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Predicting partition coefficients of multi-charged solutes in aqueous two-phase systems

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Abstract

A mathematical model incorporating the influence of a pH difference between the phases is developed to predict the partitioning of charged compounds in aqueous two-phase systems. The partition coefficients of three model compounds, terephthalic acid, benzenedimethanol and xylylenediamine, are examined in poly(ethylene glycol)–potassium phosphate aqueous two-phase systems over the pH range 5.5–9.2. The model predicts that in this particular phase system, negatively charged solutes partition higher and positively charged solutes partition lower than an otherwise identical neutral solute. The partition coefficients of the dipeptides tyrosine–tyrosine, glycine–tyrosine and leucine–phenylalanine are also examined in poly(ethylene glycol)–potassium phosphate systems, and their observed behaviour agrees with the model prediction. The addition of an alkali metal halide to a poly(ethylene glycol)–potassium phosphate system was observed to result in a decrease in the pH difference between the phases. As the model predicts for decreasing pH difference between the phases, the observed partition coefficients of negatively charged compounds decrease. The results indicate that charge and hydrophobic effects each play important roles in the partitioning of biological compounds.

1. Introduction

Aqueous solutions of two soluble but mutually incompatible components, which as poly(ethylene glycol) (PEG) and dextran, or PEG and certain salts, often form aqueous two-phase systems. Albertsson [1] showed that two liquid "aqueous" phases form when a threshold concentration of either component is exceeded with each phase tending to be enriched by one of the incompatible components. A solute such as a protein added to a two-phase system distributes between the phases, and the partition coefficient is defined as this solute's upper phase concentration divided by its lower phase concentration. Since such systems are composed primarily of water, they have received attention for the liquid–liquid extraction of biomaterials [2–6]. More recent developments have been reviewed [7].

In order to select a particular aqueous two-phase system for a given separation, models are needed to predict partition coefficients. Numerous studies have therefore focused on the general prediction of partition coefficients in aqueous two-phase systems. Partition coefficients depend on several factors including solute hydrophobicity [8,9], molecular mass [10], temperature [2], pH [11–13], solute charge [14] and the presence of additional salts [15,16].

For over 20 years, solute or particle charge has been recognized as one important factor which influences the partition coefficient. Reitherman *et al.* [17] measured an electric potential between

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phases and correlated the partitioning of negatively charged human erythrocytes with this difference in potential. Johansson [18] showed that the partitioning of proteins could be correlated with salt partitioning. Johansson [19] and Albertsson [20] developed equations to express protein partition coefficients as a function of the protein's net charge and the difference in potential between the phases. However, the mechanism and magnitude of charge effects have remained poorly understood. For example, researchers refer to "charge-sensitive" and "non-charge-sensitive" phase systems and effects when describing behavior that appears to be related in some way to charge [21,22].

A related phenomenon that has been observed is the decrease in the partition coefficients of negatively charged compounds in PEG–phosphate or PEG–sulfate systems as the alkali metal halide concentration is increased [23]. No satisfactory explanation has been advanced for this observation, and it is thus not possible to predict the quantity of a halide needed for a desired reduction in the partition coefficient of a particular solute.

Eiteman and Gainer [24] showed that a measured pH difference between the phases of an aqueous two-phase system affects the partition coefficients of charged solutes. Using a mass balance for all species (charged and uncharged) in a phase system, equations were derived to predict the partition coefficient of a charged solute relative to the partition coefficient of the neutral species alone or an uncharged, but structurally analogous, solute. Specifically, the partition coefficient of a charged solute depends upon the partition coefficient of the uncharged analog, the dissociation of the solute and the pH of each phase. The objectives of this present study were to derive a general expression for the partition coefficients of charged solutes and to compare the predicted behavior with results obtained from partitioning of several solutes.

2. Mathematical model

As noted in the Introduction, the partition coefficient of a neutral solute, K_0, is defined as the concentration of that solute in the upper phase divided by that in the lower phase. This ratio of solute concentrations (c) is related to the ratio of solute mole fractions (x) by a proportionality constant:

$$K_0 = \frac{c_0'}{c_0''} = k \cdot \frac{x_0'}{x_0''} \tag{1}$$

In Eq. 1, a single prime refers to the upper phase and a double prime to the lower phase. The subscript zero emphasizes that the solute is uncharged.

In contrast, the overall (*i.e.*, measured) partition coefficient of a charged solute depends on the partitioning of all charged and uncharged species. For example, the partition coefficient of a solute which may have one positive charge depends upon the partitioning of both the positively charged species (+) and the neutral species:

$$K = k \cdot \frac{x_+' + x_0'}{x_+'' + x_0''} = K_0 \cdot \frac{\frac{x_+'}{x_0'} + 1}{\frac{x_+''}{x_0''} + 1} \tag{2}$$

The partition coefficient of the neutral species alone is again denoted by K_0. The actual measured partition coefficient, K, does not have a subscript, emphasizing that its value includes contributions from the charged and neutral species. At low pH the solute being considered will be exclusively positively charged and, as Eq. 2 indicates, its predicted partition coefficient becomes $K = kx_+'/x_+''$. Similarly, at high pH this solute's measured partition coefficient becomes equal to the partition coefficient of the uncharged species, $K = K_0$.

The solution pH and the charge of any solute are related by an equilibrium. For the positively charged compound (A^0 in the neutral form), the equilibrium is described by

$$A^+ \rightleftharpoons A^0 + H^+ \tag{3}$$

An equilibrium constant, K_{b1}, is defined by

$$K_{b1} = \frac{a_0 a_{H^+}}{a_+} \tag{4}$$

where the subscript b indicates equilibria of

positively charged compounds and the subscript 1 indicates that an uncharged species and a species having a net charge of unity are in equilibrium.

Partitioning occurs at equilibrium, and therefore Eq. 4 must be satisfied for both phases. For the upper phase, Eq. 4 may be rewritten as

$$K_{b1}\gamma'_{+}x'_{+} = \gamma'_{0}x'_{0}a'_{H^0} \tag{5}$$

where each species activity (a) is expressed as the product of its activity coefficient (γ) and mole fraction (x). For convenience, an activity ratio, Λ_+ is defined for each phase as the activity coefficient of the neutral species divided by that of the positive species. Substituting Eq. 5 for each phase into Eq. 2 and noting that $pX = -\log_{10}X$ yields

$$\frac{K}{K_0} = \frac{1 + \Lambda'_{+} \cdot 10^{(pK_{b1} - pH')}}{1 + \Lambda''_{+} \cdot 10^{(pK_{b1} - pH'')}} \tag{6}$$

This equation does not directly predict the partition coefficient of a positively charged solute, but rather predicts the ratio of the solute's partition coefficient to that of the uncharged species alone. This partition ratio depends on the pH in each phase (or $\Delta pH = pH' - pH''$), the activity ratio and the equilibrium constant. Several important practical limitations of this equation have been previously discussed [25].

Eq. 6 may be used in two different ways. It may be used directly to predict the partition coefficient of a charged solute relative to the partition coefficient of an uncharged analog [24,25]. Alternatively, one may predict the partition coefficient of the uncharged species, K_0, using an additional model and then use Eq. 6 to predict the partitioning in systems in which the solute is charged.

2.1. General expressions

In general, a multi-charged solute may have up to m positive charges and up to n negative charges, existing in solution as a distribution of charged species depending on the pH of the solution. The measured partition coefficient is the sum of the concentrations of all the species in the upper phase divided by those of all the species in the lower phase. In terms of mole fractions:

$$K = k \cdot \frac{x'_0 + \sum_{i=1}^{m} x'_{i+} + \sum_{j=1}^{n} x'_{j-}}{x''_0 + \sum_{i=1}^{m} x''_{i+} + \sum_{j=1}^{n} x''_{j-}} \tag{7}$$

At low pH, the general solute has its maximum number of positive charges and minimum number of negative charges. At this pH, the solute's net positive charge will be m, and the solute will be referred to as A^{m+}. As the pH increases, the net charge decreases until the net charge is zero at the isoelectric pH. The solute at this pH, denoted by A^0, has an equal number of positive and negative charges. Increasing pH beyond this point increases the net negative charge to the maximum of n, at which point the solute will be denoted as A^{n-}. These equilibria will be described by

$$\begin{aligned} A^{m+} &\overset{K_{bm}}{\rightleftharpoons} A^{(m-1)+} + H^+ \overset{K_{b(m-1)}}{\rightleftharpoons} A^{(m-2)+} + 2H^+ \\ &\overset{K_{b(m-2)}}{\rightleftharpoons} \cdots \overset{K_{b2}}{\rightleftharpoons} A^{1+} + (m-1)H^+ \\ &\overset{K_{b1}}{\rightleftharpoons} A^0 + mH^+ \overset{K_{c1}}{\rightleftharpoons} A^{1-} + (m+1)H^+ \\ &\overset{K_{c2}}{\rightleftharpoons} \cdots \overset{K_{c(n-1)}}{\rightleftharpoons} A^{(n-1)-} + [m + (n-1)]H^+ \\ &\overset{K_{cn}}{\rightleftharpoons} A^{n-} + (m+n)H^+ \end{aligned} \tag{8}$$

The general equilibrium constants are given by

$$K_{bi} = \frac{a_{(i-1)+}a_{H+}}{a_{i+}} \tag{9}$$

$$K_{cj} = \frac{a_{j-}a_{H+}}{a_{(j-1)-}}. \tag{10}$$

In Eq. 9, i will refer to the equilibrium between a solute of i net positive charges and $i-1$ net positive charges with $1 \leq i \leq m$. Similarly, j indicates the equilibrium between a solute of j net negative charges and $j-1$ net negative charges with $1 \leq j \leq n$. Subscripts b and c refer to equilibria of positively and negatively charged solutes, respectively.

For convenience, a general activity ratio will be defined as the activity coefficient of the

neutral species divided by the activity coefficient of a species of particular charge, q:

$$\Lambda_q = \frac{\gamma_0}{\gamma_q} \tag{11}$$

In order to derive a general expression analogous to Eq. 5, this definition of the activity ratio and the four expressions for species mole fractions (one for each phase and charge) may be substituted into Eq. 7 to obtain

$$\frac{K}{K_0} = \frac{1 + \sum_{i=1}^{m} \Lambda'_{i+} \dfrac{a'^{i}_{H+}}{\prod_{l=1}^{i} K_{bl}} + \sum_{j=1}^{n} \Lambda'_{j-} \dfrac{\prod_{l=1}^{j} K_{cl}}{a'^{j}_{H+}}}{1 + \sum_{i=1}^{m} \Lambda''_{i+} \dfrac{a''^{i}_{H+}}{\prod_{l=1}^{i} K_{bl}} + \sum_{j=1}^{n} \Lambda''_{j-} \dfrac{\prod_{l=1}^{j} K_{cl}}{a''^{j}_{H+}}} \tag{12}$$

Eq. 12 is the general charge expression which may be simplified for particular solutes of interest. For example, for the case of a solute with up to two negative charges (*e.g.*, a dicarboxylic acid such as malic acid), $i = 0$ and $j = 2$. For this particular solute, setting the activity ratios (Λ) equal to unity for simplicity and noting that $pX = -\log_{10} X$, Eq. 12 becomes

$$\frac{K}{K_0} = \frac{1 + \dfrac{K_{c1}}{a'_{H+}} + \dfrac{K_{c1}K_{c2}}{a'^{2}_{H+}}}{1 + \dfrac{K_{c1}}{a''_{H+}} + \dfrac{K_{c1}K_{c2}}{a''^{2}_{H+}}} = \frac{1 + 10^{pH' - pK_{c1}} + 10^{2pH' - pK_{c1} - pK_{c2}}}{1 + 10^{pH'' - pK_{c1}} + 10^{2pH'' - pK_{c1} - pK_{c2}}} \tag{13}$$

Since the equilibrium constants for solutes are often available or measureable, Eq. 13 requires only that the pH be measured in each phase. One important trend predicted by Eq. 12 is that in systems having a positive pH difference between the phases, the partition ratio for a negatively charged solute is greater than unity [24]. In other words, a negatively charged solute will have a greater partition coefficient than a neutral analog in such phase systems. Similarly, a positively charged solute will have a lower partition coefficient than a neutral analog. In order to calculate the actual partition coefficient of a charged solute, the partition coefficient of the uncharged species alone must be measured or calculated by a model which considers all non-charge effects.

The objectives of this study were (1) to compare qualitatively the partitioning of uncharged and multicharged analogs, (2) to predict with Eq. 12 the partition coefficients of several dipeptides over a range of pH (using an additional model to predict K_0) and (3) to determine the effect that addition of sodium chloride has on the properties of a PEG–potassium phosphate system and the predicted and observed partition coefficients of uncharged and charged solutes.

3. Experimental

A series of 1.00 *M* potassium phosphate solutions was prepared as described elsewhere [24,26]. The phase-forming polymer used in these solutions was PEG with a molecular mass of 8000, and 2.00 g of this polymer were used per 10 ml of phosphate solution. The resulting phase systems at 25.0°C had a positive pH difference between the phases, that is, the measured pH of the upper phase was greater than the pH of the lower phase, as reported previously [24].

Phase systems with sodium chloride were prepared by mixing 5 ml of 2.00 *M* potassium phosphate solution with 5 ml of NaCl solution prior to adding 2.00 g of PEG 8000. The final salt concentration in the phase systems (prior to the addition of polymer) is the value reported. The pH of each phase of these PEG–phosphate–chloride systems was measured as before [24].

The following solutes were used for partitioning studies: tryptamine, indole-3-acetic acid, tyrosine–tyrosine, glycine–tyrosine, leucine–phenylalanine (Sigma, St. Louis, MO, USA), benzene-1,4-dimethanol, terephthalic acid and 1,4-xylylenediamine (Aldrich, Milwaukee, WI, USA).

Approximately 5 mg of a single solute were added to 10 ml of two-phase solutions. The phases were adjusted to 25.0 ±0.05°C, thorough-

ly mixed for 2 days, allowed to equilibrate for 3 days, then carefully separated with Pasteur pipets immediately before analysis. The partition coefficients of other solutes at 25.0°C were determined by HPLC. The HPLC system consisted of a Gilson Model 306 pump, a Model 231 Autosampler and an Applied Biosystems Model 759A UV–Vis detector. The column was a Waters Radial-Pak C_8, with eluent and detector settings appropriate to separate and quantify the pure solute of interest from impurities arising from the PEG and solute sample.

4. Results and discussion

One objective of this research was to observe whether a multi-charged solute has a different partition coefficient than an analogous uncharged solute. Since solute hydrophobicity influences its partitioning, charged and uncharged analogs having an identical hydrophobicity were selected. Fig. 1 shows the partition coefficients of three compounds in the PEG–potassium phosphate system: terephthalic acid (which is negatively charged), benzenedimethanol (neutral), and 1,4-xylylenediamine (positively charged). The carboxylic acid, methylene-ol, and methylene-amine functional groups have approximately the same hydrophobicity [27]. The partition coefficient of the negatively charged diacid was observed to be greater than that of the neutral

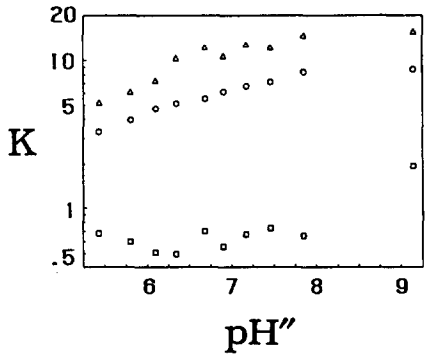

Fig. 1. Observed partition coefficients of (○) benzenedimethanol, (△) terephthalic acid and (□) 1,4-xylylenediamine in a series of PEG–potassium phosphate aqueous two-phase systems

analog, which itself was observed to be greater than those of the positively charged diamine. The results qualitatively agree with those predicted by Eq. 12 for a phase system of positive pH difference between the phases.

A second goal was to predict the partition coefficients of peptides over a range of pH. Several dipeptides were selected for this study: tyrosine–tyrosine, leucine–phenylalanine and glycine-tyrosine. Assuming that all activity ratios in Eq. 12 are equal to unity, the parameters required to predict the partition coefficients for these peptides in the PEG–potassium phosphate systems are the pH in each phase (previously measured [24]), the equilibrium constants of these solutes (shown in Table 1) and the values for the partition coefficients of the uncharged species, K_0. The value of K_0 will be estimated by an additional model.

A model to predict the partition coefficients of hypothetical uncharged amino acids and peptides in the PEG–potassium phosphate systems was advanced by Eiteman and Gainer [26]:

$$\ln K_0 = D\,\Delta w_2 \log(P/P_0) \qquad (14)$$

This simple model contains two parameters which describe a phase system. The discrimination factor, D, and the intrinsic hydrophobicity, $\log(P_0)$, have been previously determined for the PEG–potassium phosphate system [26]. Log P is the hydrophobicity (a theoretical distribution between octanol and water) of the particular solute, and Δw_2 is the PEG concentration difference between the phases and have been measured previously [26] for the PEG–potassium phosphate system. Values for the hydrophobicity of each of the three dipeptides studied are listed in Table 1.

The value of K_0 for each phase system was calculated by Eq. 14 for these three solutes. Eq. 12 was then used to predict the partition coefficient of each dipeptide in the phase systems. Figs. 2, 3 and 4 show the observed and predicted partition coefficients for tyrosine–tyrosine, leucine–phenylalanine and glycine–tyrosine, respectively. The partition coefficient of all three solutes was greatest at high pH. The model

Table 1
Hydrophobicity (log P) and equilibrium (dissociation) constants for the dipeptides used in this study

Solute	Log P	pK_{b1}	pK_{c1}	pK_{c2}	pK_{c3}
Tyr–Tyr	1.27[a]	3.52[b]	7.68[b]	9.80[b]	10.26[b]
Leu–Phe	0.06[c]	2.85	8.41[d]	–	–
Gly–Tyr	−1.12[c]	2.93[b]	8.45[b]	10.49[b]	–

[a] Ref. 9.
[b] Ref. 28.
[c] Ref. 29.
[d] Ref. 30.

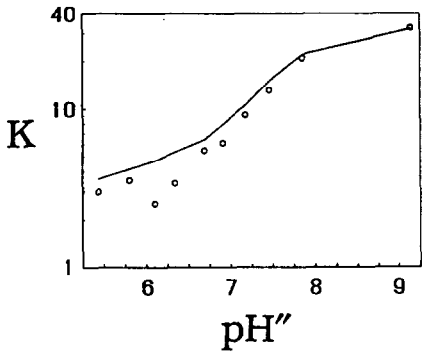

Fig. 2. Observed and predicted partition coefficients of the dipeptide tyrosine–tyrosine in a series of PEG–potassium phosphate aqueous two-phase systems. Predictions using Eq. 12 are indicated by the solid curve.

predicted this observation and the magnitudes of the partition coefficients very well. The observed partition coefficients of tyrosine–tyrosine were predicted most closely. The observed partition coefficients of leucine–phenylalanine showed an inexplicable maximum at a pH of *ca.* 8. Partition coefficients lower than unity were observed and predicted for glycine–tyrosine, although the partition coefficient at the highest pH was underpredicted.

As noted in the Introduction, the addition of alkali metal halides to phase systems has been known to influence partition coefficients of charged compounds significantly. Another objective of this study was to determine if these observed changes in partition coefficients could be explained in terms of a pH difference between the phases. The addition of what amounts to another component to a phase system may also have several unavoidable consequences. Considering Eq. 14, the addition of salt may alter the concentration difference between the

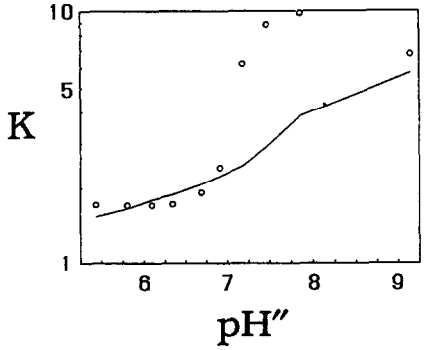

Fig. 3. Observed and predicted partition coefficients of the dipeptide leucine–phenylalanine in a series of PEG–potassium phosphate aqueous two-phase systems. Predictions using Eq. 12 are indicated by the solid curve.

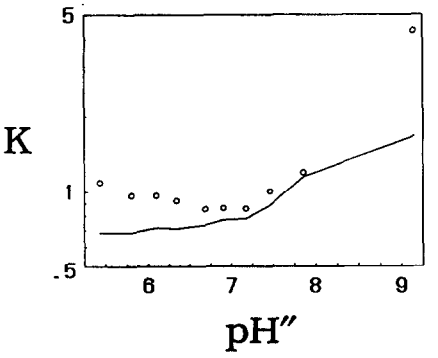

Fig. 4. Observed and predicted partition coefficients of the dipeptide glycine–tyrosine in a series of PEG–potassium phosphate aqueous two-phase systems. Predictions using Eq. 12 are indicated by the solid curve.

phases, the discrimination factor and the instrinsic hydrophobicity of the phase system. New values for each of these parameters would affect the partition coefficient calculated for uncharged compounds. Naturally, the addition of salt might also alter the pH difference between the phases, a consequence which, by itself, would alter only the partitioning of charged compounds.

To examine the effect of an alkali metal halide addition on the model parameters, a single PEG–phosphate phase system (without alkali metal halide) was selected having a lower phase pH of 8.0. Sodium chloride was added to this phase system, resulting in six PEG–phosphate–chloride phase systems with NaCl concentrations ranging from 0.0 to 2.0 *M*. In order to remove at least one variable, all systems were prepared to have identical PEG concentration differences between the phases. This continuity was accomplished by reducing the concentration of potassium phosphate (to a minimum of about 0.8 *M*) as the sodium chloride concentration was increased.

Fig. 5 shows the pH measured in each phase of the six phase systems. As the concentration of salt was increased, the pH in each phase decreased. More importantly, as the concentration of sodium chloride was increased, the pH difference between the phases decreased. At a concentration of 2.0 *M* sodium chloride, the pH difference between the phases was essentially zero. The pH difference decreased most quickly at low NaCl concentrations: between 0.0 and 0.2 *M* NaCl, the value of ΔpH decreased from 0.54 to 0.17, while between 1.0 and 2.0 *M* it decreased from only 0.05 to 0.02. Additional studies with PEG–phosphate–chloride phase systems all having a 1.00 *M* phosphate concentration showed similar pH difference behavior. Therefore, the decrease in pH difference was due to the increase in NaCl concentration, not the slight reduction in phosphate concentration required to maintain an identical PEG concentration difference between the phases.

Eq. 12 predicts that if a positive pH difference between the phases is reduced, the partition coefficient of negatively charged compounds will decrease, while the partition coefficient of positively charged compounds will increase. In order to test this prediction, two analogous compounds, indoleacetic acid and tryptamine, were partitioned in these six phase systems. These two compounds have approximately identical hydrophobicities [27], but the acid is negatively charged at pH 7.5–8.0, while the amine is positively charged in solutions in this pH range. Fig. 6 shows the results of partitioning these two compounds in the six PEG–phosphate–chloride systems.

As predicted, the partition coefficient of the negatively charged acid decreased while the partition coefficient for the positively charged amine increased. Furthermore, the rate of change in these partition coefficients decreased

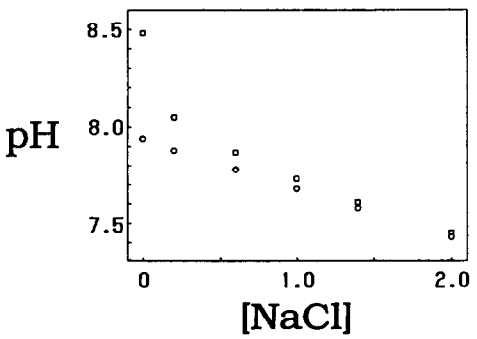

Fig. 5. Observed pH of phases (○ = lower and □ = upper phase) in a PEG–potassium phosphate–sodium chloride aqueous two-phase system (90% dibasic) as a function of sodium chloride concentration.

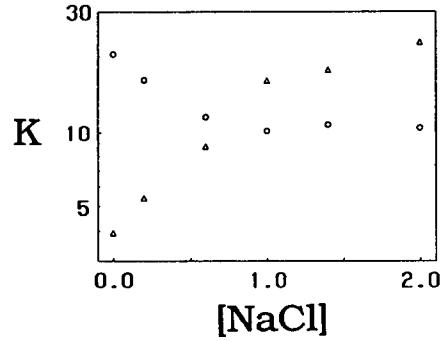

Fig. 6. Observed partition coefficients of (△) tryptamine and (○) indole-3-acetic acid in a PEG–potassium phosphate–sodium chloride aqueous two-phase system (90% dibasic) as a function of sodium chloride concentration.

at higher salt concentrations, where the rate of decrease in the pH difference between the phases was reduced. The difference between the partition coefficients of these two compounds at the highest NaCl concentration, where the charge effects should be negligible since $\Delta pH \approx 0$, might be due to a slight difference in the hydrophobicities of the two compounds or to other interactions between the solutes and components.

The partitioning of the three dipeptides was next reconsidered in these PEG–phosphate–chloride phase systems. Again, Eq. 14 was used to predict the theoretical partition coefficients of the uncharged dipeptide species. In this case, the values for the two parameters, the discrimination factor and the intrinsic hydrophobicity, were assumed to remain constant in phase systems of varying sodium chloride concentration. This simplification means that the value of K_0 calculated by Eq. 14 is the same for all sodium chloride concentrations. As before, with the value of K_0, the pH measured in each phase and the equilibrium constants, Eq. 12 may be used to predict the partition coefficients of these dipeptides as sodium chloride is added.

Figs. 7, 8 and 9 show the measured and predicted partition coefficients for tyrosine–tyrosine, leucine–phenylalanine and glycine–tyrosine, respectively, in PEG–potassium phosphate systems of increasing sodium chloride concentration. The measured partition coefficients for tyrosine–tyrosine were observed to decrease from about 22 to 14 as the concentration of sodium chloride in the phase system was increased. The model successfully predicted that the partition coefficients of this dipeptide will decrease initially with increasing sodium chloride concentration, but generally underestimated the partition coefficients. The predicted partition coefficients agreed with the observation that the partition coefficients change more slowly as more salt was added.

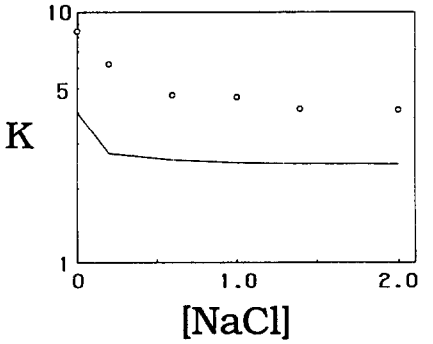

Fig. 8. Observed and predicted partition coefficients of leucine–phenylalanine in a PEG–potassium phosphate–sodium chloride aqueous two-phase system (90% dibasic) as a function of sodium chloride concentration.

The measured partition coefficients for leucine–phenylalanine shown in Fig. 8 similarly decreased with increasing sodium chloride concentration, in this case from about 8.5 to 4. The model prediction again underestimated the partition coefficients, but the trend agreed with the

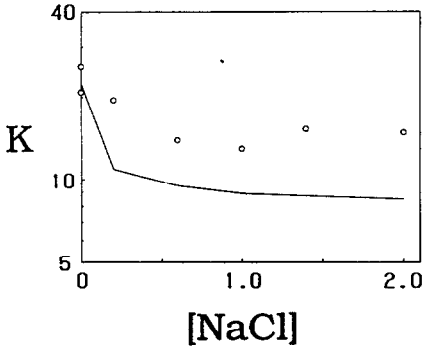

Fig. 7. Observed and prediction partition coefficients of tyrosine–tyrosine in a PEG–potassium phosphate–sodium chloride aqueous two-phase system (90% dibasic) as a function of sodium chloride concentration.

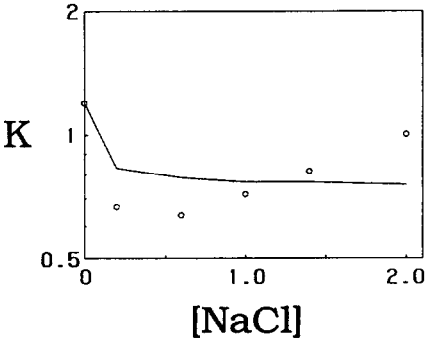

Fig. 9. Observed and predicted partition coefficients of glycine–tyrosine in a PEG–potassium phosphate–sodium chloride aqueous two-phase system (90% dibasic) as a function of sodium chloride concentration.

observation. In this case, the partition coefficient predicted at zero salt concentration was also significantly underestimated (as noted in Fig. 3). The predicted and observed partition coefficients again decreased more slowly as more salt was added.

The measured partition coefficients for glycine–tyrosine (Fig. 9) decreased with increasing sodium chloride up to a concentration of about 0.6 *M*, then increased slowly with a further increase in salt concentration. At this point, glycine–tyrosine also shifted from partitioning predominantly into the upper phase to partitioning predominantly into the lower phase. The model correctly predicted the decrease in partition coefficient from above to below unity, but did not predict the subsequent increase in partition coefficient as the sodium chloride concentration was further increased.

Discrepancies between the observed and predicted partition coefficients could be explained as resulting from the assumption that the discrimination factor and intrinsic hydrophobicity remained constant as sodium chloride was added, or from the assumption that the activity ratios were equal to unity. The observed increase in partition coefficient for glycine–tyrosine suggests another mechanism. One should note that the two equations used in the prediction of partition coefficients of the dipeptides, Eqs. 12 and 14, contain no adjustable parameters. Eq. 12 contains exclusively measurable quantities: the pH and the equilibrium constants. Eq. 14 contains quantities that are determined independently (log P, log P_0, D) and one which is directly measured (Δw_2).

5. Conclusions

Solutes of different charge do not necessarily partition preferentially into different phases. Rather, changing the charge of a solute shifts the partition coefficient of the compound. The size of this shift depends on the pH difference between the phases and the dissociation constants for the two charged compounds. As Eq. 12 predicts for a system having a positive pH difference between the phases, a negatively charged solute has a greater partition coefficient than an otherwise identical neutral solute, which in turn is greater than that for a positively charged solute. The partition coefficients of solutes may be predicted by considering models which account for both a charge effect (as discussed here) and a hydrophobic effect (as discussed elsewhere).

Addition of an alkali metal halide to the PEG–phosphate phase system decreased the positive pH difference between the phases and, as the model predicted, decreased the partition coefficients of negatively charged compounds and increased the partition coefficients of positively charged compounds. Observed partition coefficients of glycine–tyrosine did not follow this prediction at high pH. The partition coefficient of this solute also was predicted and observed to shift from a value greater than unity to a value lower than unity as this salt was added. These results suggest that the proper selection of pH, phase system and salt concentration could greatly enhance the separation of solutes.

6. Acknowledgements

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