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Predicting partition coefficients in polyethylene glycol–potassium phosphate aqueous two-phase systems

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ABSTRACT

Partition coefficients in polyethylene glycol–potassium phosphate aqueous two-phase systems are predicted using a previously developed mathematical model. The model is based on a simplification of equations which arise from an osmotic pressure virial expansion and relates the partition coefficient to the concentration difference between phases of one of the phase-forming components and to the solute hydrophobicity. The predicted partition coefficients are compared to experimental values for several different solutes in this phase system over the range of pH of 5.5 to 9.2. The predictions are generally good for uncharged solutes, but show disagreement with experimental values for charged solutes.

INTRODUCTION

An aqueous two-phase system may occur when two mutually incompatible components, such as polyethylene glycol (PEG) and dextran, or PEG and certain salts, are dissolved together in water. Two liquid phases form, with each of the incompatible components tending to enrich one or the other phase. A solute added to such a system partitions between the phases, and its partition coefficient, K, is defined as the solute concentration in the upper phase divided by the solute concentration in the lower phase. Numerous studies have focussed on the general prediction of partition coefficients in aqueous two-phase systems. For example, the partition coefficient is thought to depend on solute hydrophobicity [1,2], molecular weight [3], temperature [4], pH [5–7], solute charge [8], and the presence of additional salts [9–12]. Since such two-phase systems are composed primarily of water, they provide a gentle environment for the fractionation of biomaterials [4,13–15], and a detailed review of the use of two-phase systems for the recovery of proteins has recently been published by Huddleston and Lyddiatt [16].

Several models and correlations have been developed to predict partition coefficients in aqueous two-phase systems. Diamond and Hsu [17,18] simplified the lattice model of Flory [19] and Huggins [20] to correlate the partitioning of peptides and proteins. Baskir *et al.* [21] modified a spherical lattice model to predict the distribution of particulates between two polymer–polymer phases. Kang and Sandler [22,23] extended the UNIQUAC equation to predict the binodal phase diagram, while King *et al.* [24] combined a term for electrostatic effects with the osmotic pressure virial expansion to predict protein partitioning. Cabezas *et al.* [25] proposed a model derived from the Hill solution theory, and Forciniti and Hall [26] have used statistical mechanical models for predicting phase diagrams and partition coefficients of proteins. All of these approaches have provided insights into polymer solution behavior and partitioning, but many are of limited use

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because of the difficulty in predicting the pertinent parameters. However, one result has been that, to a good approximation, the logarithm of a solute's partition coefficient is proportional to the concentration difference between the phases of one of the phase-forming components (Δw_2):

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

Obviously, if a suitable expression is found for the proportionality constant, then eqn. 1 can be very convenient and useful for predicting partition coefficients. Although no insight is gained into the actual molecular mechanisms of phase formation and solute partitioning, eqn. 1, nevertheless, permits *a priori* predictions of partition coefficients in aqueous two-phase systems.

For several years, Zaslavsky and co-workers [27–30] have studied the effect that the number of methylene groups on the solute molecule has on its partition coefficient. In all systems studied, they found that a linear relationship exists between the logarithm of the partition coefficient and the number of methylene groups on the aliphatic chain of the solute. Moreover, the hydrophobic properties of aqueous two-phase systems were quantified by a Δg^{CH2} value [31], which has been defined as the free energy for a methylene group transfer between the phases. The introduction of such a hydrophobicity scale permits comparison of different phase systems.

One method to describe solute hydrophobicity in terms which are independent of the two-phase system has recently been advanced by Eiteman and Gainer [32]. Consistent with observations of Zaslavsky and co-workers [27–30], the logarithm of the partition coefficient of a solute in an aqueous two-phase system has been shown to be linearly related to the solute hydrophobicity (as measured by its log P value [33,34]). Since a value for log P for a solute may be calculated using a group contribution method [35], this approach allows an *a priori* estimation of partition coefficients. Specifically, the partition coefficient may be correlated by:

$$\ln K = D\Delta w_2 \log(P/P_0), \quad (2)$$

where D has been termed the discrimination factor and log P_0 the intrinsic hydrophobicity of the phase system.The value of the intrinsic hydrophobicity marks the boundary on the hydrophobicity scale above which solutes will partition into the upper phase and below which solutes will partition into the lower phase. Both of these parameters should be constant for any given two-phase system. Their values for a particular phase system (constant Δw_2) may be readily determined by partitioning a series of normal alcohols. The partition coefficients will be related to the hydrophobicity of the solutes:

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

If the value of Δw_2 is also measured for the specific system of interest, the D and log P_0 may be calculated directly by comparing eqns. 2 and 3:

$$D = \Delta w_2/m \quad (4)$$

$$\log P_0 = -B/m \quad (5)$$

The model (eqn. 2) has previously been applied to the prediction of partition coefficients in the PEG–$MgSO_4$ system, at a single pH [32]. The same model should apply for simple compounds in other systems, such as a system which spans several units of pH. In this study the PEG–potassium phosphate system, without additional buffering, was selected to examine the applicability of eqn. 2.

MATERIALS AND METHODS

PEG molecular weight 8000, potassium phosphate monobasic (KH_2PO_4) and potassium phosphate dibasic (K_2HPO_4) were each purchased from Sigma, St. Louis, USA. Stock solutions of 1.00 *M* monobasic and dibasic salts were each prepared with distilled deionized water. Ten phase systems covering a range of pH values (but each of 1.00 *M* phosphate ion concentration) were prepared by combining proportions of the monobasic and dibasic stock solutions. The proportion of the monobasic salt solution used varied between 0 and 90%. An amount of 2.00 g PEG was added to each of the 10 ml 1.00 *M* phosphate solutions. For pH, density, PEG concentration measurements, and partitioning experiments the capped solutions were equilibrated at 25°C (±0.05°C) in a constant temperature bath (Brinkmann Instruments) for about one week.

Amounts of 10–50 mg of various solutes were

added to each of the separate systems. After equilibration, the phases were then carefully separated with glass Pasteur pipets. Gas or liquid chromatography, as appropriate, was employed to determine the solute concentration in each phase. The partition coefficients for small molecules were found to be independent of solute concentration for the range of dilute solutions prepared. The two-phase systems used for the determination of pH were placed in capped tubes under nitrogen, and the analysis performed on the lower of separated phases at 23°C using an Orion Sureflow pH electrode and a Corning general purpose combination electrode. A 10-ml pycnometer was used to measure density (ρ) at 25°C. The concentration of PEG was found by freeze-drying each phase, then extracting PEG from the residue with warm acetone.

The gas chromatographic (GC) column selected was a 6 ft. × 2 mm I.D. glass column packed with Chromosorb 101 (80/100) from Alltech, Deerfield, USA. The instrument itself was a Hewlett-Packard 5890A fitted with a flame ionization detector. The operating temperatures, carrier gas (helium) flowrate and pressure were adjusted depending upon the particular solute analyzed. The high-performance liquid chromatographic (HPLC) system comprised a Whatman 5-μm Partisphere C_8 column, Waters pumps Model 510, with a Gilson Model 231 sample injector, a Waters UV detector (Model 481), and Hewlett-Packard 3392A integrator. The standard error of the mean from the analyses did not exceed 6%.

RESULTS AND DISCUSSION

Ten different two-phase systems, each containing 1.00 *M* phosphate ion concentration, were prepared over the pH range of 5.5 to 9.2, and Table I lists their properties. The effect of pH on the PEG concentration difference between the phases is shown in Fig. 1. The value of the PEG concentration difference is greatest at the highest values of pH. Since eqn. 1 suggests that the logarithm of the partition coefficient is proportional to the PEG concentration difference, one might anticipate that any hydrophobic solute will exhibit the greatest partition coefficient at high pH values. (Conversely, a hydrophilic solute will partition more into the lower phase the higher the pH.)

Initially, a series of normal alcohols (from ethanol to hexanol) was studied, and the partition coefficients are shown in Fig. 2 as a function of pH. The partition coefficients for each alcohol were lowest at acidic pH values. This observation should not be attributed to any change in the solute with pH, but rather to a change in the phase system itself since the PEG concentration difference between the phases (Δw_2) is greatest at the highest pH values. Furthermore, the more hydrophobic the alcohol, the greater its partition coefficient. Therefore, the partitioning behavior observed qualitatively agrees with eqn. 2.

The parameters D and log P_0 may be calculated from the data shown in Fig. 2. These two parameters will have different values for each solution.

TABLE I

SUMMARY OF THE PEG–1.00 *M* POTASSIUM PHOSPHATE AQUEOUS TWO-PHASE SYSTEM AT 25°C.

pH$^-$ denotes lower phase pH, while ρ' and ρ'' denote upper and lower phase densities, respectively.

% Dibasic (1.00 *M*)	PEG (wt.%)	K (wt.%)	PO_4^{3-} (wt.%)	pH″	ρ' (g/ml)	ρ'' (g/ml)	Δw_2
100	0.150	0.0586	0.0712	9.17	1.0839	1.1773	0.64
90	0.150	0.0559	0.0714	7.88	1.0827	1.1736	0.60
80	0.151	0.0531	0.0717	7.44	1.0819	1.1699	0.53
70	0.151	0.0504	0.0720	7.17	1.0813	1.1660	0.52
60	0.152	0.0476	0.0722	6.90	1.0809	1.1622	0.48
50	0.153	0.0447	0.0724	6.63	1.0810	1.1587	0.46
40	0.153	0.0419	0.0727	6.39	1.0814	1.1540	0.44
30	0.154	0.0390	0.0730	6.13	1.0825	1.1499	0.40
20	0.154	0.0362	0.0732	5.83	1.0848	1.1454	0.35
10	0.155	0.0333	0.0735	5.52	1.0889	1.1400	0.30

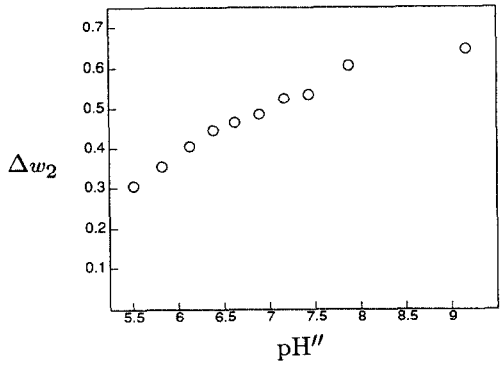

Fig. 1. PEG concentration difference between the phases (Δw_2) *versus* the lower phase pH in the PEG–1.00 *M* potassium phosphate aqueous two-phase system at 25°C.

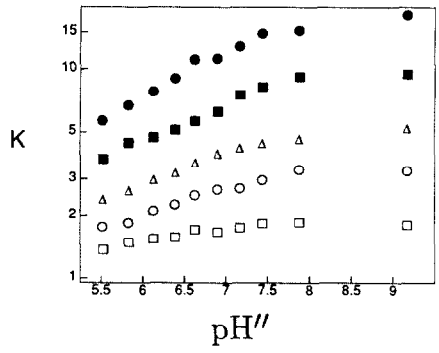

Fig. 2. Measured partition coefficients (K) of normal alcohols *versus* the lower phase pH in the PEG–1.00 *M* potassium phosphate aqueuos two-phase system at 25°C. ● = Hexanol; ■ = pentanol; △ = butanol; ○ = propanol; □ = ethanol.

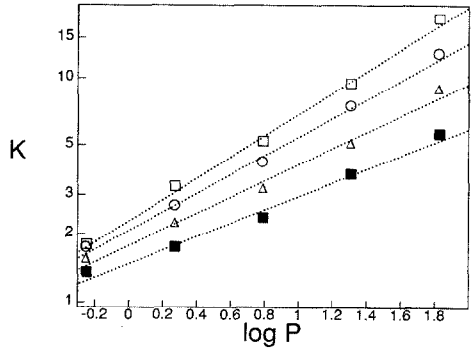

Fig. 3. Measured partition coefficients (K) of normal alcohols *versus* their hydrophobicity (log P) as calculated by the method of Rekker and DeKort [35]. Dotted lines show least squares fit of data to eqn. 3 (numeric values of constants shown in Table II). Lower phase pH: □ = 9.17; ○ = 7.17; △ = 6.39; ■ = 5.52.

The parameters are determined using eqn. 3 by plotting the logarithm of the observed partition coefficient *versus* the calculated hydrophobicity. Fig. 3 shows such plots for four of the solutions, while Table II lists the calculated values of the slope (m), intercept (B) and correlation coefficient (R) for all of the systems studied. In general, the slopes and intercepts (of eqn. 3) are larger the higher the pH. The values of these slopes and intercepts may be used in eqn. 4 and 5, along with the measured concentration differences to calculate the discrimination factor and intrinsic hydrophobicity for each phase system. Fig. 4 shows the values for the paramaters D and log P_0 for each solution. The value of each parameter generally decreases with increasing pH, so that the phosphate system having the lowest pH is the most intrinsically hydrophobic.

With these calculated values for the discrimination factor and intrinsic hydrophobicity, as well as the measured concentration difference, eqn. 2 may be used to predict partition coefficients for other solutes. Fig. 5 shows the predicted and observed partition coefficients for three organic solutes in the PEG–potassium phosphate aqueous two-phase system. The partition coefficients for methyl pentanone and butanediol are very well predicted by the model, while the predicted partition coefficients for phenylpropanol are consistently about 15% below the observed values.

TABLE II

CALCULATED SLOPES (m), INTERCEPTS (B) AND CORRELATION COEFFICIENTS (R) OF PARTITION COEFFICIENTS OF NORMAL ALCOHOLS IN THE PEG–1.00 *M* POTASSIUM PHOSPHATE AQUEOUS TWO-PHASE SYSTEM AT 25°C

Data was fit to the Eqn. 3.

pH″	Slope (m)	Intercept (B)	R
9.17	1.0978	0.8351	0.999
7.88	1.0081	0.8484	0.996
7.44	0.9987	0.7905	0.997
7.17	0.9636	0.7341	0.998
6.90	0.8973	0.6985	0.998
6.63	0.8790	0.6652	0.991
6.39	0.8320	0.5804	0.995
6.13	0.7809	0.5396	0.995
5.83	0.7501	0.4615	0.991
5.52	0.6909	0.3957	0.994

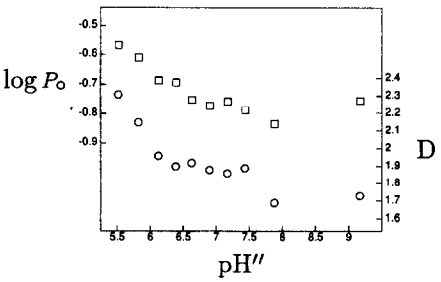

Fig. 4. Intrinsic hydrophobicity (log P_0) and discrimination factor (D) in the PEG–1.00 M potassium phosphate aqueous two-phase system at 25°C. □ = Intrinsic hydrophobicity; ○ = discrimination factor.

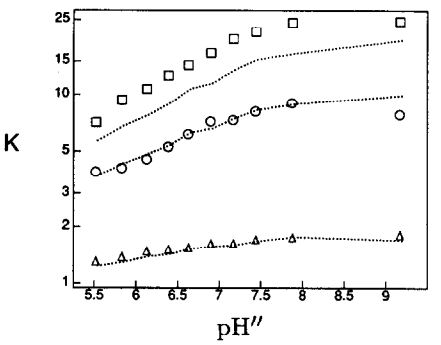

Fig. 5. Predicted (dotted curves) and observed partition coefficients *versus* the pH of the lower phase in the PEG–1.00 M potassium phosphate aqueous two-phase system at 25°C. Hydrophobicities calculated by the method of Rekker and DeKort [35]. □ = Phenylpropanol (log P = 1.93); ○ = 4-methyl 5-pentanone (log P = 1.32); △ = 2,3-butanediol (log P = −0.286).

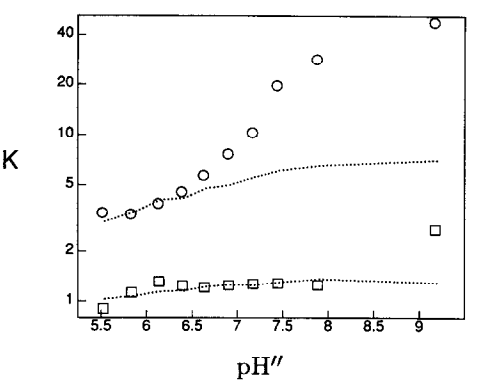

Fig. 6. Predicted (dotted curves) and observed partition coefficients *versus* the pH of the lower phase in the PEG–1.00 M potassium phosphate aqueous two-phase system at 25°C. Hydrophobicities calculated by partition in PEG–magnesium sulfate system [32,36]. ○ = Tyrosine-phenylalanine (log P = 1.01); □ = tyrosine (log P = −0.539).

The solutes shown in Fig. 5 are all uncharged, while Fig. 6 shows the application of the model to two charged compounds, tyrosine and the peptide tyrosine-phenylalanine. The model predicts the partitioning behavior of tyrosine at low pH; however, at the highest pH the observed partition coefficient is twice as high as the prediction. Since these partition coefficients are close to one and a relatively small change may be difficult to detect, a more hydrophobic peptide was selected. As Fig. 6 shows, the partition coefficients of tyrosine-phenylalanine are predicted well by the model only at low pH, which are solutions in which the peptide will be uncharged. When the pH increases and the peptide becomes increasingly negatively charged, the observed partition coefficients increasingly exceed the model predictions. Although limited, these results suggest that in the PEG–phosphate system, a negatively charged solute will tend to have a greater partition coefficient at a particular pH than it would have if it were uncharged at that same pH. An additional term appears to be necessary in the model to account for these charge effects.

CONCLUSIONS

Partition coefficients of uncharged solutes have been successfully predicted in PEG–potassium phosphate aqueous two-phase systems over the pH range of 5.5–9.2. However, partitioning results with charged solutes indicate that, in this phase system, the observed partition coefficients become greater than predicted when solutes become more negatively charged.

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