms of environmental impact. Systems employed in liquid–liquid separations processes frequently involve the use of toxic and volatile organic compounds [1]. Thus, aqueous biphasic systems (ABS) may represent relatively benign systems for extractive processes for the separation of small organic molecules in industrial separations and environmental redemption applications [2]. In ABS, the major component in each of the two phases is water, thus providing an extraction technology that eliminates the need for volatile organic compounds [3,4]. However, the partitioning behavior in ABS of such molecules is poorly understood at present, especially for the

potentially most useful polymer–salt systems whose physical (and probably extractive) properties are most nearly similar to current aqueous–organic systems. Such data is essential for the efficient evaluation and application of these systems.

The partitioning behavior of some small, uncharged aromatic compounds has been examined as a function of the salt cation and anion, and the total salt concentration in polymer–salt ABS formed with poly(ethylene glycol) (PEG) of molecular mass 2000 g/mol. This partitioning behavior may be understood in terms of the Gibbs free energy of hydration (ΔG_{hyd}) of the salt used and of the concentration of the salt stock solution used to form the biphasic system. The distribution data of these molecules in ABS has been compared with similar published values for their distribution in 1-octanol–water systems (log *P* values [5]). This aspect of the work is also compared to previous observations on the distribution of dinit-

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rophenylated amino acids and other small organic molecules in ABS [6–10]. The correlation observed may be developed as a plausible way to predict the partitioning behavior of many small organic molecules in ABS. In the future, we hope to be able to predict the partition coefficient in organic solvent–water systems from fundamental (albeit partly parameterized) quantum-mechanical calculations of the solvent free energy of solvation made solely by reference to the molecular structure of the solute, from which it should also be possible to derive the partition coefficient in ABS.

2. Experimental

The chemicals, $(\text{NH}_4)_2\text{SO}_4$, K_2CO_3 , NaOH and poly(ethylene glycol), were obtained from Aldrich (Milwaukee, WI, USA) and were of reagent grade. ^{14}C tracer solutions of benzene, toluene, chlorobenzene, 1,4-dichlorobenzene and 1,2,4-trichlorobenzene were purchased from Sigma (St. Louis, MO, USA) and upon receipt, all solutes were diluted with benzene purchased from Aldrich. All water was purified using commercial deionization systems. 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.

The polymer and salt stock solutions were prepared on a mass percent or molar basis. The compositions referred to are for pre-equilibrium stock solution concentrations. The experiments were carried out using the methods from reference [11]. Liquid–liquid distribution ratios (D) were determined by mixing 1 ml of a 40% (w/w) PEG-2000–water solution with 1 ml of a salt stock solution of known concentration. The systems thus prepared were Vortex mixed for 2 min and then centrifuged (2000 g) for 2 min. ^{14}C tracers were diluted with benzene to an activity of approximately 0.06–0.08 $\mu\text{Ci}/\mu\text{l}$ for use as the ‘spike’ in the partitioning experiment. 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 contact time was experimentally deter-

mined to be sufficient for these systems to reach equilibrium.) The phases were disengaged by 2 min of centrifugation (2000 g) and were carefully separated. Equal aliquots of each phase were then removed for standard liquid scintillation analysis. All measurements were carried out at least in duplicate.

The distribution ratio represents the total concentration of tracer in the upper PEG-rich phase divided by the concentration of the total tracer concentration in the lower salt-rich phase. Since equal aliquots of each phase were analyzed, and the activity of the ^{14}C tracer is directly proportional to its concentration, D can be defined as:

$$D = \frac{\text{Activity in counts per minute PEG - rich phase}}{\text{Activity in counts per minute salt - rich phase}}$$

3. Results and discussion

Previous work from our group has indicated a preference for molecules with sulfonated aromatic rings to partition to the PEG-rich phase in PEG–salt ABS [12,13]. Given the current needs for removal of aromatic compounds (e.g., benzene, PCBs, etc.) from both the environment and from industrial effluents, and the ready availability of ^{14}C -labeled benzene derivatives of controlled substitution, we chose to investigate simple benzene-like molecules in ABS. Five, uncharged, substituted benzene compounds were chosen for this initial study: benzene, toluene, chlorobenzene, 1,4-dichlorobenzene and 1,2,4-trichlorobenzene. The partitioning behavior of these five solutes was studied in ABS prepared from stock solutions of 40% (w/w) PEG-2000 and increasing concentrations of four water-structuring salts: potassium phosphate, potassium carbonate, ammonium sulfate and sodium hydroxide.

Figs. 1–5 show the variation in the distribution ratios with increase in salt stock solution concentration for each solute in each of the four salt systems. For each solute, in each ABS, formed with each different salt, the distribution ratios increase with increasing salt stock solution concentration. In common with macromolecular and ionic solutes, the distribution becomes increasingly one-sided the greater the difference in composition between the two phases [14,15]. Thus, these results approximately confirm the widely cited relationship Eq. (1):

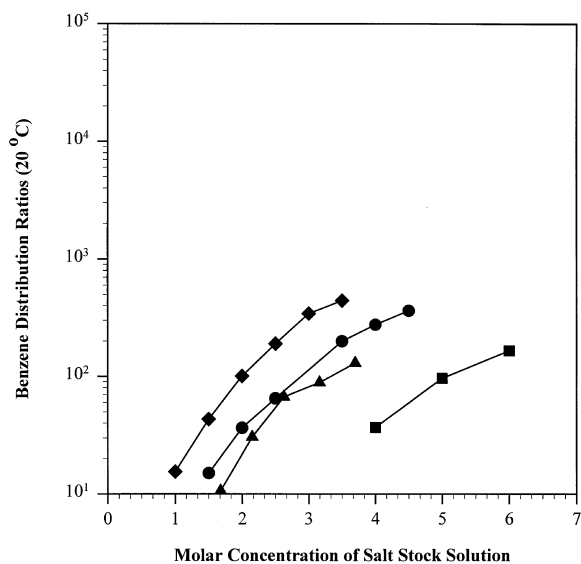


Fig. 1. Distribution ratios for benzene in 40% (w/w) PEG-2000- X M salt ABS as a function of increasing salt stock solution concentration. Symbols refer to X M as: (◆) K_3PO_4 ; (●) K_2CO_3 ; (▲) $(NH_4)_2SO_4$; (■) NaOH.

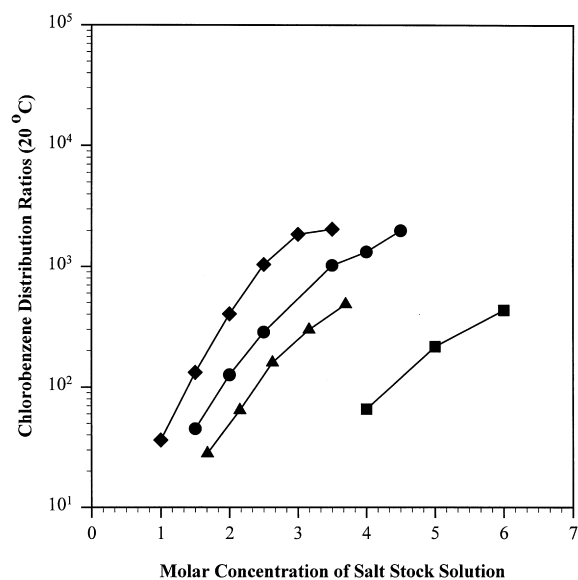


Fig. 3. Distribution ratios for chlorobenzene in 40% (w/w) PEG-2000- X M salt ABS as a function of increasing salt stock solution concentration. Symbols refer to X M as: (◆) K_3PO_4 ; (●) K_2CO_3 ; (▲) $(NH_4)_2SO_4$; (■) NaOH.

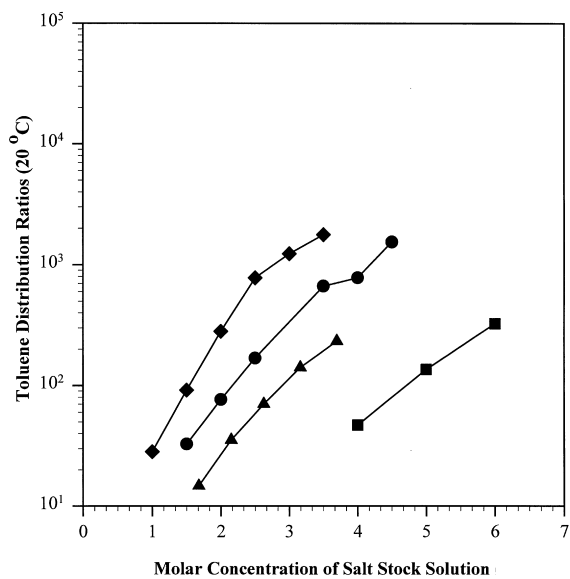


Fig. 2. Distribution ratios for toluene in 40% (w/w) PEG-2000- X M salt ABS as a function of increasing salt stock solution concentration. Symbols refer to X M as: (◆) K_3PO_4 ; (●) K_2CO_3 ; (▲) $(NH_4)_2SO_4$; (■) NaOH.

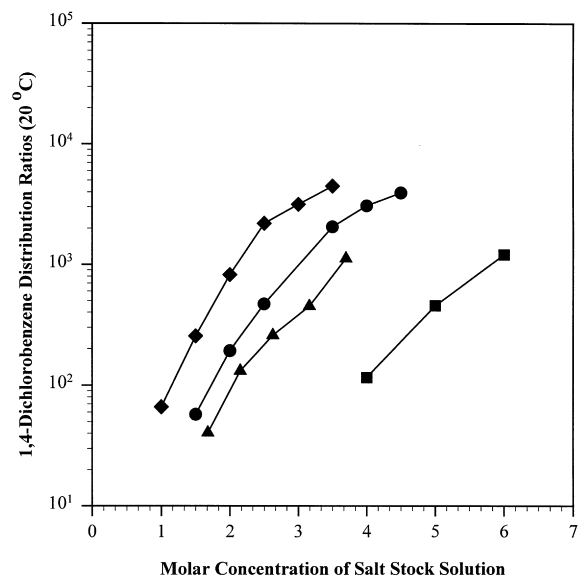


Fig. 4. Distribution ratios for 1,4-dichlorobenzene in 40% (w/w) PEG-2000- X M salt ABS as a function of increasing salt stock solution concentration. Symbols refer to X M as: (◆) K_3PO_4 ; (●) K_2CO_3 ; (▲) $(NH_4)_2SO_4$; (■) NaOH.

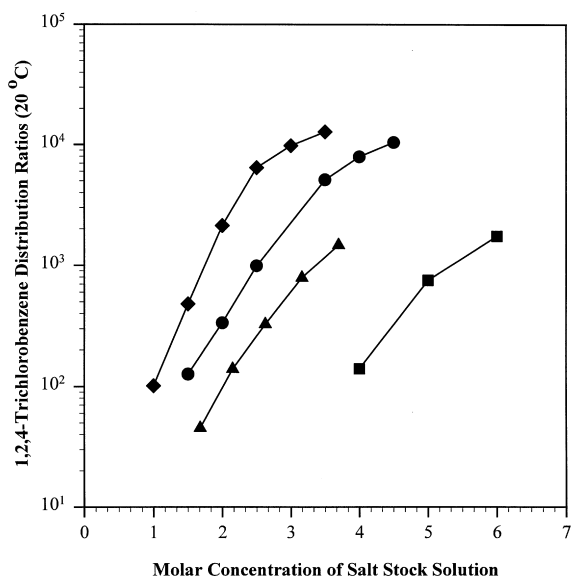


Fig. 5. Distribution ratios for 1,2,4-trichlorobenzene in 40% (w/w) PEG-2000- $X M$ salt ABS as a function of increasing salt stock solution concentration. Symbols refer to $X M$ as: (◆) K_3PO_4 ; (●) K_2CO_3 ; (▲) $(NH_4)_2SO_4$; (■) NaOH.

$$\ln K = k\Delta w_2 \quad (1)$$

(see for instance references [9,10,16]) where K is the partition coefficient, k is a constant, and Δw_2 is the concentration difference between the phases of one of the phase-forming components. It should be noted, however, that Figs. 1–5 show that this relationship appears to saturate at the highest concentrations of added salt.

Examination of Figs. 1–5 also reveals that for a defined concentration of salt, the partition coefficient is (or would be) higher in systems formed with these salts in the following anion series, $PO_4^{3-} > CO_3^{2-} > SO_4^{2-} > OH^-$. Thus, for all solutes and salt systems, the partition coefficient at a given salt concentration follows the lyotropic or Hofmeister series of the salt. This relationship has been noted previously in terms of the weakening of the tendency of these anions to promote phase separation in the presence of PEG [17]. This may also be expressed in terms of the Gibbs free energy of hydration (ΔG_{hyd}) of the salt. The more negative the ΔG_{hyd} of the salt, the higher the distribution ratio for the solute and the greater the salting-out effect on the solute. This conclusion

confirms previous findings utilizing the pertechnetate anion as the solute [4,14,18,19]. The effect of salt type seems to be largely dependent on the anion, which makes the dominant contribution [14].

At a defined salt concentration, for a given salt, the partition coefficients of the solutes follow the series benzene < toluene < chlorobenzene < 1,4-dichlorobenzene < 1,2,4-trichlorobenzene. Fig. 6 illustrates this trend for all five solutes in the K_2CO_3 ABS. (Similar trends are observed for the other three salt systems as well.) Table 1 indicates that the ordering of increasing D values does not strictly follow the decreasing order of the calculated ΔG_{hyd} of the solute, as toluene is out of place. Table 1 also shows that this is the case when molecular volume (a parameter affecting the free energy of cavity formation in the solution) is considered. Once again toluene is out of place.

The increase in the distribution ratios for these solutes does appear, however, to follow the overall increase in their hydrophobicity. This suggests that the partitioning of small molecules in ABS formed by admixture of PEG and different salts, should be

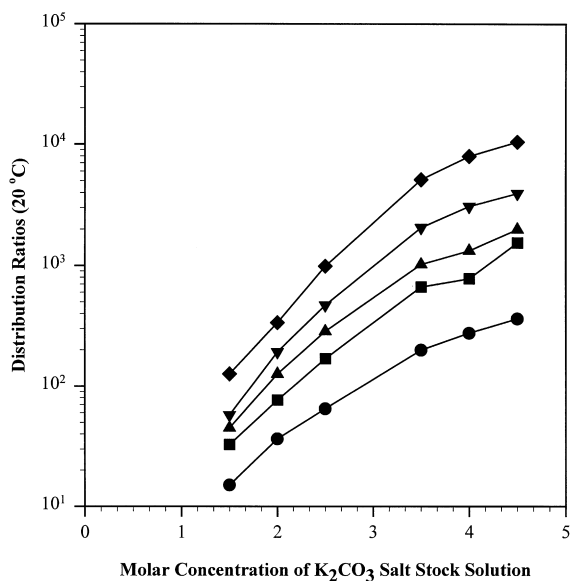


Fig. 6. Distribution ratios for all five organic solutes in five 40% (w/w) PEG-2000- K_2CO_3 ABS as a function of increasing concentration of K_2CO_3 stock solution. (●) Benzene; (■) toluene; (▲) chlorobenzene; (▼) 1,2-dichlorobenzene; (◆) 1,2,4-trichlorobenzene.

Table 1
Parameters associated with the five solutes studied

Solute	Log P	ΔG_{hyd} (kcal/mol)	Molar volume (\AA^3)
Benzene	2.13	−0.516	85.22
Toluene	2.73	−0.273	102.04
Chlorobenzene	2.84	−1.135	99.09
1,4-Dichlorobenzene	3.44	−1.355	112.79
1,2,4-Trichlorobenzene	4.02	−1.621	125.61

related to similar partitioning in aqueous–organic systems as has been shown in other studies [6–10]. Figs. 7–10 relate published values for 1-octanol–water partition coefficients (P) [5] for all five solutes to the distribution values found at each salt concentration in each of the four different PEG–salt ABS examined here. It is of interest to note that the free energy of transfer of some −1200 to −2400 cal/mol involved in the distribution of these solutes in the 1-octanol system can be equalled, and in some cases exceeded, for individual solutes in these ABS.

Additionally, the free energy of transfer and thus, the selectivity of the system can be reduced well below these levels by manipulation of the system composition as may be seen in Figs. 7–9.

For each D vs. P plot, for each salt and salt concentration at which the distribution coefficient was measured in the ABS, the regression constants were obtained and are given in Table 2. For the most part, the correlation coefficients in Table 2 are not less than 0.90 and are generally better than 0.97, except in the $(\text{NH}_4)_2\text{SO}_4$ system where the correla-

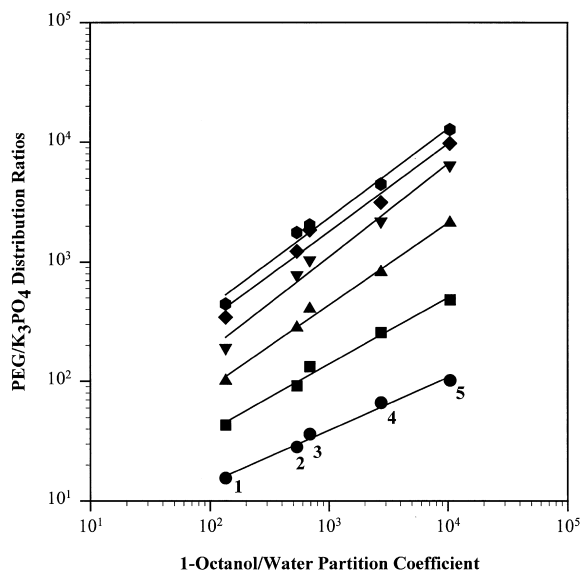


Fig. 7. Correlation between distribution ratios (D) in the 40% (w/w) PEG– K_3PO_4 ABS and the partition coefficient (P) for the solutes in 1-octanol–water. The five solutes are: 1, benzene; 2, toluene; 3, chlorobenzene; 4, 1,4-dichlorobenzene; 5, 1,2,4-trichlorobenzene. Symbols refer to the salt concentration of the added K_3PO_4 stock solution as: (●) 1.0 M; (■) 1.5 M; (▲) 2.0 M; (▼) 2.5 M; (◆) 3.0 M; (●) 3.5 M.

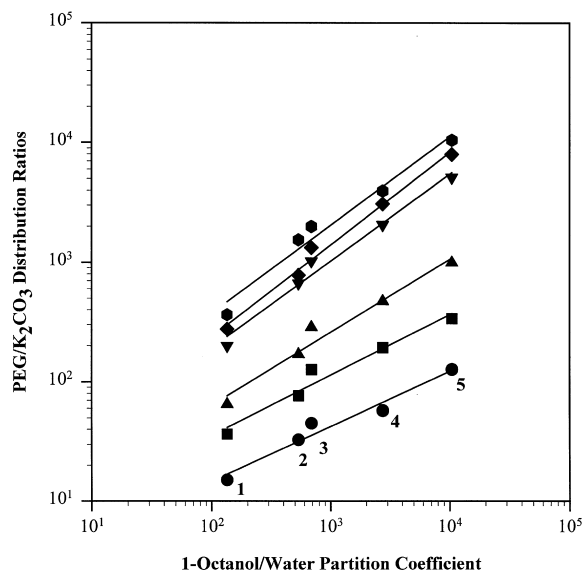


Fig. 8. Correlation between distribution ratios (D) in the 40% (w/w) PEG– K_2CO_3 ABS and the partition coefficient (P) for the solutes in 1-octanol–water. The five solutes are: 1, benzene; 2, toluene; 3, chlorobenzene; 4, 1,4-dichlorobenzene; 5, 1,2,4-trichlorobenzene. Symbols refer to the salt concentration of the added K_2CO_3 stock solution as: (●) 1.5 M; (■) 2.0 M; (▲) 2.5 M; (▼) 3.0 M; (◆) 4.0 M; (●) 4.5 M.

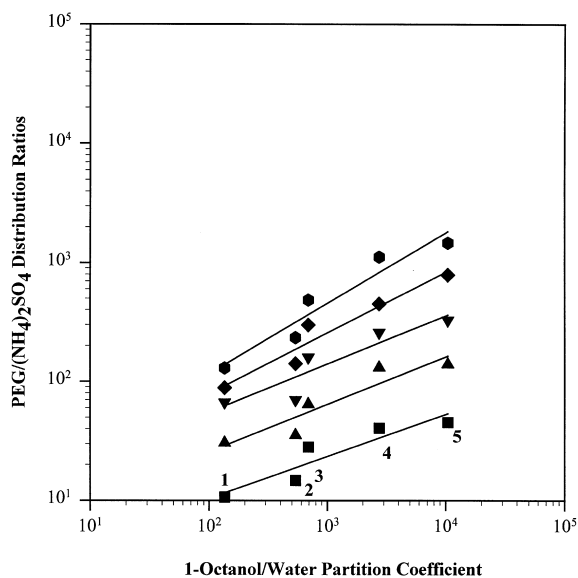


Fig. 9. Correlation between distribution ratios (D) in the 40% (w/w) PEG– $(\text{NH}_4)_2\text{SO}_4$ ABS and the partition coefficient (P) for the solutes in 1-octanol–water. The five solutes are: 1, benzene; 2, toluene; 3, chlorobenzene; 4, 1,4-dichlorobenzene; 5, 1,2,4-trichlorobenzene. Symbols refer to the salt concentration of the added $(\text{NH}_4)_2\text{SO}_4$ stock solution as: (■) 1.68 M; (▲) 2.15 M; (▼) 2.63 M; (◆) 3.16 M; (●) 3.70 M.

tions are noticeably weaker. The cause of this is not yet clear.

These correlations of the partition of substituted and unsubstituted phenyl moieties in PEG–salt and 1-octanol–water biphasic systems (Figs. 7–10) are in general agreement with previously published accounts of small organic molecule partitioning in ABS [6–10]. Such relationships have been described by the equivalent expressions:

$$\ln K = C + En_c \quad (2)$$

$$\ln K = B + m \log P \quad (3)$$

where, K is the partition coefficient and in Eq. (2) [6–8], E is a constant related to the free energy of transfer of a methylene group between the phases and C is a constant which has been related to the differing hydration properties of the two phases [6]. In Eq. (3) [9], B and m are constants which have been related to the intrinsic hydrophobicity of the phase system [9], $\log P_0$ by:

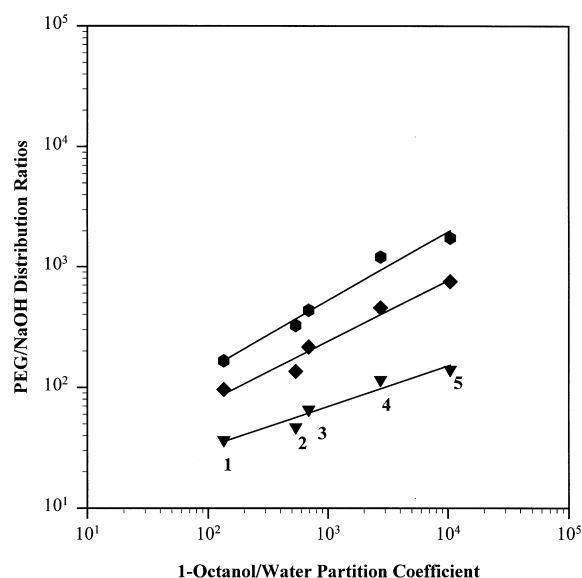


Fig. 10. Correlation between distribution ratios (D) in the 40% (w/w) PEG–NaOH ABS and the partition coefficient (P) for the solutes in 1-octanol–water. The five solutes are: 1, benzene; 2, toluene; 3, chlorobenzene; 4, 1,4-dichlorobenzene; 5, 1,2,4-trichlorobenzene. Symbols refer to the salt concentration of the added NaOH stock solution as: (▼) 4.0 M; (◆) 5.0 M; (●) 6.0 M.

$$\log P_0 = -B/m \quad (4)$$

$\log P_0$ indicates the value of the 1-octanol–water partition coefficient above which solutes will partition to the upper, PEG-rich, phase of the ABS and below which they will partition to the lower, salt-rich phase [9]. Values of the constants m and B and the calculated value of $\log P_0$ for this solute series at each different added salt concentration in all four of the ABS are shown in Table 2.

Zaslavsky et al. [6] have shown that in PEG– $(\text{NH}_4)_2\text{SO}_4$ ABS composed of different molecular masses of PEG, the parameter E of Eq. (2) is dependent only on the difference in PEG concentration between the co-existing phases comprising the ABS. On the other hand, parameter C of Eq. (2) was found to be dependent not only on the PEG concentration difference between the phases but also on the molecular mass of PEG used to form the system. For reasons of experimental expediency connected with the handling of radioactive materials, the present data has been collected with imprecise knowledge of the equilibrium composition of the

Table 2

Correlation coefficients for the distribution ratio (ABS) vs. partition coefficient (1-octanol–water) relationship

Salt	Concentration of salt stock solution (M)	M/M_0 (see text)	Correlation coefficient for D vs. P	Slope (m)	Intercept (B)	Log P_0 ($-B/m$)
K_3PO_4	1.0	1.21	0.988	0.436	0.278	-0.635
K_3PO_4	1.5	1.82	0.989	0.555	0.479	-0.863
K_3PO_4	2.0	2.42	0.992	0.687	0.576	-0.838
K_3PO_4	2.5	3.03	0.982	0.776	0.714	-0.920
K_3PO_4	3.0	3.63	0.973	0.731	1.061	-1.451
K_3PO_4	3.5	4.24	0.984	0.740	1.148	-1.551
K_2CO_3	1.5	1.28	0.962	0.459	0.249	-0.542
K_2CO_3	2.0	1.82	0.957	0.502	0.548	-1.092
K_2CO_3	2.5	2.42	0.967	0.610	0.583	-0.956
K_2CO_3	3.5	3.03	0.980	0.724	0.834	-1.152
K_2CO_3	4.0	3.63	0.988	0.768	0.838	-1.091
K_2CO_3	4.5	4.24	0.972	0.735	1.104	-1.502
$(NH_4)_2SO_4$	1.7	1.05	0.857	0.353	0.311	-0.881
$(NH_4)_2SO_4$	2.2	1.36	0.868	0.398	0.614	-1.543
$(NH_4)_2SO_4$	2.6	1.61	0.847	0.405	0.932	-2.301
$(NH_4)_2SO_4$	3.2	1.98	0.934	0.511	0.875	-1.712
$(NH_4)_2SO_4$	3.7	2.29	0.926	0.594	0.875	-1.473
NaOH	4.0	1.14	0.942	0.334	0.840	-2.515
NaOH	5.0	1.43	0.965	0.501	0.876	-1.748
NaOH	6.0	1.71	0.973	0.572	1.003	-1.753

ABS. In order to discover whether similar relationships apply in the systems under study, a means of normalizing the salt concentration data is required. This may be achieved by determining a parameter M_0 from the phase diagram of each PEG–salt ABS as shown in Fig. 11. The partitioning data may now be expressed in terms of M/M_0 instead of the concentration of added salt stock solution. This measure is proportional to other measures of the relative divergence of the composition of the phases such as tie line length [15] or system stability [20].

Fig. 12a depicts the change in slope (m) of the correlation between $\ln D$ and $\log P$ with increase in concentration of the salt stock solution, normalized by the parameter M/M_0 , for each PEG–salt ABS. In agreement with the findings of Zaslavsky et al. [6], the slope of the correlation between $\ln D$ and $\log P$ is dependent only on the relative concentration of the salt solution forming the biphasic, as expressed by M/M_0 and not at all on the salt type. This indicates that the difference in the distribution coefficients of the solutes as a function of hydrophobicity, depends only on the increasing divergence of the phase

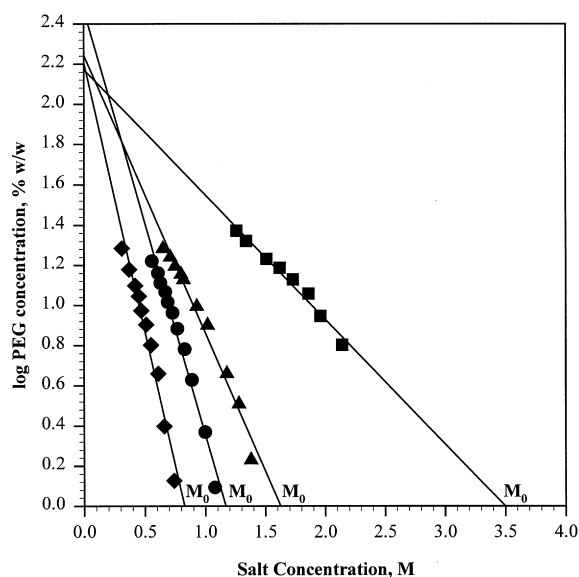


Fig. 11. Phase diagrams for the four ABS used in this study showing the determination of the parameter M_0 as the salt concentration extrapolated to a log PEG concentration of 0. Symbols refer to PEG-2000 systems with addition of the salts: (◆) K_3PO_4 ; (●) K_2CO_3 ; (▲) $(NH_4)_2SO_4$; (■) NaOH.

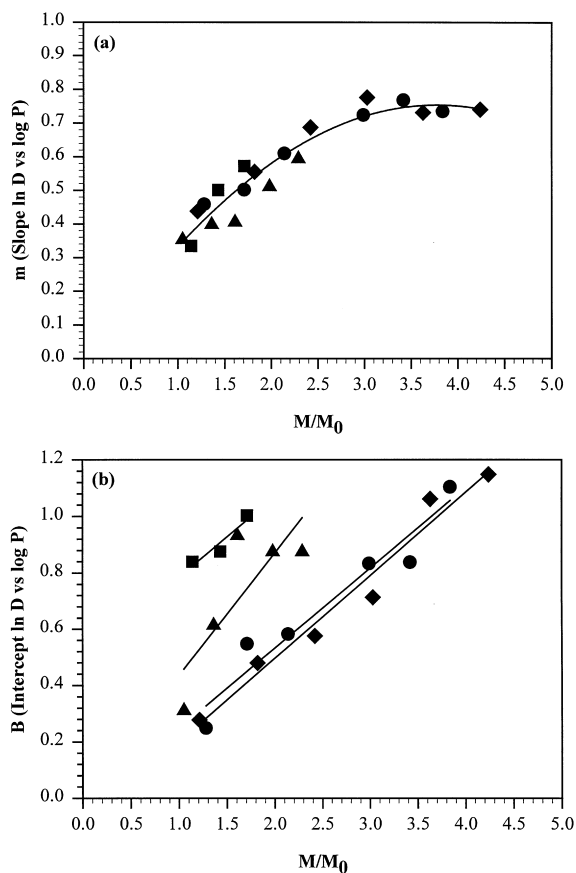


Fig. 12. (a) Slope (m) of the correlation between distribution ratios (D) in the 40% (w/w) PEG–salt ABS and the partition coefficient (P) as a function of the normalized salt composition parameter M/M_0 . (b) Intercept (B) of the correlation between distribution ratios (D) in the 40% (w/w) PEG–salt ABS and the partition coefficient (P) as a function of the normalized salt composition parameter M/M_0 . Symbols refer to the salts forming the ABS: (◆) K₃PO₄; (●) K₂CO₃; (▲) (NH₄)₂SO₄; (■) NaOH.

compositions as the concentration of the added salt stock solution increases. In other words, the resolution of the biphasic systems for these small organic molecules on the basis of hydrophobicity, increases with increasing concentration of the salt stock solution. At the highest salt concentrations, in the most strongly salting-out systems (phosphate and carbonate), there is evidence that this tendency decreases. It appears that no further improvements in resolution are possible beyond a certain K₃PO₄ or K₂CO₃ concentration, whereas this limit has not been reached for the concentrations of (NH₄)₂SO₄ or

NaOH studied. Fig. 12b shows that the intercepts of the $\ln D / \log P$ correlations are also dependent on the concentration of the added salt solution, but are additionally dependent on the salt type used to form the biphasic.

As a consequence of the dependency of m on the difference in phase composition, and B on the composition and salt type, the parameter $\log P_0$ also seems to vary with salt type and composition, as shown in Table 2. Variation of $\log P_0$ with salt type seems reasonable and consideration of the range of values associated with each salt in Table 2, seems to imply that salts having less negative ΔG_{hyd} are capable of resolving more hydrophilic species than salts having more negative ΔG_{hyd} . However, variation of $\log P_0$ with salt concentration seems counterintuitive. The dependence of solute partition on Eq. (1) and the dependence of the partition of an approximately homologous series of solutes on Eq. (3) implies that a solute having $\log P = \log P_0$ should maintain $\ln D = 1$ with increase in divergence of phase composition. It may be that the parameter is affected by the salting-out tendency of the lower phase but the matter is presently unresolved. The effect of salt concentration on the $\log P_0$ parameter can also be estimated visually in Figs. 7–10 from the changing intersection of the $\ln D / \log P$ correlations with the $\ln D$ axis.

4. Conclusions

The quantitative partitioning of a number of substituted benzene compounds has been demonstrated. Distribution values ranging from 10 to more than 10 000 have been found. Even for the solute with the lowest preference for the PEG-rich phase (benzene), values of D in excess of 100 were observed. This indicates that ABS extraction processes should have utility for the recovery, purification and extraction of these small organic molecules. It may be possible to replace currently operating extraction schemes utilizing volatile and toxic organic chemicals with extraction schemes based entirely on aqueous–aqueous technology.

For these uncharged, substituted benzenes, partitioning behavior in PEG–salt ABS was found to be strongly correlated with their partitioning in 1-oc-

tanol–water biphasic systems. This suggests the possibility of a facile means of predicting the partitioning behavior of a vast array of organic species whose log P is known or can be calculated by group contribution methods.

Further research is underway to determine the group contribution effects on partitioning behavior. Such information should be valuable in understanding the driving forces for solute partitioning. With this understanding it should be possible to model and predict the behavior of pollutants such as dyes and PCBs in ABS and thus, evaluate the applicability of ABS for the analysis or clean-up of these important classes of molecules.

Acknowledgements

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