Amino-Acid Extraction Using D2EHPA: New Description of Equilibrium Behavior

G. L. Tulasi and Sanjeev Kumar
Dept. of Chemical Engineering, Indian Institute of Science, Bangalore 560 012, India

A new reaction mechanism to explain the equilibrium behavior of amino-acid extraction using di(2-ethylhexyl)phosphoric acid (D2EHPA) is proposed. This mechanism represents the interaction between amino acid and D2EHPA through a set of two reactions, one at the aqueous–organic interface and the other in the bulk of the organic phase. It quantitatively predicts the hitherto unexplained dependence of apparent equilibrium constant on a single parameter — loading ratio (ratio of concentration of amino acid in the organic phase at equilibrium to initial concentration of carrier). At low loading ratios, reaction in the bulk plays an insignificant role, and interfacial reaction alone can explain the equilibrium behavior. At high loading ratios, both reactions need to be considered. The new mechanism is tested for the extraction of two amino acids, phenylalanine and tryptophan, for which the equilibrium data have been published. New data were also obtained for phenylalanine to test it over a much wider range of parameter values. The proposed mechanism can predict all the data very well.

Introduction

Recent advances in membrane technology have spurred interest in membrane-based separations, as they offer many advantages over the conventional separation processes. Increased interfacial area provided by porous walls of hollow fibers allows gas treatment to be carried out ten times faster than that in packed towers (Zhang and Cussler, 1985). Similarly, liquid–liquid extraction in hollow-fiber modules (HFM) can be over one hundred times faster than that in spray towers (Kiani et al., 1984; Kim, 1984).

More interesting applications of membranes, however, come about when an organic liquid containing carrier molecules is used as membrane (Cussler and Evans, 1980). The solute, if insoluble in the organic phase, can be transported across the membrane, from feed side to the strip side, only through formation of a complex with carrier molecules dissolved in the organic phase. A judicious choice of carrier molecules can thus result in (1) selective separation of the desired species from a mixture, and/or (2) transport of species against their concentration gradient.

Di(2-ethylhexyl)phosphoric acid (D2EHPA) is a well-known carrier for the extraction of metals ions (Huang and Juang, 1986). Recently, D2EHPA also has been found to be a good carrier for the extraction of amino acids (Itoh et al., 1990; Teramoto et al., 1991; Hong and Yang, 1994; Chan and Yang, 1995; Shi et al., 1997). Separation and concentration of amino acids from aqueous solutions and fermentation broths using HFM thus holds potential for commercialization. A first step in this direction is taken by knowing the maximum extraction that can be affected. In other words, an understanding of the equilibrium behavior of the reaction between amino acids and the D2EHPA carrier is needed.

The objective of this article is to provide a quantitative understanding of the equilibrium behavior of amino-acid–D2EHPA systems, as the previous efforts made in this direction (Itoh et al., 1990; Teramoto et al., 1991; Shi et al., 1997) do not explain the observed behavior.

Previous Work

Extraction of amino acids using D2EHPA has been studied by many investigators in the literature; however, only a few studies have appeared on the equilibrium behavior of the reaction between amino acid and D2EHPA. The two commonly investigated amino acids in these studies are phenyl-

Correspondence concerning this article should be addressed to S. Kumar.

alanine and tryptophan (Itoh et al., 1990; Teramoto et al., 1991; Shi et al., 1997). In this section, we review the current understanding of the equilibrium behavior of the amino-acid–D2EHPA system, with phenylalanine as a representative amino acid.

In experimental studies on equilibrium behavior, an aqueous solution of phenylalanine (Phe) is brought in contact with an organic solvent having carrier D2EHPA dissolved in it. Extraction of Phe into the organic phase containing D2EHPA occurs only in a certain range of pH. The reason for this behavior is that phenylalanine, like other amino acids, exists in different forms depending on the pH of the aqueous solution—cationic form Phe^+, zwitterionic form $Phe^{\pm}$, and the anionic form Phe^-—and it's only the cationic form that can be extracted using D2EHPA. The equilibrium between the three forms can be represented as

$$Phe^+ \overset{K_p}{\rightleftharpoons} Phe^{\pm} + H^+ \tag{1}$$

$$Phe^{\pm} \overset{K'_p}{\rightleftharpoons} Phe^- + H^+ \tag{2}$$

$$K_p = \frac{[Phe^{\pm}][H^+]}{[Phe^+]} \tag{3}$$

$$K'_p = \frac{[Phe^-][H^+]}{[Phe^{\pm}]}, \tag{4}$$

where K_p and K'_p are dissociation constants for phenylalanine ($K_p = 1.479 \times 10^{-2}$ M and $K'_p = 7.413 \times 10^{-10}$ M; Lehninger, 1993).

As the phenylalanine present in the aqueous phase is insoluble in the organic phase and the aqueous solubility of D2EHPA is very low, the reaction between Phe cation and D2EHPA takes place at the aqueous–organic interface. Itoh et al. (1990) have proposed the following reaction mechanism

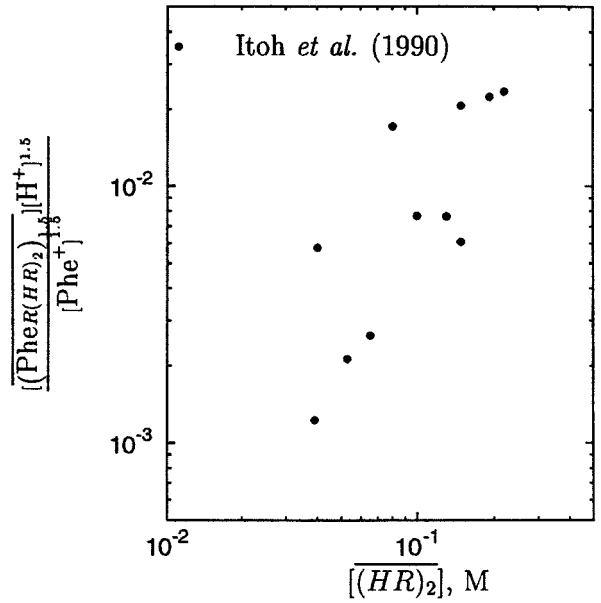

Figure 1. Test of the reaction mechanism proposed by Itoh et al. (1990).

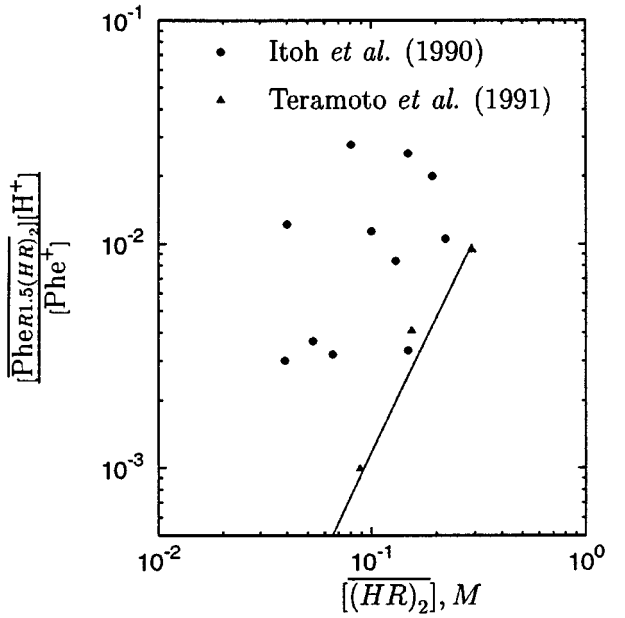

Figure 2. Test of the reaction mechanism proposed by Teramoto et al. (1991).

for the reaction on the interface:

$$1.5\,Phe^+ + 2.25\overline{(HR)_2} \rightleftharpoons \overline{[Phe\,R(HR)_2]_{1.5}} + 1.5\,H^+. \tag{5}$$

The overbar indicates that the species is present in the organic phase. $\overline{(HR)_2}$ is the dimer of D2EHPA, a form in which it is reported to exist in the nonpolar organic phases (Huang and Juang, 1986). The concentration of complex $[\overline{(Phe\,R(HR)_2)_{1.5}}]$ cannot be measured, but it is related to the concentration of amino acid in the organic phase, $[\overline{(Phe)}_{org}]$, which can be obtained by making a mass balance on the amino acid present in the system. Thus, according to this reaction mechanism, a plot of $\log[\overline{(Phe\,R(HR)_2)_{1.5}}]$ $([H^+]/[Phe^+])^{1.5}$ vs. $\log[\overline{(HR)_2}]$ should yield a straight line. Figure 1 shows a plot for the data reported by Itoh et al. (in Figure 11 of their paper). The figure shows that the mechanism proposed by the authors fails to explain their experimental data.

Teramoto et al. (1991) have proposed a different reaction mechanism:

$$Phe^+ + 2\overline{(HR)_2} \rightleftharpoons \overline{Phe\,R1.5(HR)_2} + H^+. \tag{6}$$

According to this mechanism, a plot of $\log[\overline{Phe\,R1.5(HR)_2}]$ $[H^+]/[Phe^+]$ vs. $\log[\overline{(HR)_2}]$ should result in a straight line. Figure 2 shows a plot for the data produced by Itoh et al. and Teramoto et al. The figure shows that the experimental data of Itoh et al. is described poorly by this mechanism. The experimental data of Teramoto et al., however, falls on a straight line. The significant deviation observed for one data point is attributed to scatter in the experimental data, as their mechanism is otherwise able to explain most of their data very well. The best-fit value of equilibrium constant (K_{eq}) for their data, as reported by them, is 0.117 dm^3/mol.

It is interesting to note that the enormous difference between the two sets of experimental data shown in Figure 2 is caused by the different ranges of amino-acid concentrations employed by the two groups of investigators; Teramoto et al. have used very low concentrations of amino acids.

Figures 1 and 2 clearly show that both mechanisms proposed in the literature fail to explain the available data. Interestingly, the same data, when plotted as apparent equilibrium constant, K_a, vs. loading ratio, L, falls on a single curve (the details are provided later) irrespective of the values of other parameters such as initial carrier and amino-acid concentrations. The dependence of K_a on L alone has been known for metals and amino-acid–D2EHPA systems (Komasawa et al., 1981; Huang and Juang, 1986; Shi et al., 1997), but a quantitative explanation is still not available.

In this article, we propose a new reaction mechanism involving two reaction steps to quantitatively explain the equilibrium behavior of amino-acid–D2EHPA reaction systems. The proposed mechanism also explains the hitherto unexplained dependence of apparent equilibrium constant K_a on the loading ratio alone.

Proposed Mechanism

We consider a system consisting of an aqueous phase containing an amino acid, and an organic phase containing the carrier D2EHPA. The amino acid has negligible solubility in the organic phase and can get extracted into the organic phase only by reaction with D2EHPA. Since the solubility of D2EHPA in the aqueous phase is also very low, D2EHPA and the amino acid can react to form a complex only through an interfacial reaction on the aqueous–organic interface. This complex, denoted as $\overline{C}$, then goes into the bulk of the organic phase. Previous studies (Itoh et al., 1990; Teramoto et al., 1991) have considered that this reaction only occur in the system, and it is shown that their mechanisms are not able to explain the equilibrium behavior of amino-acid extraction using D2EHPA.

We propose that complex $\overline{C}$ can undergo further reaction in the bulk of the organic phase by combining with another molecule of $\overline{C}$ to make a larger complex $\overline{D}$. A free-carrier molecule is released in the process. The release of a carrier molecule implies that the number of carrier molecules per molecule of amino acid is less in $\overline{D}$ than in $\overline{C}$. Thus, more amino acid can enter the organic phase for the same concentration of the carrier via the formation of complex $\overline{C}$ on the interface. As the proposed reaction is second order with respect to $\overline{C}$, at a low concentration of $\overline{C}$ ($[\overline{C}] \to 0$), the reaction in the bulk does not affect the equilibrium behavior. The experimental data of Teramoto et al. (1991) that corresponds to the same limit ($[\overline{C}] \to 0$) was explained very well by their reaction mechanism (Eq. 6). We have therefore retained their reaction as the interfacial reaction in the set of reactions that we propose for a complete description. The proposed reactions are:

At the interface:

$$\mathrm{Phe}^+ + 2\overline{(HR)_2} \overset{K_{e1}}{\rightleftharpoons} \overline{\mathrm{Phe}\,R1.5(HR)_2} + H^+; \qquad (7)$$

In the bulk of organic phase:

$$2\overline{\mathrm{Phe}\,R1.5(HR)_2} \overset{K_{e2}}{\rightleftharpoons} \overline{[\mathrm{Phe}\,R(HR)_2]_2} + \overline{(HR)_2}. \qquad (8)$$

The complexes $\overline{C}$ and $\overline{D}$ are $\overline{\mathrm{Phe}\,R1.5(HR)_2}$ and $\overline{[\mathrm{Phe}\,R(HR)_2]_2}$, respectively. Representing amino acid Phe^+ by A^+ for generality, and the free carrier $\overline{(HR)_2}$ by $\overline{B}$, the equilibrium constants K_{e1} and K_{e2} can be defined in terms of the concentration of various species at equilibrium as:

$$K_{e1} = \frac{[\overline{C}][H^+]}{[A^+][\overline{B}]^2} \qquad (9)$$

$$K_{e2} = \frac{[\overline{D}][\overline{B}]}{[\overline{C}]^2}. \qquad (10)$$

Teramoto et al. (1991) have reported the value of equilibrium constant K_{e1} to be 0.117 $\mathrm{dm^3/mol}$. The value of K_{e2} needs to be determined.

If the reaction mechanism proposed in Eqs. 7 and 8 is valid, the value of K_{e2} could be obtained by plotting $\log[\overline{C}]$ vs. $\log([\overline{D}]\cdot[\overline{B}])$. The data points should fall on a straight line with a slope of 0.5. The intercept will then yield the equilibrium constant K_{e2}. Unfortunately, concentrations of species $\overline{C}$, $\overline{D}$, and $\overline{B}$ cannot be measured experimentally.

The following strategy was adopted to test the validity of the proposed mechanism and estimation of K_{e2}. In a typical experiment, a known volume of aqueous phase V_{aq}, with a known initial concentration of amino acid $[A]_i$, is contacted with an organic phase of known volume V_{org} with a known initial carrier concentration $[\overline{B}]_i$. After equilibrium has been reached, the final concentration of the amino acid present in all forms, $[A]$, and the pH of the aqueous phase are measured. Only the cationic form of amino acid reacts with D2EHPA and its concentration can be obtained from the measured concentration of amino acid, $[A]$. When the hydrogen-ion concentration is much higher than the dissociation constant K'_p, the formation of phenylalanine anion can be neglected. A relationship between the concentration of the cationic form of amino acid and the concentration of the amino acid present in all forms can then be obtained from Eq. 3 and the mass balance of amino acid present in all forms:

$$\frac{[A^+]}{[H^+]} = \frac{[A]}{K_p + [H^+]}. \qquad (11)$$

According to the proposed mechanism, amino acid in the organic phase, $\overline{A}_{\mathrm{org}}$, is present in the form of complexes $\overline{C}$ and $\overline{D}$. Hence, a mass balance for amino acid requires that

$$V_{\mathrm{aq}}([A]_i - [A]) = V_{\mathrm{org}}[\overline{A}_{\mathrm{org}}] = V_{\mathrm{org}}([\overline{C}] + 2[\overline{D}]). \qquad (12)$$

A similar mass balance for the carrier requires

$$[\overline{B}]_i = [\overline{B}] + 2[\overline{C}] + 3[\overline{D}], \qquad (13)$$

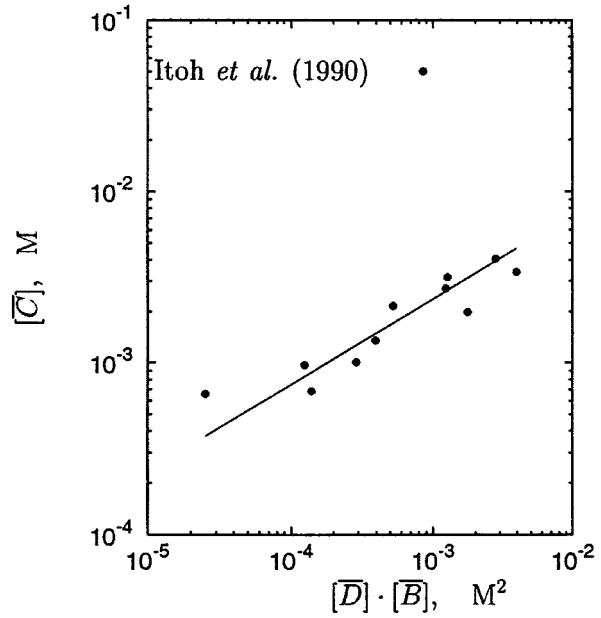

Figure 3. Estimation of equilibrium constant K_{e2}.

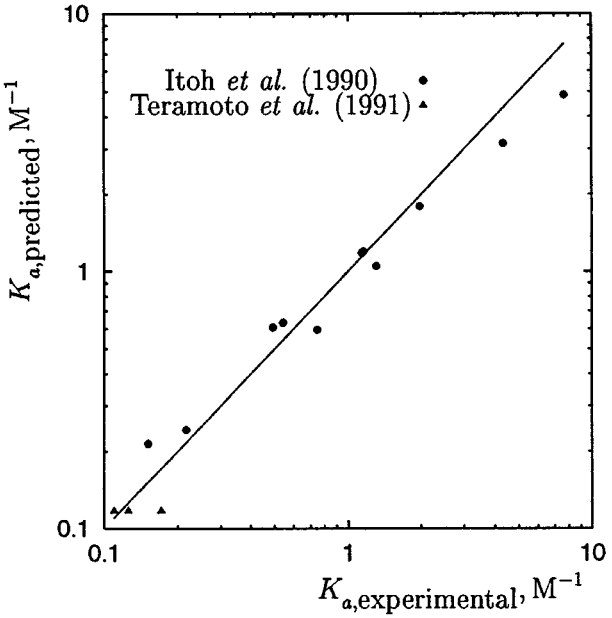

Figure 4. Experimentally obtained apparent equilibrium constant K_a vs. predicted values for the data reported in the literature.

where $[\bar{B}]$ is the concentration of the free carrier at equilibrium. The coupled nonlinear equations (Eqs. 9 and 11–13) are solved using Newton's method to obtain the unknowns $[\bar{C}]$, $[\bar{D}]$, and $[\bar{B}]$. A plot of $[\bar{C}]$ vs. $[\bar{D}]\cdot[\bar{B}]$ for the data of Itoh et al. (1990) is shown in Figure 3. The figure shows that the experimental data fall on a straight line with a slope of 0.5. The intercept of the best-fit line passing through these data points yields a value of 180 for equilibrium constant (K_{e2}) for extraction of Phe using D2EHPA.

In order to test the proposed mechanism, we represent the reaction system using just the interfacial reaction (Eq. 7) and let it be characterized by an apparent equilibrium constant K_a, instead of the true equilibrium constant K_{e1}. In contrast with K_{e1}, which is independent of the concentration of the species participating in the reaction, K_a, which is not a true equilibrium constant, is expected to depend on them. The apparent equilibrium constant K_a is thus defined as

$$K_a = \frac{[\bar{C}]_a[H^+]}{[A^+][\bar{B}]_a^2} = \frac{[\bar{A}_{\text{org}}][H^+]}{[A^+][\bar{B}]_a^2}. \qquad (14)$$

According to this definition, the amino acid transferred from the aqueous side to the organic side is expected to appear as complex $\bar{C}$ alone. Hence,

$$V_{\text{org}}[\bar{C}]_a = V_{\text{org}}[\bar{A}_{\text{org}}] = V_{\text{aq}}([A]_i - [A]) \qquad (15)$$

and

$$V_{\text{org}}[\bar{B}]_a = V_{\text{org}}[\bar{B}]_i - 2V_{\text{aq}}([A]_i - [A]). \qquad (16)$$

The experimentally observed K_a can now be obtained by solving Eqs. 11–16. The model-predicted K_a values are obtained in an identical manner by replacing the experimentally measured value of $[A^+]/[H^+]$, with the model-predicted one. The predicted values of $[A^+]/[H^+]$ were obtained by solving Eqs. 9–13 simultaneously for four unknowns $[A^+]/[H^+]$, $[\bar{B}]$, $[\bar{C}]$, and $[\bar{D}]$.

Figure 4 shows a comparison of K_a values obtained for the experimental data of Itoh et al. (1990) and Teramoto et al. (1991) with the theoretical predictions of the proposed mechanism. The figure shows that the experimental data are predicted well. The agreement between the predicted values and the data of Teramoto et al. appears to be poor. This, however, is misleading. The mechanism proposed by Teramoto et al. explains most of their data, which is obtained for $L \to 0$. The proposed mechanism will also explain all their data, as in the limit $L \to 0$ it reduces to the mechanism of Teramoto et al.

Dependence of K_a on L

As discussed in the previous section, the apparent equilibrium constant K_a can be a function of concentrations of all the species involved in the reaction. The literature on metal extraction, however, shows that K_a is a function of only loading ratio L (Komasawa et al., 1981; Komasawa and Otake, 1983; Li et al., 1986; Huang and Juang, 1986), which for the amino-acid–D2EHPA system is defined as

$$L = \frac{[\bar{A}_{\text{org}}]}{[\bar{B}]_i}. \qquad (17)$$

Shi et al. (1997) have shown that the dependence of K_a on L alone holds for amino acid (tryptophan)-D2EHPA as well. We have plotted the data available for the Phe–D2EHPA system using the same procedure. The data points in Figure 5 show that for the Phe–D2EHPA system also K_a is a function of L alone.

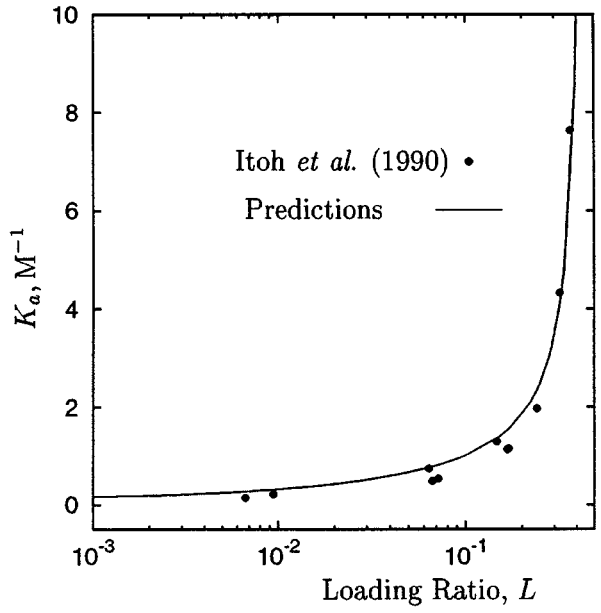

Figure 5. Apparent equilibrium constant K_a vs. loading ratio L for the phenylalanine-D2EHPA system for the data of Itoh et al. (1990).

Although the observed dependence of K_a on L alone has been used by many investigators to show the limit of the validity of their equilibrium reactions, no attempt is made in the literature to explain why the apparent equilibrium constant should depend on just one parameter. In the following, we show that the mechanism that we proposed quantitatively explains the dependence of K_a on L alone.

Following Eqs. 7 and 8, which contain the reaction mechanism, the amino acid in organic phase is present in two forms: complex $\overline{C}$ and complex $\overline{D}$. The total amino acid in the organic phase is therefore given as

$$\left[\overline{A}_{\text{org}}\right] = \overline{C} + 2\overline{D}. \tag{18}$$

Because concentrations $[\overline{\text{C}}]$ and $[\overline{\text{D}}]$ cannot be measured experimentally, they are eliminated using equilibrium relations defined through Eqs. 9 and 10. Thus,

$$\frac{\left[\overline{A}_{\text{org}}\right][H^+]}{[A^+]} = K_{e1}[\overline{B}]^2 + 2K_{e2}K_{e1}^2\frac{[A^+]}{[H^+]}[\overline{B}]^3. \tag{19}$$

Using Eqs. 14, 15, 16, 17 and 19,

$$K_a = \frac{K_{e1}r^2}{(1-2L)^2} + \frac{2K_{e2}K_{e1}^2Lr^3}{K_a(1-2L)^4}, \tag{20}$$

where r is the ratio of concentration of D2EHPA at equilibrium to that taken initially:

$$r = \frac{[\overline{B}]}{[\overline{B}]_i}. \tag{21}$$

A mass balance on carrier molecules yields

$$[\overline{B}]_i = [\overline{B}] + 2[\overline{C}] + 3[\overline{D}], \tag{22}$$

which can be used to obtain r as

$$r = 1 - \frac{2K_{e1}Lr^2}{(1-2L)^2K_a} - \frac{3K_{e2}K_{e1}^2r^3L^2}{K_a^2(1-2L)^4}. \tag{23}$$

Since the values of equilibrium constants K_{e1} and K_{e2} are already obtained for the Phe−D2EHPA system, Eqs. 20 and 23 can be solved simultaneously to obtain the unique relationship between K_a and L. These predictions are shown by the solid curve in Figure 5. It shows that the proposed mechanism quantitatively explains the observed dependence of K_a on L alone.

At low values of the loading ratio, K_a approaches a constant value. As argued before, this is because the second reaction is second order (Eq. 8) with respect to $\overline{C}$. At low values of L, the concentration of $\overline{C}$ remains very low, hence the second reaction has almost zero yield. The interfacial reaction (Eq. 7) plays a dominant role, and therefore K_a reduces to K_{e1}. As loading ratio increases, the second reaction becomes increasingly important, and more and more carrier is released for further extraction of the amino acid. The apparent equilibrium constant therefore becomes larger than K_{e1}; in fact, at high loading ratios, most of the phenylalanine is present in the form of complex $\overline{D}$.

Itoh et al. (1990) have conducted their experiments for a somewhat narrow range of parameters: initial carrier concentration was varied between 0.075 and 0.225 M, and initial phenylalanine concentration was varied between 0.006 and 0.12 M. In all their experiments, the starting hydrogen concentration was kept at 0.001 M, for which the equilibrium $[\text{H}^+]$ is expected to lie in the range $0.081-10^{-3}$ M.

To test the proposed mechanism over a wider range of parameters, we have conducted new shake-flask experiments. The concentration of $[\text{H}^+]$ at equilibrium in our experiments was varied over a large range of 2.2×10^{-5} to 0.316 M. The initial concentrations of carrier and phenylalanine were varied in ranges 0.012−0.737 M and 0.05−0.45 M, respectively. The materials used in experiments are as follows: D2EHPA of purity 98% was obtained from Merck; phenylalanine with a purity of 98% was obtained from Sigma Chemicals; *n*-heptane (diluent) used was of analytical reagent grade. The phenylalanine concentration in the aqueous phase was analyzed by UV-spectrophotometer (Shimadzu) at a wavelength of 257.5 nm. The hydrogen-ion concentration was measured by using an Orion Ion Analyser. The experimental procedure followed was similar to that given by Itoh et al. (1990), with minor variations that can be found elsewhere (Tulasi, 1999). Figure 6 shows our experimental data on a K_a vs. L plot along with the predictions of the mechanism proposed by us. The largest value of K_a obtained in the present work is ~ 90 $(\text{mol/dm}^3)^{-1}$ (shown in the inset), which is ten times greater than ~ 8 $(\text{mol/dm}^3)^{-1}$, the largest value for the data of Itoh et al. (1990). The figure shows that the agreement between the theoretical predictions and the experimental data for the expanded parameter range investigated in this work is also very good.

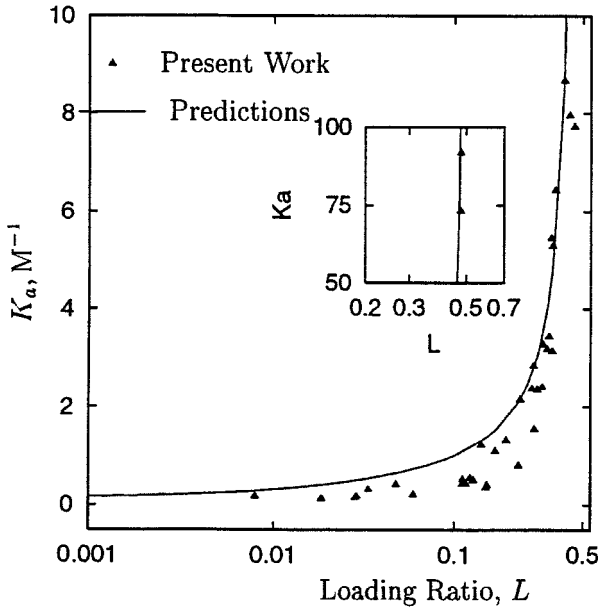

Figure 6. Apparent equilibrium constant K_a vs. loading ratio L for the phenylalanine-D2EHPA system for the data obtained in the present work.

Inset shows very high values of K_a that could be obtained in the present work and the predictions.

Tryptophan D2EHPA System

Shi et al. (1997) have reported experimental data for low to high loading ratios for tryptophan (Trp)-D2EHPA system. These data points have been replotted here in Figure 7. The mechanism proposed by us is readily extended to this reaction system as well. Trp reacts on the interface to form a complex similar to complex $\overline{C}$ as discussed in the case of the Phe−D2EHPA system. The complex formed on the interface undergoes further reaction in the bulk to form a bigger complex like the complex $\overline{D}$.

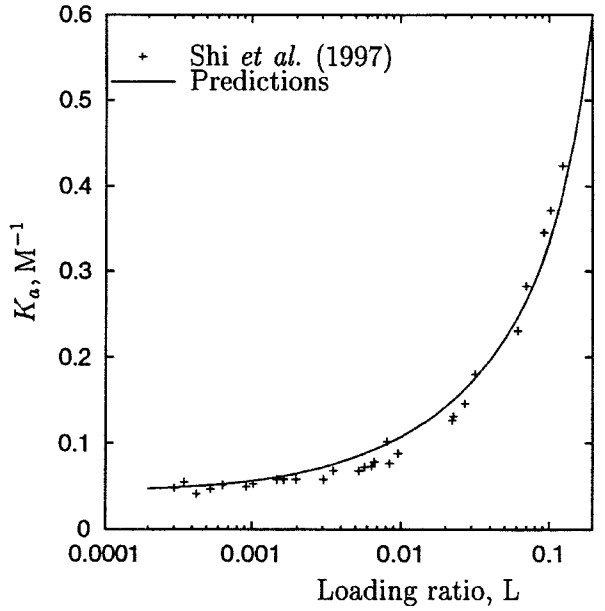

Figure 7. Apparent equilibrium constant K_a vs. loading ratio L for tryptophan-D2EHPA system.

To make predictions for this system using the mechanism proposed in this work, information on interfacial reaction is needed. As discussed before, this information can be obtained through experimental data at very low loading ratios. Teramoto et al. (1991) and Shi et al. (1997) have already reported that one mole of Trp reacts with two moles of dimer of D2EHPA at the interface. The equilibrium constant for this reaction is reported to be 0.045 dm^3/mol by Shi et al. (1997). The value of K_{e1} for this system is therefore taken to be 0.045 dm^3/mol.

The value of equilibrium constant K_{e2} is obtained as the best fit value for K_a vs. L data shown in Figure 7. The best-fit value is found to be 160. The predicted values of K_a corresponding to this value of K_{e2} are shown by the solid line. The figure shows that the proposed mechanism is able to explain the entire set of experimental data very well.

Discussion

The apparent equilibrium constant K_a, defined by Eq. 20, is valid up to a loading ratio of 0.5. Since the stoichiometric coefficient for both the amino acids is 2 for the interfacial reaction, K_a becomes infinity at a loading ratio of 0.5; the definition of K_a does not hold for $L > 0.5$. For $L > 0.5$, which are allowed by the proposed mechanism, the data cannot be represented on K_a vs. L plots; the proposed mechanism can, however, be tested against the experimental data by comparing the measured concentration of amino acid at equilibrium with the predicted values.

The mechanism proposed by us envisages formation of larger aggregates at higher loading ratios through reaction in the bulk. Formation of larger aggregates at high loading ratios has also been suggested by Huang and Juang (1986) and Li et al. (1986) in the extraction of metal ions. Shi et al. (1997) have, however, attempted a qualitative explanation based on the breakdown of the complex of type C into smaller components at high loading ratios. The sequence of reactions proposed by them for the Trp−D2EHPA system are

$$A^+ + 2\overline{(HR)_2} \overset{K_{ex1}}{\rightleftharpoons} \overline{AR1.5(HR)_2} + H^+ \tag{24}$$

$$A^+ + \overline{AR1.5(HR)_2} \overset{K_{ex2}}{\rightleftharpoons} 2\overline{ARHR} + H^+ \tag{25}$$

$$A^+ + \overline{ARHR} \overset{K_{ex3}}{\rightleftharpoons} 2\overline{AR} + H^+. \tag{26}$$

Their expression for apparent equilibrium constant in terms of the equilibrium constants for the proposed reactions is reproduced here:

$$K_a = K_{ex1} + \frac{(K_{ex1}K_{ex2})^{1/2}}{[(HR)_2]} + \frac{K_{ex3}^{1/2}(K_{ex1}K_{ex2})^{1/4}}{\left[\overline{(HR)_2}\right]^{3/2}}. \tag{27}$$

As the concentration of $\overline{(HR)_2}$ at equilibrium decreases with increasing loading ratio L, they concluded that the preceding equation qualitatively explains the increase in K_a at increas-

ing values of L. The values of K_{ex2} and K_{ex3} were not estimated and no quantitative comparisons were made.

We have found that this mechanism cannot explain the dependence of K_a on just one parameter—loading ratio L, as it predicts K_a to be a function of initial D2EHPA and amino-acid concentrations as well. Furthermore, in obtaining the preceding expression, Shi et al. (1997) have incorrectly taken $[\overline{(HR)_2}]$ to be $[\overline{(HR)_2}]_i - 2[\bar{A}_{org}]$. Correcting for this, Eq. 27 modifies to

$$K_a = \frac{K_{ex1}\left[\overline{(HR)_2}\right]^2}{\left[\overline{(HR)_2}\right]_i^2(1-2L)^2} + \frac{K_{ex2}K_{ex1}^{1/2}\left[\overline{(HR)_2}\right]}{\left[\overline{(HR)_2}\right]_i^2(1-2L)^2} + \frac{K_{ex3}^{1/2}(K_{ex1}K_{ex2})^{1/4}\left[\overline{(HR)_2}\right]^{1/2}}{\left[\overline{(HR)_2}\right]_i^2(1-2L)^2}. \quad (28)$$

As $L \to 0$, the reactions represented through Eqs. 25 and 26 should not play a role, as they are projected to become operative only at high loading ratios. The apparent rate constant K_a is therefore expected to approach K_{ex1}, the equilibrium constant for the first reaction (Eq. 24). On the contrary, Eq. 28, in the limit of $L \to 0$, reduces to

$$K_a = K_{ex1} + \frac{K_{ex2}K_{ex1}^{1/2}}{\left[\overline{(HR)_2}\right]_i} + \frac{K_{ex3}^{1/2}(K_{ex1}K_{ex2})^{1/4}}{\left[\overline{(HR)_2}\right]_i^{3/2}}, \quad (29)$$

which shows that (1) K_a does not approach K_{ex1}, but instead depends on equilibrium constants for breakage of large complexes, and (2) K_a also depends on the initial carrier concentration. The latter is not in agreement with the experimental data of Teramoto et al. (1991), which was plotted earlier in Figure 2. Their data show that in the limit of the low loading ratio, K_a does not depend on initial carrier concentration. In fact, Teramoto et al. (1991) could obtain an equilibrium constant for their reaction only for the reason just given. The mechanism proposed by Shi et al. (1997) is therefore not able to explain both of the features shown by the experimental data.

Although our mechanism can quantitatively explain (1) the data reported in the literature as well as the data collected in the present work over a wide range of parameter values, (2) the dependence of apparent equilibrium constant K_a on just one parameter L, and (3) K_a approaching a constant value for $L \to 0$, for the two amino-acid–D2EHPA systems for which the data are available, more direct evidence in favor of the formation of bigger complexes is welcome. The phosphorous–NMR method was attempted, but due to continuous formation and breakage of weak hydrogen-bond-mediated structures, it was not successful. Colligative property determination methods such as vapor-phase osmometry, which can differentiate between the formation of larger and smaller complexes, could not be tried due to the nonavailability of facilities.

Conclusions

A new reaction mechanism is proposed to predict the equilibrium behavior of amino-acid extraction using D2EHPA. The proposed reaction mechanism involves formation of a complex on the interface and formation of larger aggregates in the bulk by association of the complex molecules formed at the aqueous–organic interface.

The proposed mechanism can quantitatively explain the equilibrium behavior of the phenylalanine–D2EHPA and tryptophan–D2EHPA systems for which the experimental data are reported. The mechanism also quantitatively predicts the hitherto unexplained dependence of the apparent equilibrium constant on just one parameter—the loading ratio. New data were also obtained for the phenylalanine–D2EHPA system to test the proposed mechanism over a wide range of parameter values. All the data were successfully predicted using the proposed mechanism.

Acknowledgments

We thank Prof. A. K. Mukherjee for initiating us into this field of research. G. L. Tulasi acknowledges the Council for Scientific and Industrial Research (CSIR), India, for financial assistance through grant number 9/79(615)/97-EMR-I.

Literature Cited

Chan, C. C., and J. F. Yang, "Separation and Concentration of Phenylalanine by Emulsion Liquid Membrane in a CSTR Extraction Process," *Sep. Sci. Technol.*, **30**, 3001 (1995).

Cussler, E. L., and D. F. Evans, "Liquid Membranes for Separations and Reactions," *J. Membr. Sci.*, **6**, 113 (1980).

Hong, S.-A., and J.-W. Yang, "Process Development of Amino Acid Concentration by a Liquid Emulsion Membrane Technique," *J. Memb. Sci.*, **86**, 181 (1994).

Huang, T. C., and R. S. Juang, "Extraction Equilibrium of Zinc from Sulfate Media with Bis(2-ethylhexyl) Phosphoric Acid," *Ind. Eng. Chem. Fundam.*, **25**, 752 (1986).

Itoh, H., M. P. Thien, T. A. Hatton, and D. I. C. Wang, "Water Transport Mechanism in Liquid Emulsion Membrane Process for the Separation of Amino Acids," *J. Membr. Sci.*, **51**, 309 (1990).

Kiani, A., R. Bhave, and K. K. Sirkar, "Solvent Extraction with Immobilized Interfaces in Microporous Membranes," *J. Memb. Sci.*, **20**, 125 (1984).

Kim, B. M., "Membrane-Based Solvent Extraction for Selective Removal and Recovery of Metals," *J. Memb. Sci.*, **21**, 5 (1984).

Komasawa, I., T. Otake, and Y. Higaki, "Equilibrium Studies of the Extraction of Divalent Metals from Nitrate Media with Di(2-Ethylhexyl) Phosphoric Acid," *J. Inorg. Nucl. Chem.*, **43**, 3351 (1981).

Komasawa, I., and T. Otake, "Kinetic Studies of the Extraction of Divalent Metals from Nitrate Media with Bis(2-Ethylhexyl) Phosphoric Acid," *Ind. Eng. Chem. Fundam.*, **22**, 367 (1983).

Lehninger, A. L., D. L. Nelson, and M. M. Cox, *Principles of Biochemistry*, Chap. 5, CBS, New York, p. 113 (1993).

Li, Z. C., W. Furst, and H. Renon, "Extraction of Zinc(II) from Chloride and Perchlorate Aqueous Solutions by Di(2-Ethylhexyl) Phosphoric Acid in Escaid 100: Experimental Equilibrium Study," *Hydrometallurgy*, **16**, 231 (1986).

Shi, Q.-H., Y. Sun, L. Liu, and S. Bai, "Distribution Behaviour of Amino Acids by Extraction with Di(2-Ethylhexyl) Phosphoric Acid," *Sep. Sci. Technol.*, **32**, 2051 (1997).

Teramoto, M., T. Yamashiro, A. Inoue, A. Yamamoto, H. Matsuyama, and Y. J. Miyake, "Extraction of Amino Acids by Emulsion Liquid Membranes Containing Di(2-Ethylhexyl) Phosphoric Acid as a Carrier Biotechnology; Coupled, Facilitated Transport; Diffusion," *J. Memb. Sci.*, **58**, 11 (1991).

Tulasi, G. L., "Extraction of Amino Acids Using D2EHPA," PhD Thesis, Dept. of Chemical Engineering, Indian Inst. of Science, Bangalor, India (1999).

Zhang, Q., and E. L. Cussler, "Microporous Hollow Fibers for Gas Absorption," *J. Memb. Sci.*, **23**, 321 (1985).

Manuscript received March 30, 1999, revision received July 30, 1999.