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A Model to Predict the Partition Coefficients of Amino Acids in PEG/Salt Aqueous Two-Phase Systems

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ABSTRACT

A mathematical model incorporating both the influence of pH and solute hydrophobicity is presented to describe the partitioning of charged compounds in aqueous two-phase systems. The model is applied to the partitioning of amino acids in PEG/potassium phosphate aqueous two-phase systems in which the pH lies between 6.1 and 11.9. The model predicts the minimum in the partition coefficient of several amino acids which is observed in phase systems of intermediate pH.

Key Words. Poly(ethylene glycol); Purification; Potassium phosphate; Partitioning; Charge; Amino acids

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INTRODUCTION

Aqueous two-phase systems are the biphasic solutions formed when two polymers such as poly(ethylene glycol) (PEG) and dextran, or a polymer and a salt, are dissolved together in water. Such systems offer the possibility of separating compounds in downstream processes because different solutes may distribute unequally between the phases. The partition coefficient of a solute in an aqueous two-phase system is defined as this solute's upper phase concentration divided by its lower phase concentration. In order to select the optimal possible 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 have been shown to depend on several factors including solute hydrophobicity (7, 17), molecular weight (4), temperature (3), pH (1, 6, 19), solute charge (12), and the presence of additional salts (5, 20).

Solute charge has clearly been identified as one important factor influencing the partition coefficient. For example, Reitherman et al. (14) measured an electric potential between phases and correlated the partitioning of negatively charged human erythrocytes with this difference in potential. Johansson (10) showed that the partitioning of proteins could be correlated with salt partitioning. Johansson (11) and Albertsson (2) developed equations to predict protein partition coefficients as functions of the protein's net charge and the difference in potential between the phases. However, the effects due to charge have been poorly understood. There is a need to quantify this charge effect in order to use its influence to modify partitioning and hence optimize separations.

Recently, Eiteman and Gainer (8) showed that a measured pH difference between the phases of an aqueous two-phase system has a predictable effect on the partition coefficients of charged solutes. One important recognition is that charged solutes generally occur as a distribution of individual, uniquely charged species. Each species is therefore influenced by the pH difference between the phases. 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 uncharged species. Specifically, the partition coefficient of a charged solute was shown to depend upon the partition coefficient of the uncharged species alone (or that of a hypothetical uncharged analog of the solute), the dissociation of the solute, and the pH in each phase.

Since the partition coefficient of a solute does not depend solely on charge effects, a predictive model incorporating other factors is needed to predict the partition coefficient of the uncharged species. The goal of this present study is to predict the partition coefficients of simple amino

acids by incorporating a previously-derived model which accounts for hydrophobic effects into the charge model.

MATHEMATICAL MODEL

The partition coefficient of a neutral solute, K_0, is defined as the concentration of that solute in the upper phase divided by the concentration of the solute in the lower phase:

$$K_0 = C_0'/C_0'' \tag{1}$$

In Eq. (1), the prime (') refers to the upper phase while a double prime (") refers to the lower phase. The subscript (0) denotes that the solute is uncharged.

The partition coefficient of an uncharged compound, K_0, has been shown [modified from Eiteman and Gainer (7)] to be related to the properties of the phase system and the solute by

$$RT \ln K_0 = b(\alpha_P + \Delta f)\Delta w_2 \tag{2}$$

where Δf is the solute hydrophobicity and Δw_2 is the PEG concentration difference between the phases, an approximation of the tie line length (7). The parameters b and α_P are assumed to be constants for a particular phase system (i.e., same components) at a given temperature. In this study these parameters will also be assumed to be unaffected by the pH of the phase system.

Equation (2) does not consider the effect of a solute's charge on its partitioning. In general, a solute may have up to m positive charges and n negative charges, and it exists in solution as a distribution of charged species depending on the pH of the solution. This distribution may be quantified at a given pH by the dissociation constants for the equilibria of the particular solute.

Naturally, the measured partition coefficient of a charged solute is related to the individual partitioning of each of the charged and uncharged species. A previously-derived relationship (8) expresses the ratio of the partition coefficient of a charged compound to the partition coefficient of its uncharged analog. The general partition ratio for a multicharged solute is

$$\frac{K}{K_0} = \frac{1 + \sum_{i=1}^{m} \Lambda_{i+}' \dfrac{a_{H^+}'^{\,i}}{\prod_{l=1}^{i} K_{bl}} + \sum_{j=1}^{n} \Lambda_{j-}' \dfrac{\prod_{l=1}^{j} K_{cl}}{a_{H^+}'^{\,j}}}{1 + \sum_{i=1}^{m} \Lambda_{i+}'' \dfrac{a_{H^+}''^{\,i}}{\prod_{l=1}^{i} K_{bl}} + \sum_{j=1}^{n} \Lambda_{j-}'' \dfrac{\prod_{l=1}^{j} K_{cl}}{a_{H^+}''^{\,j}}} \tag{3}$$

In Eq. (3), i refers to the equilibrium between a solute of i net positive charges and $i - 1$ net positive charges where $1 \leq i \leq m$. Similarly, j indicates the equilibrium between a solute of j net negative charges and $j - 1$ net negative charges with $1 \leq j \leq n$. The general dissociation constants for positively and negatively charged solutes are:

$$K_{bi} = \frac{a_{(i-1)^+} a_{H^+}}{a_{i^+}}, \qquad K_{cj} = \frac{a_{j^-} a_{H^+}}{a_{(j-1)^-}} \tag{4}$$

Subscripts b and c are reserved for dissociation constants of positively and negatively charged solutes, respectively. Also, a general activity ratio (Λ) has been defined in either phase as the activity coefficient of the neutral species divided by the activity coefficient of a species of charge q:

$$\Lambda_q = \gamma_0/\gamma_q \tag{5}$$

According to Eq. (3), the partition coefficient of any charged solute (all species existing in solution) depends on the partition coefficient of the neutral species, the pH in each phase, the activity ratio, and the dissociation constants for the solute. This general expression may be simplified greatly for many solutes. For example, $i = 1$ and $j = 1$ for a solute with one positive and one negative charge (e.g., many common amino acids). Setting the activity ratios (Λ) equal to unity and noting that $pX = -\log_{10} X$, Eq. (3) becomes

$$\frac{K}{K_0} = \frac{1 + \dfrac{a'_{H^+}}{K_{b1}} + \dfrac{K_{c1}}{a'_{H^+}}}{1 + \dfrac{a''_{H^+}}{K_{b1}} + \dfrac{K_{c1}}{a''_{H^+}}} = \frac{1 + 10^{(pK_{b1} - pH')} + 10^{(pH' - pK_{c1})}}{1 + 10^{(pK_{b1} - pH'')} + 10^{(pH'' - pK_{c1})}} \tag{6}$$

One method to estimate the partition coefficient of a charged solute such as an amino acid is to use Eq. (2) to estimate the partition coefficient of the uncharged species, K_0. With this value, Eq. (6) may then be used to estimate the partition coefficient of the charged solute, K.

In order to use Eq. (2) to account for the hydrophobic contribution to amino acid partitioning, values for α_P and b are needed. Equation (6) can then be used to account for charge contribution to the partitioning. If one assumes that the activity ratios are unity, Eq. (6) requires only the measurement of the pH in each phase since the dissociation constants for amino acids are known.

Fortunately, the two required parameters may be determined by a simple experiment. Equation (2) is readily normalized by adopting a hydrophobicity scale such that $\Delta f = 0$ for the simplest amino acid, glycine. Each member in a series of analogous amino acids, differing from glycine

only by chain length, would then have a hydrophobicity given by $n\Delta f_{CH_2}$, where n is the number of methylene groups on the amino acid, and Δf_{CH_2} is the hydrophobicity of one methylene group, assumed to have a value of 500 cal·mol^{-1} (13). If these amino acids are partitioned at their (nearly identical) isoelectric point, charge effects are removed since by Eq. (6), $K \approx K_0$. For these analogous amino acids, then, Eq. (2) becomes

$$RT \ln K_0 = b(\alpha_P + n\Delta f_{CH_2})\Delta w_2 \tag{7}$$

The procedure for determining the values of α_P and b is to partition the series of analogous amino acids in a phase system at their isoelectric point with any PEG concentration difference, denoted D. The logarithm of the observed partition coefficient of each of these amino acids is then plotted as a function of the chain length, n. The slope of this plot will provide the value of parameter b:

$$b = \frac{RT(\text{slope})}{\Delta f_{CH_2} D} \tag{8}$$

and the intercept of the ordinate will provide the value for the phase constant, α_P:

$$\alpha_P = \frac{RT(\text{intercept})}{bD} \tag{9}$$

If these two parameters are assumed to be constant for systems at other pH values, Eqs. (2) and (6) may be used to predict the partitioning of any amino acid.

MATERIALS AND METHODS

A series of six 0.95 M potassium phosphate solutions was prepared by combining stock aqueous solutions of phosphoric acid, potassium hydroxide, and a solution of each solute. One gram of poly(ethylene glycol) (PEG) having a molecular weight of 8000 was added to each 10 mL solution. The phases were placed at 35.0°C (± 0.1°C), thoroughly mixed for 2 days, allowed to equilibrate for 3 days, then carefully separated. The pH (8) and the PEG concentration (18) were determined in each phase of these systems.

The following amino acids were selected for partitioning studies: glycine, alanine, α-aminobutyric acid, *nor*-valine, *nor*-leucine, α-aminocaprylic acid, and glutamic acid (Sigma Chemical Co., St. Louis, Missouri). Phase systems were prepared such that the final concentration of each solute was 50 μM. Dissociation constants for the selected amino acids are provided in Table 1.

TABLE 1
Dissociation Constants for Amino Acids Selected for Partitioning Studies (9)

Amino acid	n	pK_{c2}	pK_{c1}	pK_{b1}
Glycine	0		2.34	9.60
Alanine	1		2.34	9.69
α-Aminobutyric acid	2		2.55	9.60
nor-Valine	3		2.30	9.78
nor-Leucine	4		2.39	9.76
α-Aminocaprylic acid	6		2.3	9.8
Glutamic acid		2.19	4.25	9.67

The partition coefficients of these amino acids were determined by high performance liquid chromatography (HPLC) using a modified ortho-phthalaldehyde (OPA) derivatization method (16). The HPLC system comprised a Waters gradient controller, UV detector model 481, and pumps model 510, with a Gilson model 231 sample injector and model 121 fluorometer, in addition to a Whatman C_{18} 5 μm Partisphere column (12.5 × 4 mm). Since the derivatization method requires a pH of 9.5–10.5, individual phases were diluted five- to tenfold with a 400-mM sodium borate buffer at pH 10.5 prior to analysis.

RESULTS AND DISCUSSION

Table 2 shows the properties determined which were later used to predict partition coefficients in the six phase systems. At 35.0°C, each system was observed to have 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.

TABLE 2
Properties of Six 0.95 M Potassium Phosphate Phase Systems Selected for Studying Amino Acid Partitioning

pH′	pH″	Δw_2
6.22	6.17	0.058
6.88	6.75	0.136
7.64	7.45	0.217
9.20	8.81	0.267
11.30	10.72	0.279
11.90	11.18	0.291

Before partition coefficients could be determined using Eqs. (2) and (6), values were required for the parameter b and the phase constant α_P. Both parameters were determined by partitioning analogous amino acids in an isoelectric phase system. The phase system listed in Table 2 having a lower phase pH of 6.17 was selected for this experiment, and Fig. 1 shows the results of partitioning six of the series of amino acids in this particular phase system, which had a PEG concentration difference (D in Eqs. 8 and 9) of 0.058. From the slope of these data, the value of parameter b was found to be 2.75. From the intercept of these data, the value of the phase constant was found to be -2650 cal·mol^{-1}. Throughout this study, these values were assumed to be constant for PEG/potassium phosphate systems having other pH values.

Equation (2) was then used to estimate the (theoretical) partition coefficient of the neutral amino acids in other phase systems at other pH values even though the solutes are not actually neutral at other pH values. With this calculated value of K_0, Eq. (6) could then be used to estimate the actual partition coefficient (i.e., including charge effects). Figures 2–7 show the observed and predicted partition coefficients of the six members in the analogous series of amino acids: glycine, alanine, α-aminobutyric acid, *nor*-valine, *nor*-leucine, and α-aminocaprylic acid. For the smaller five of these amino acids, the partition coefficients at first decreased with

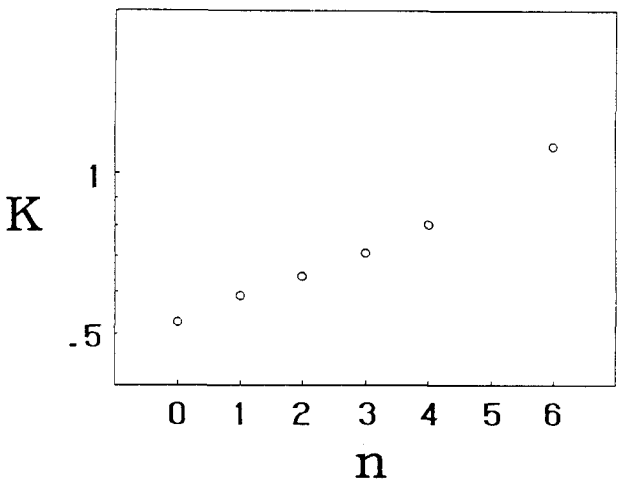

FIG. 1 Observed partition coefficients of an analogous series of amino acids (i.e., glycine, alanine, etc.) in an isoelectric PEG/potassium phosphate aqueous two-phase system at 35.0°C versus the number of methylene groups (n) on the amino acid.

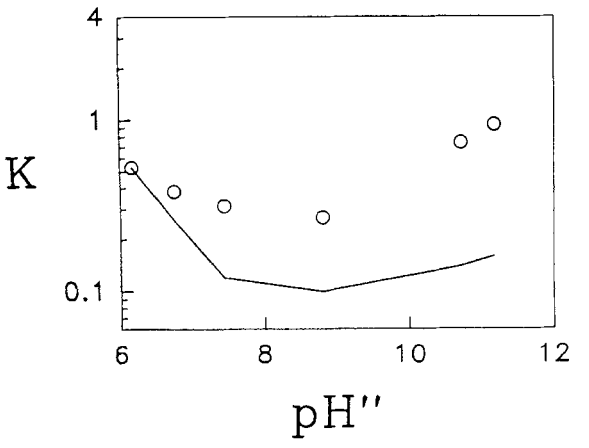

FIG. 2 Observed and predicted partition coefficients of glycine versus the lower phase pH in PEG/potassium phosphate aqueous two-phase system at 35.0°C.

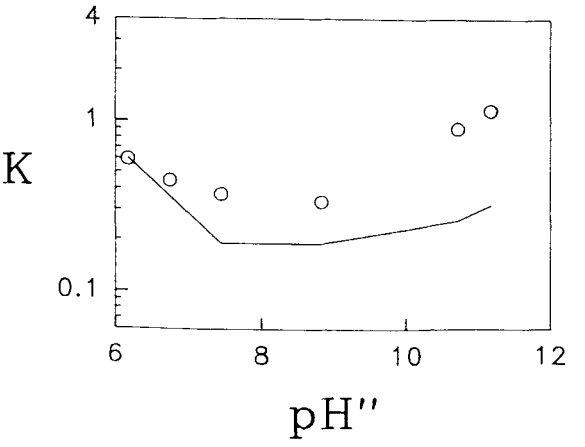

FIG. 3 Observed and predicted partition coefficients of alanine versus the lower phase pH in PEG/potassium phosphate aqueous two-phase systems at 35.0°C.

increasing pH, achieved a minimum, and then increased. The pH corresponding to this minimum was greatest for glycine (about 9.0) and decreased slightly with increasing chain length. Moreover, the rate of initial decline in the partition coefficient with pH decreased with increasing chain length. The observed partition coefficient of glycine decreased from 0.53

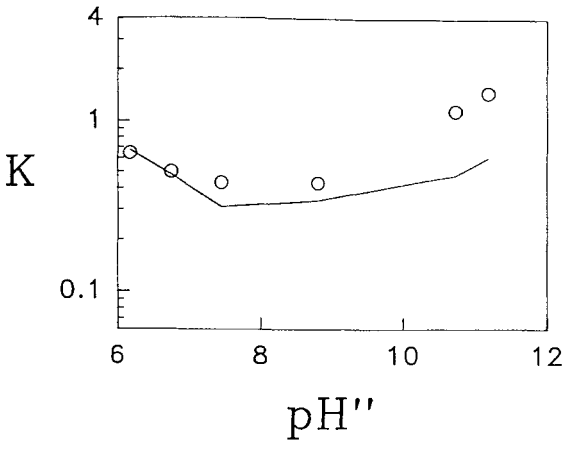

FIG. 4 Observed and predicted partition coefficients of α-aminobutyric acid versus the lower phase pH in PEG/potassium phosphate aqueous two-phase systems at 35.0°C.

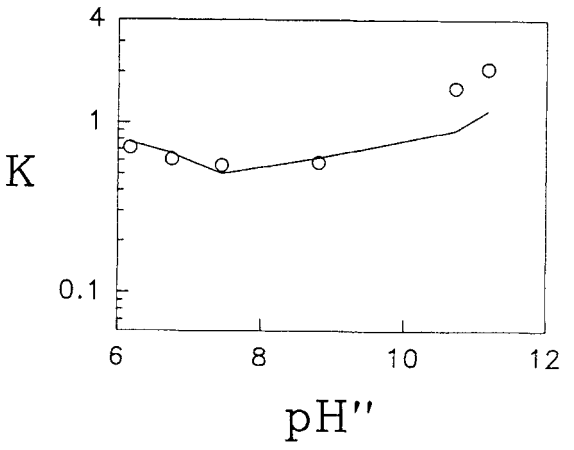

FIG. 5 Observed and predicted partition coefficients of *nor*-valine versus the lower phase pH in PEG/potassium phosphate aqueous two-phase systems at 35.0°C.

to 0.27, while the partition coefficient of *nor*-leucine decreased only from 0.81 to 0.75. The largest amino acid studied, α-aminocaprylic acid, did not exhibit a decrease in the observed partition coefficient.

The model (depicted by the solid line in each figure) predicted minima in the partition coefficients for the five smaller amino acids. It correctly

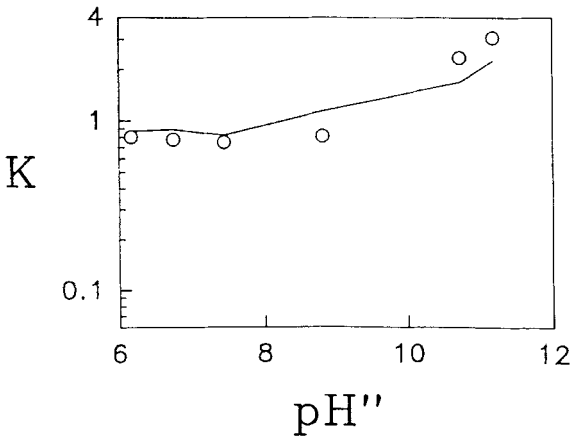

FIG. 6 Observed and predicted partition coefficients of *nor*-leucine versus the lower phase pH in PEG/potassium phosphate aqueous two-phase systems at 35.0°C.

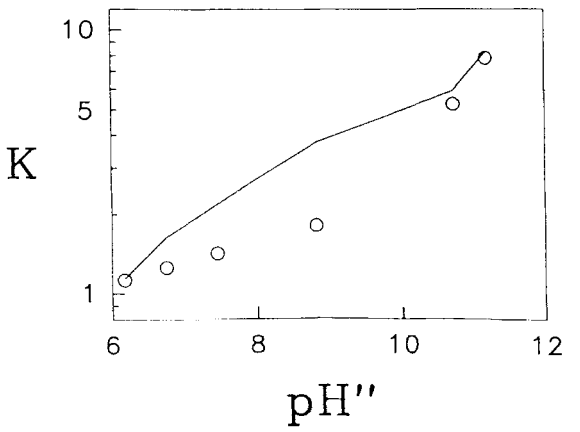

FIG. 7 Observed and predicted partition coefficients of α-aminocaprylic acid versus the lower phase pH in PEG/potassium phosphate aqueous two-phase systems at 35.0°C.

predicted that the minimum for glycine should be at a higher pH than the minimum for *nor*-leucine. The figures indicate that the rate of initial decrease for glycine should be greater than the decrease for *nor*-leucine. While the general shape of the curves differ, the model does predict that α-aminocaprylic acid should not exhibit a minimum in its partition coeffi-

cient with increasing pH. The prediction is in agreement with the observation that the order of the partition coefficients was from glycine to α-aminocaprylic acid. Although the model was less successful at predicting the exact value of the partition coefficients, particularly for glycine and alanine, the trend of the predictions agreed with all the observations.

To determine if the model was applicable to other amino acids not in the analogous series, glutamic acid was selected for study. In order to predict the partition coefficient of the neutral species by Eq. (2), a value is needed for the relative hydrophobicity of this amino acid. Relative to glycine, the hydrophobicity of glutamic acid was calculated to be -80 cal·mol^{-1} (15). This is the only new value required to predict the partition coefficients using Eq. (3). Figure 8 shows the observed and predicted partition coefficients of glutamic acid in the six PEG/phosphate phase systems. The model agreed with the observations that this solute would partition predominantly into the lower phase, and that a minimum in the partition coefficient would occur at intermediate pH. The fact that the actual observed values increasingly deviated from the predicted values may in large part be due to the assumptions that the phase constant and parameter b were constant over the pH range studied.

The model predictions required only the estimated hydrophobicity of the neutral amino acid, the measured pH difference and PEG concentration difference between the phases, and the dissociation constants of each amino acid. The major and limiting assumption in the model was that the

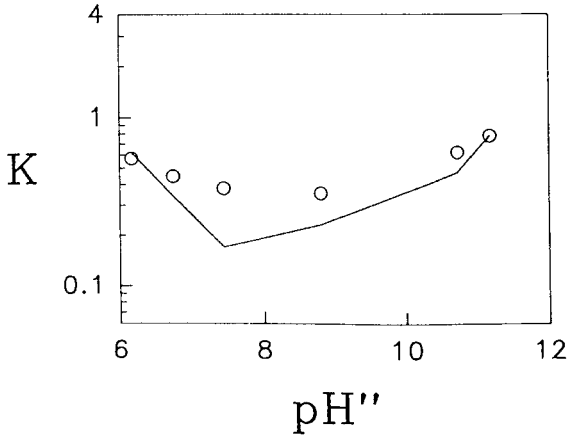

FIG. 8 Observed and predicted partition coefficients of glutamic acid versus the lower phase pH in PEG/potassium phosphate aqueous two-phase systems at 35.0°C.

phase constant and parameter b were constant for all phase systems studied. These constants might be determined at pH values for which amino acids are charged by partitioning an analogous series of neutral compounds such as normal alcohols in the studied systems.

CONCLUSIONS

Despite the several assumptions made in using Eqs. (2) and (3), the measured partition coefficients of several amino acids where shown to agree qualitatively with model predictions. These equations provide a quick means to estimate the partition coefficients of small charged compounds. The equations also appear to be particularly useful in answering some fundamental questions such as to which phase a solute predominantly partitions, and how the pH should be altered to affect the partition coefficient. Additional studies are needed to determine if the model is suitable for use with other amino acids and more complicated solutes such as peptides and proteins.

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