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Distribution Behavior of L-Phenylalanine by Extraction with Di(2-Ethylhexyl) Phosphoric Acid

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ABSTRACT

In the separation processes of amino acids, zwitterionic species are prevented from being exchanged because of the spatial proximity of two opposite charges on the molecule. There would be a high chemicals cost due to the need to change the solution pH. It is therefore desirable to develop a novel recovery technology for amino acids in the middle pH range. A series of extraction equilibrium experiments for L-phenylalanine (L-phe) with di(2-ethylhexyl) phosphoric acid (D2EHPA) dissolved in *n*-octane was carried out. The effects of L-phe concentration, D2EHPA concentration, and pH on the distribution ratio were discussed in detail. The infrared spectrogram of the organic-phase-loaded solute illustrated that the pH condition had little effect on the structure of the complex. There are proton-transfer and ion-exchange reactions in the extraction. One L-phe molecule is extracted by forming a complex with two dimeric D2EHPA molecules. An expression of the equilibrium distribution was proposed.

INTRODUCTION

Amino acids are valuable bioproducts. They find their principal commercial applications in human foods, animal feed additives, and in the pharmaceutical field (intravenous solutions, medicine, cosmetics). They are also used as intermediates for the synthesis of special chemicals like hypocaloric sweeteners, chelating agents, and pharmaceutical peptides. Most of the amino acids are produced in commercial quantities by microbial fermentation. The down-

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stream processing technologies for the separation and recovery of amino acids are very complicated. In the last decades there has been a continous interest in developing processes that will improve the selectivity and yield of downstream processing steps for the separation and recovery of amino acids (1–9). Since the 1980s the application of di(2-ethylhexyl) phosphoric acid (D2EHPA) in an emulsion liquid membrane (ELM) as a carrier has attracted much attention from researchers and engineers. It has been reported that such amino acids as lysine (10), tryptophan (15), and phenylalanine (9, 15) can be separated and concentrated by ELM containing D2EHPA as a carrier. D2EHPA can also separate amino acids as extractant dissolved in different diluents. Liao et al. (11) studied the extraction mechanism of isolesine with D2EHPA as the extractant and proposed an extraction equilibrium constant. Teramoto et al. (12) studied the extraction of several amino acids and their related compounds with D2EHPA dissolved in benzene. The exponent m in the relation $D \propto [(\mathrm{D2EHPA})_2]^m$ was in the 1.7 to 2.0 range for alanine, valine, leucine, isoleucine, phenylalanine, and tryptophan. They suggested that one amino acid molecule coordinates to 1.7 to 2.0 dimeric D2EHPA. Q.-H. Shi et al. (13) studied the distribution equilibrium of L-tryptophan by extraction with D2EHPA dissolved in *n*-hexane. A general extraction formula and supposed structure of L-Trp-D2EHPA were proposed. However, the extraction mechanism of amino acids by D2EHPA was limited to a cation-exchange reaction in all of the above works; the extraction equilibria were in the pH < 3 range. To date, there is no published report on the extraction of amino acids in the middle pH range. Because amino acids change their charged state depending on the pH, the extraction equilibrium mechanism for different species of amino acids may be different from each other, and the structures of amino acid–D2EHPA complexes may differ too. Therefore, it is very important to determine the extraction equilibrium at various pH conditions.

In this work, using L-phenylalanine (L-phe) as a model amino acid, the extraction mechanism at various pH conditions was studied. The relation between pH and the structure of the L-phe–D2EHPA complex was determined. The effects of amino acid and extractant concentrations on equilibrium are discussed.

MATERIALS AND METHODS

Materials

D2EHPA is a product of Chemical Reagent Factory of Beijing, People's Republic of China. It is further purified by recrystallization with copper hydroxide, as described by Patridge and Jensen (14). L-Phe is of chromatographic grade. Other reagents, such as *n*-octane, sulfuric acid (H_2SO_4), sodium sulfate (Na_2SO_4), and sodium hydroxide (NaOH), are all of analytical grade.

Extraction Experiments

All of the extraction experiments were carried out in a bath shaker at 298 ± 0.5 K, 200 ± 2 rpm. Organic solutions, prepared by dissolving D2EHPA in *n*-octane, contained 0.745–1.787 $\mathrm{mol{\cdot}L^{-1}}$ D2EHPA. Aqueous solutions contained 0.00606–0.02276 $\mathrm{mmol{\cdot}L^{-1}}$ L-phe; the ionic strength was fixed at 0.2 $\mathrm{mol{\cdot}L^{-1}}$. The organic phase (10 mL) and the aqueous phase (10 $\mathrm{mol{\cdot}L^{-1}}$. The organic phase (10 mL) and the aqueous phase (10 mL) were stirred in the bath shaker for 1 hour. After equilibration, the phases were separated by centrifugation.

Analysis

The pH of the aqueous phase was measured with a digital pH meter (model SA 520, USA). The concentration of L-phe was determined using an UV spectrophotometer (model Hp8452, USA) at a wavelength of 258 nm. According to Beer's law, the molar absorption coefficient, ξ is

$$\xi = A/LC \tag{1}$$

where A is the absorbance, L is the width of the cell (equal to 1 cm), and C is the concentration of the sample. From the calibration curve of A vs C (Fig. 1), the slope $(\xi L)^{-1}$ is found to be 880.25 ppm. The wavelength of peak and the molar absorption coefficient do not vary with the pH. The concentration of L-phe in the organic phase was calculated according to the mass balance of L-phe. Experiments proved that the error is less than 3%. To examine the effect

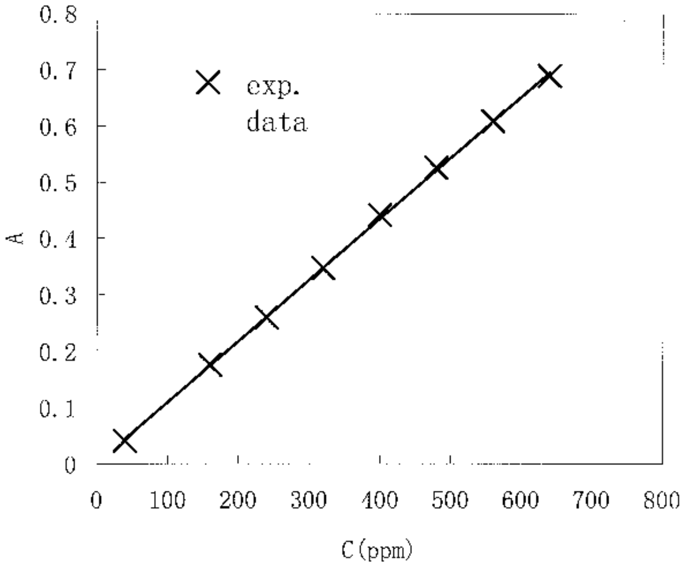


FIG. 1 The calibration curve of absorbance vs concentration of L-phe aqueous solution.

of pH on the structure of the complex in the organic phase, infrared spectra (model FIR8200, Japan) of the organic-samples-loaded solute at different equilibrium pH values of the aqueous phase were analyzed.

RESULTS AND DISCUSSION

Behavior of Aqueous Solution of L-Phe

L-Phe has one carboxylic group, one amino group, and a side chain. Two dissociation equilibria exit in aqueous solutions:

$$\mathrm{PhCH_2(NH_3^+)CHCOOH} \xrightleftharpoons{K_{a1}} \mathrm{PhCH_2(NH_3^+)CHCOO^-} + \mathrm{H^+} \quad (2)$$

$$\mathrm{PhCH_2(NH_3^+)CHCOO^-} \xrightleftharpoons{K_{a2}} \mathrm{PhCH_2(NH_2)CHCOO^-} + \mathrm{H^+} \quad (3)$$

The two dissociation constants for Eqs. (2) and (3) can be described as:

$$K_{a1} = \frac{[A^{\pm}][H^+]}{[A^+]} \quad (4)$$

$$K_{a2} = \frac{[A^-][H^+]}{[A^{\pm}]} \quad (5)$$

where A^+, $A^{\pm}$, A^- are the cation, zwitterion, and anion of L-phe, respectively. In aqueous solutions, pK_{a1} and pK_{a2} of L-phe are 1.83 and 9.13, respectively.

Effects of Extractant Concentration and Equilibrium pH on Distribution Ratio

If the extraction of L-phe with *n*-octane is negligible, the distribution ratio of L-phe between the organic and the aqueous is defined as

$$D = [A]_{(o)}/[A]_{(w)} \quad (6)$$

where $[A]_{(o)}$ is the concentration of L-phe in the organic phase, and $[A]_{(w)}$ is the concentration of L-phe in the aqueous phase. As shown Fig. 2, at a constant extractant concentration (B_0) the distribution ratio increases with an increase of pH in the range $1 < \mathrm{pH} < 3$. However, the effect of pH on the distribution ratio is not as obvious in the range $3 < \mathrm{pH} < 5$. An increase of the initial extractant concentration increases the distribution ratio; this is ascribed to enhancement of the extraction capacity of the organic phase.

At the same equilibrium pH of the aqueous phase, the difference in the total concentration of L-phe (C_0) has little effect on the distribution ratio, as can be seen in Fig. 3. Because different C_0 values result in different equilibrium concentrations in the aqueous phase, the distribution ratio is obtained differently.

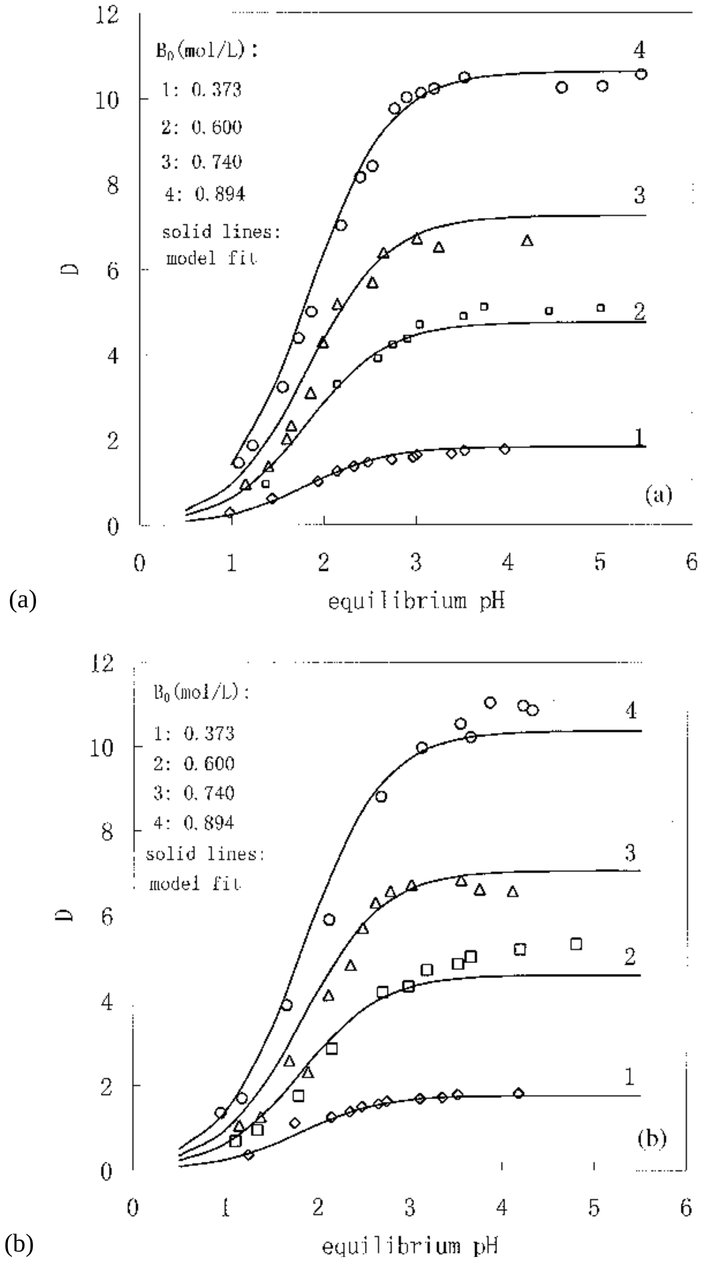
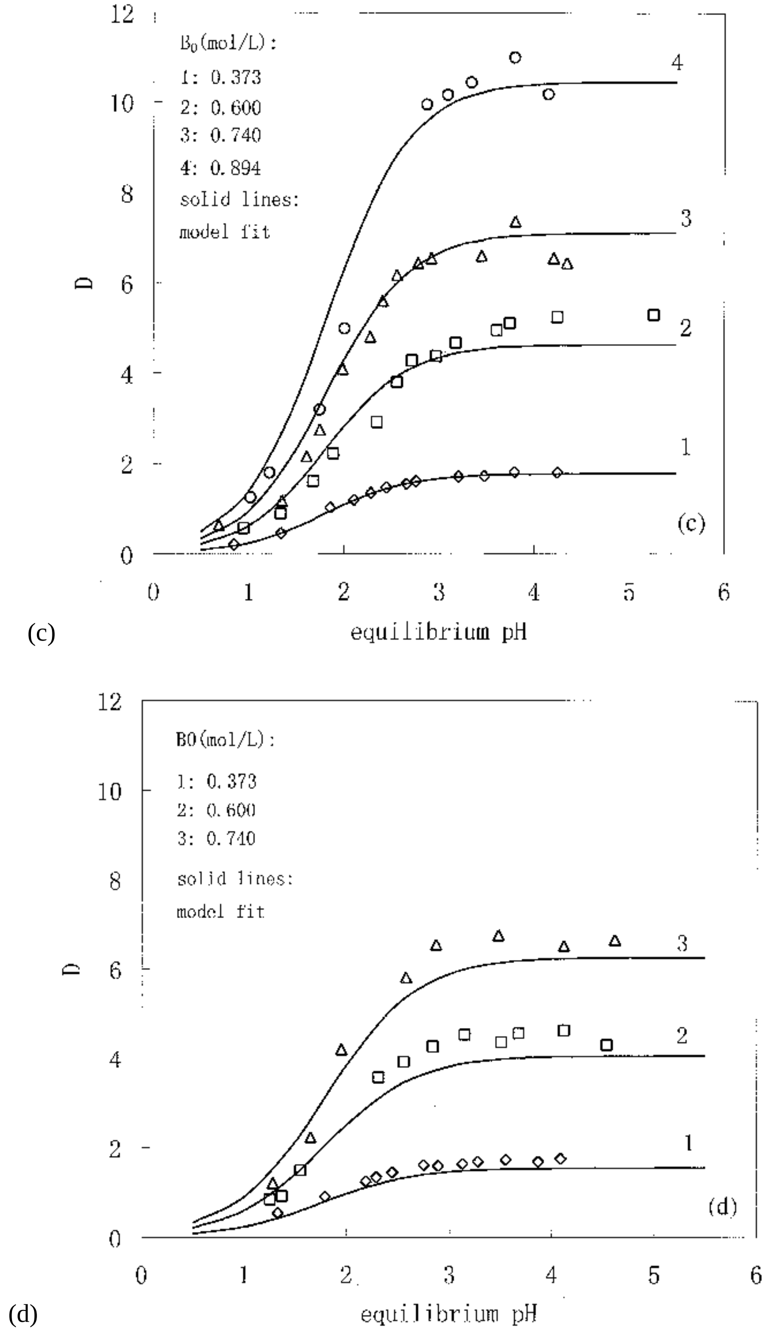


FIG. 2 Effect of concentrations of D2EHPA on D. C_0 (mmol·L^{-1}): (a) 6.06; (b) 8.10; (c) 17.76; (d) 22.76.

(continued)

Determination of the Complex Structure

Many workers (13, 15) considered that the mechanism of the extraction of amino acid with D2EHPA was a cation-exchange reaction in the low pH range. Their investigations focused on the cation-exchange reaction in the pH



(c)

(d)

FIG. 2 Continued

< 3.0 range. In the pH < 5.0 region the predominant species are A^+ and $A^\pm$. The presence of A^- can be ignored. In the 3.0 < pH < 5.0 range the molar fraction of $A^\pm$ is over 94%, but that of A^+ is less than 6%. It can be concluded from Fig. 2 that the distribution ratio for the extraction of $A^\pm$ is higher. The

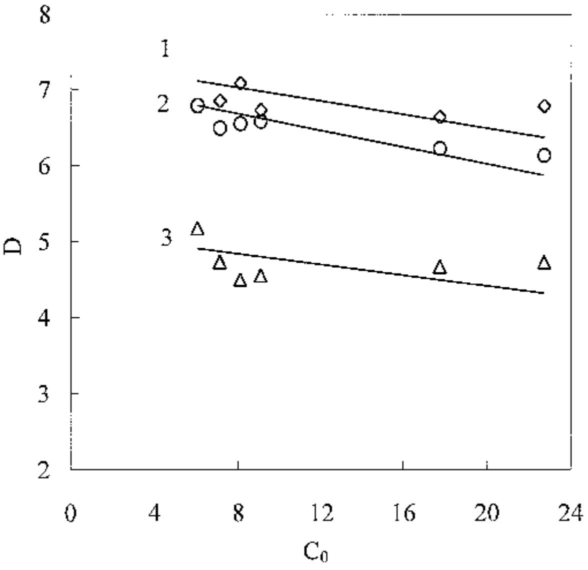
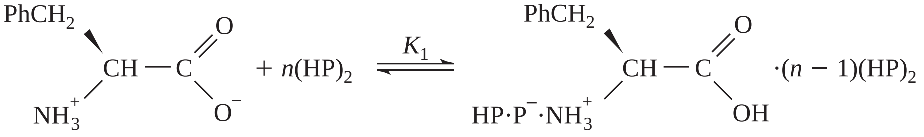


FIG. 3 Effect of C_0 on D at the same aqueous pH. 1: pH 3.80; 2: pH 3.01; 3: pH 2.15. Solid lines: model fit.

extraction of $A^{\pm}$ should attract more attention. The mechanism of the extraction of $A^{\pm}$ by D2EHPA should be different from that of A^{+}. It is evident that the zwitterionic molecule ($A^{\pm}$) results from proton transfer from —COOH to —NH_2 in the amino acid molecule. Lewis acid–D2EHPA in the organic phase is likely to transfer its proton to $A^{\pm}$ to form an ion-pair complex. This is demonstrated in *the infrared spectrogram of an organic-phase-loaded solute* (ISOPLS) (Fig. 4).

The results show that pH has little effect on the structure of the complex (shown in Fig. 4). As illustrated in Fig. 5, in ISOPLS the stretching peaks of —COO^{-} at 1560.3 and 1409.9 cm^{-1} are not present, but the absorption peak of —COOH appears at wavenumber 1735.8 cm^{-1}. The characteristic peak of —NH_3^+ at 1494.7 cm^{-1} moved to a higher wavenumber, 1498.6 cm^{-1}. When —NH_3^+ combines with —$(C_3H_{17}O)_2P(O)O^{-}$, its stretching is affected by —$(C_3H_{17}O)_2P(O)O^{-}$, and the absorption peak of the group shifts to the higher wavenumber.

According to the above analysis, there are two extraction equilibria:



(7)

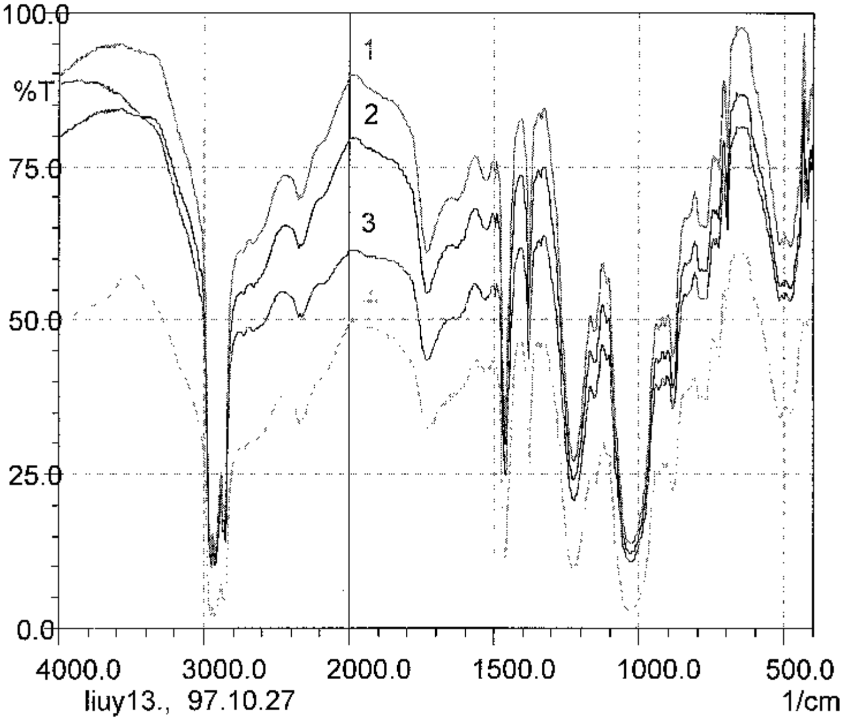


FIG. 4 Infrared spectrograms of loaded organic phase. The equilibrium pHs of the aqueous phase are (1) 1.39; (2) 2.25; (3) 3.39; (4) 3.85.

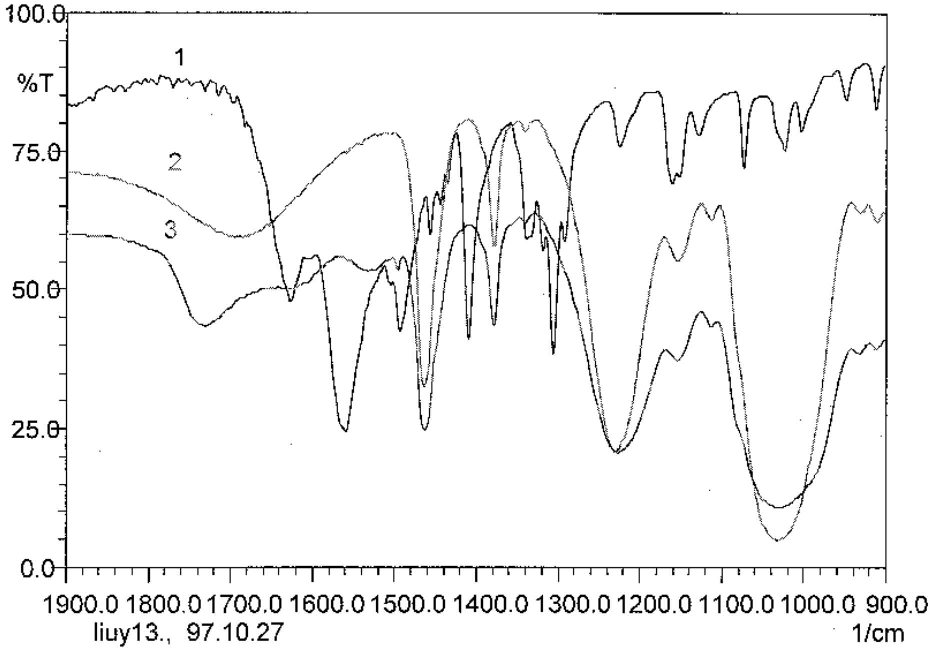
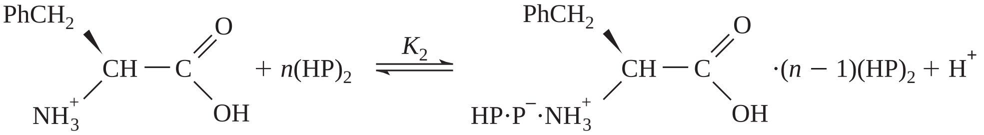


FIG. 5 Infrared spectrograms of L-phenylalanine, blank organic phase, and organic phase loaded with solute. The equilibrium pH of the aqueous phases is 3.85. (1) L-phenylalanine; (2) blank organic phase; (3) loaded organic phase.


(8)

There are two reactive mechanisms in the extraction—cation-exchange reaction and a proton-transfer reaction. In the proton-transfer reaction, D2EHPA reacts with $A^{\pm}$: One H^+ of D2EHPA transfers to -COO^- of $A^{\pm}$.

The extraction equilibrium constants (K_1, K_2) can be expressed as

$$K_1 = \frac{[\overline{PA}]}{[A^{\pm}][\overline{(HP)_2}]^n} \tag{9}$$

$$K_2 = \frac{[\overline{PA}][H^+]}{[A^+][\overline{(HP)_2}]^n} = K_1 K_{a1} \tag{10}$$

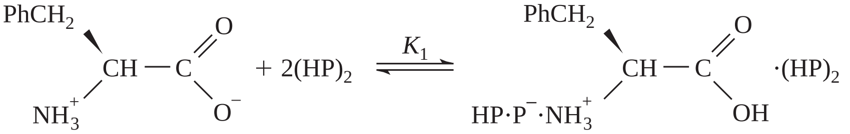
where the organic-phase species are marked with an overbar. $\overline{PA}$ is the complex. $\overline{(HP)_2}$ is dimeric D2EHPA. Equation (6) can be rewritten as

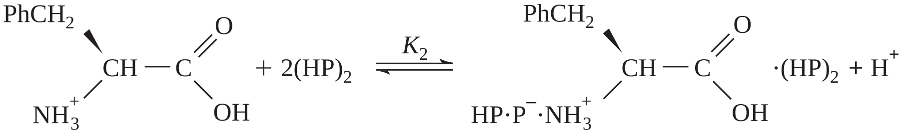
$$D = \frac{[\overline{PA}]}{[A^+] + [A^{\pm}]} \tag{11}$$

By rewriting Eq. (11) and taking the logarithm of both sides, the following equation is obtained:

$$\begin{aligned} \log D &= \log K_1 + n \log[\overline{(HP)_2}] - \log(1 + 10^{pK_{a1} - pH}) \\ &= \log K_1 + n \log[\overline{(HP)_2}] - \log(K_{a1} + [H^+)] + \log K_{a1} \end{aligned} \tag{12}$$

Plots of log D vs log$[\overline{(HP)_2}]$ are shown in Fig. 6. Linearly least-square fittings give the slopes at different pH condition, i.e., the values of n in Eq. (12). They are nearly equal to 2.0. The relationship of log$[D(1 + 10^{pK_{a1} - pH})]$ and log$[\overline{(HP)_2}]$ is shown in Fig. 7. Linearly least-square fitting gives the slope and intercept, i.e., the values of n and K_1 in Eq. (12). They are 2.02 and $12.77 L^2 \cdot mol^{-2}$, respectively. The value of n is nearly equal to 2.0, which suggests the stoichiometry of the extraction can be expressed as:


(13)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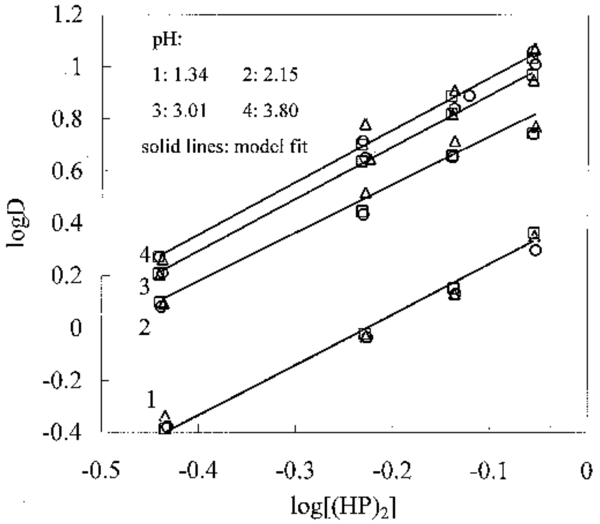

FIG. 6 Plots of log D vs $\log[\overline{(\mathrm{HP})_2}]$. $C_0(\mathrm{mmol \cdot L^{-1}})$: (△) 6.06; (○) 8.10; (□) 9.10.

According to Eq. (12), the slope of the plot of log D vs $\log(K_1 + [\mathrm{H}^+])$ is negative unity. The straight line in Fig. 8 is the expression of Eq. (12). Its slope is -1.05, nearly equal to -1.

Expression of Distribution Ratio

When the ratio of the volume of organic phase to the volume of aqueous phase is unity, the distribution ratio of L-phe between the organic and the aqueous phases (D) can be calculated by using the following equations:

$$C_0 = (1 + D)[\mathrm{A}^{\pm}](1 + 10^{\mathrm{p}K_{\mathrm{a1}} - \mathrm{pH}}) \tag{15}$$

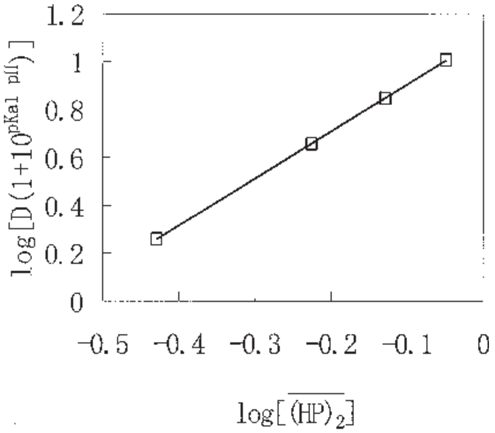

FIG. 7 Plots of $\log[D(1 + 10^{\mathrm{p}K_{\mathrm{a1}} - \mathrm{pH}})]$ vs $\log[\overline{(\mathrm{HP})_2}]$. □: Experimental data.

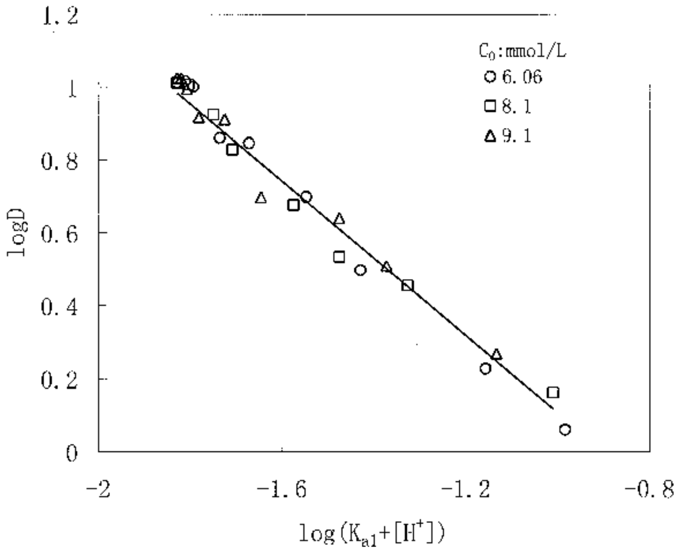

FIG. 8 Effects of pH on *D*.

$$[\overline{(HP)_2}] = B_0 - 2D[A^{\pm}](1 + 10^{pK_{a1}-pH}) \tag{16}$$

$$D = \frac{K_1[\overline{(HP)_2}]^2}{1 + 10^{pK_{a1}-pH}} \tag{17}$$

where C_0 is the initial aqueous concentration of L-phe, and B_0 is the original concentration of D2EHPA in the organic phase. The solid lines in Fig. 2 are as predicted by the model.

CONCLUSIONS

In this paper the extraction equilibrium of L-phelylalanine with D2EHPA dissolved in *n*-octane was studied in detail. In the $1.0 < pH < 3.0$ range, of *D* is very low because of Le Chatelier's effect and increases with an increase of pH, but it changes little in the $3 < pH < 5$ range. An increase of the extractant concentration increases *D*; the initial aqueous concentration of L-phe has a slight effect on *D*.

A proton transfer reaction was first proposed in the extraction of L-phe with D2EHPA. The pH condition has little effect on the structure of the complex. In the $1 < pH < 3$ range, the cation-exchange reaction and the proton-transfer reaction coexist. In the $3 < pH < 5$ range, the extraction reaction is mainly in the form of the proton transfer reaction; *D* is higher and slightly affected by pH. One L-phe is extracted by forming a complex with four monomeric D2EHPAs; the pH condition has little effect on the structure of the complex. An expression for the equilibrium distribution is proposed. The experimental data present good agreement to Eq. (12).

NOMENCLATURE

A^{+}	L-phenylalanine cation
A^{-}	L-phenylalanine anion
$A^{\pm}$	L-phenylalanine zwitterion
$[A]_{(o)}$	concentration of L-phe in organic phase ($L \cdot mol^{-1}$)
$[A]_{(w)}$	concentration of L-phe in aqueous phase ($L \cdot mol^{-1}$)
C_0	initial aqueous concentration of L-phe ($mol \cdot L^{-1}$)
D	distribution ratio (dimensionless)
K_1	equilibrium constant of proton transfer reaction ($L^{2} \cdot mol^{-2}$)
K_2	equilibrium constant of cation exchange reaction ($L \cdot mol^{-1}$)
K_{a1}, K_{a2}	dissociation constant of L-phe ($L \cdot mol^{-1}$)
$(HP)_2$	dimeric of D2EHPA
PA	complex of L-phenylalanine combined with D2EHPA

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