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Distribution Behavior of α-Amino Acids and Aminobenzoic Acid by Extraction with Trioctylamine

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ABSTRACT

The separation technique of extraction based on reversible chemical complexation can be a high-capacity and high-selectivity technology for separating polar organic solutes from dilute solution. The Lewis acid, di(2-ethylhexyl)phosphoric acid (D2EHPA), is often chosen as extractant to separate amino acids from aqueous solution because of

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its high capability in the reversible chemical complexation. In this paper, trioctylamine (TOA) is used as extractant, and three α-amino acids [L-phenylalanine (L-phe), L-tryptophane (L-trp), and L-isoleucine (L-ile)] and aminobenzoic acid (ABC) are used as model amino acids. We present a series of extraction equilibrium experiments for them with TOA dissolved in n-octane and n-octanol. The effects of initial extractant concentration, equilibrium pH value, and the polarity of bulk solvent on the distribution ratio (*D*) of L-phe, L-trp, L-ile, and ABC between the organic and aqueous phases were discussed. Expressions for the distribution ratio are proposed, and the experimental data are in good agreement with the proposed expressions.

INTRODUCTION

The separation technique of extraction based on reversible chemical complexation can be a high-capacity and high-selectivity technology for separating polar organic solutes from dilute solution.[1] Amino acids are valuable chemical products. They find their principal commercial applications in human foods, in animal feed additives, and in the pharmaceutical field (intravenous solutions, medicine, and cosmetics). In the last decades there has been a continuous interest in developing processes that will improve the selectivity and yield of downstream processing steps for the separation and recovery of amino acids.[2–7] The Lewis acid, di(2-ethylhexyl)phosphoric acid (D2EHPA), is often chosen as the extractant because of its high capability in the reversible chemical complexation.[2–6] Several years ago, the extraction of organic compounds with an acid group and an alkaline group on the molecule attracted much attention from researchers, and many interesting results were presented.[8,9]

In this work, the Lewis base, trioctylamine (TOA), is used as the extractant to investigate the distribution behavior of amino acids and aminobenzoic acid (ABC) between the organic and aqueous phases. Three α-amino acids [L-phenylalanine (L-phe), L-tryptophane (L-Trp), and L-isoleucine (L-ile)] and two aminobenzoic acids (p-ABC and o-ABC) are used as model amino acids. A series of extraction equilibrium experiments with TOA dissolved in n-octane and n-octanol were performed. The effects of extractant concentrations and pH value on equilibrium are discussed.

MATERIALS AND METHODS

Materials

TOA is of chemical grade (90%, wt). L-phe, L-trp, and L-ile are of chromatographic grade. Other reagents, such as p-aminobenzoic acid (PABC), o-aminobenzoic acid (OABC), n-octane, n-octanol, 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 ± 0.5K, 200 ± 2 rpm. TOA solutions, prepared by dissolving TOA in n-octane or n-octanol, contained 0.232–0.464 mol/L TOA. The aqueous ionic strength was fixed at 0.2 mol/L. 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. Each experiment was carried out three times in identical conditions, and the average value of the studied parameter was used.

Analysis

The pH of the aqueous phase was measured with a digital pH meter (model SA 520, USA). The concentrations of L-phe, L-trp, PABC, and OABC in the aqueous solution were measured using an UV spectrophotometer (model HP8452, USA) at wavelengths of 258, 280, 266, and 310 nm, respectively. The concentrations of L-ile in the aqueous solution were measured according to the literature.[10] The concentration of the model amino acids in the organic phase was calculated according to the mass balance of them. Experiments proved that the error is less than 2%.

RESULTS AND DISCUSSION

Behavior of Aqueous Solution of Aminobenzoic Acids

Aminobenzoic acid has one carboxylic group, one amino group, and a benzene ring that connects with the carboxylic group and the amino group.

Two dissociation equilibria exist in aqueous solutions

$$H_3{}^+NC_6H_4COOH \underset{}{\overset{K_{a1}}{\rightleftharpoons}} H_2NC_6H_4COOH + H^+ \tag{1}$$

$$H_2NC_6H_4COOH \overset{K_{a2}}{\rightleftharpoons} H_2NC_6H_4COO^- + H^+ \tag{2}$$

The two dissociation constants for Eqs. (1) and (2) can be described as:

$$K_{a1} = \frac{[A][H^+]}{[A^+]} \tag{3}$$

$$K_{a2} = \frac{[A^-][H^+]}{[A]} \tag{4}$$

where A^+, A^- are the cation and anion of aminobenzoic acid, respectively. A is the neutral molecule of aminobenzoic acid. The pK_{a1} and pK_{a2} of PABC and OABC in aqueous solutions are shown in Table 1.

Behavior of Aqueous Solution of α-Amino Acids

α-Amino acid has one carboxylic group, one amino group, and a side chain. Two dissociation equilibria exist in aqueous solutions:

$$H_3{}^+N{-}R{-}COOH \overset{K_{a1}}{\rightleftharpoons} H_3{}^+N{-}R{-}COO^- + H^+ \tag{5}$$

$$H_3{}^+N{-}R{-}COO^- \overset{K_{a2}}{\rightleftharpoons} H_2N{-}R{-}COO^- + H^+ \tag{6}$$

Table 1. Dissociation constants (pK_{a1}, pK_{a2}) of PABC and OABC.

	pK_{a1}	pK_{a2}
PABC	2.50	4.87
OABC	2.11	4.95
L-phe	1.83	9.13
L-trp	2.38	9.63
L-ile	2.36	9.62

Data from Ref.[11].

The two dissociation constants for Eqs. (5) and (6) can be described as

$$K_{a1} = \frac{[A^{\pm}][H^{+}]}{[A^{+}]} \tag{7}$$

$$K_{a2} = \frac{[A^{-}][H^{+}]}{[A^{\pm}]} \tag{8}$$

where R is the respective side chain of L-phe, L-trp, and L-ile. A^{+}, $A^{\pm}$, A^{-} are the cation, zwitterion, and anion of three α-amino acids, respectively. The pK_{a1} and pK_{a2} of them in aqueous solutions are shown in Table 1.

Distribution Ratio Definition

The distribution ratio (D) of amino acids between the organic and the aqueous is defined as

$$D = [A]_{(o)}/[A]_{(w)} \tag{9}$$

where $[A]_{(o)}$ is the concentration of amino acids in the organic phase and $[A]_{(w)}$ is the total concentration of all species of amino acids in the aqueous phase.

Physical Extraction Behavior of Amino Acid with n-Octanol

In most of the experiments, n-octanol is chosen as diluent, so its extraction characteristic will play an important role in the extraction of amino acid when TOA acts as the extractant.

The physical extraction behavior of aminobenzoic acid with n-octanol is shown in Fig. 1. With an increase of pH value, the distribution ratio (D) of OABC and PABC between the organic and the aqueous phases is represented as a peak, and this peak is at the point of $pH_{equiv} = (pK_{a1} + pK_{a2})/2$. The solid lines in Fig. 1 are as predicted by the following equation:

$$D = D_m\varphi = D_m(1 + 10^{pK_{a1}-pH} + 10^{pH-pK_{a2}})^{-1} \tag{10}$$

where $(1 + 10^{pK_{a1}-pH} + 10^{pH-pK_{a2}})^{-1}$ is the molar fraction of neutral molecule at a specific pH and D_m is the distribution ratio of aminobenzoic acid between the organic and the aqueous phases when the molar fraction of neutral molecule in the aqueous is 1.0. The values of D_m are 7.12 and 5.86 for PABC and OABC, respectively.

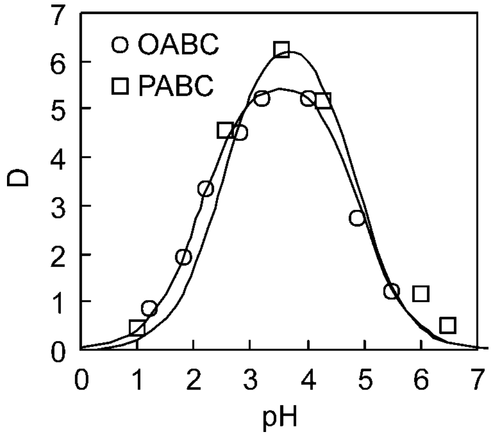

Figure 1. Physical extraction behavior of aminobenzoic acid with n-octanol (a) Initial concentrations of PABC and OABC are 7.29 mmol l^{-1}.

One can conclude from Fig. 1 and Eq. 10 that the neutral molecules of aminobenzoic acid were extracted with n-octanol.

We performed many experiments and demonstrated that the distribution ratios of L-phe, L-trp, and L-ile between the organic phase (n-octanol) and the aqueous phase are nearly equal to zero. In these experiments, the initial concentrations of L-phe, L-trp, and L-ile in the aqueous solutions are 6.06, 4.90, and 7.63 mmol/L, respectively.

Effect of TOA Concentration and Equilibrium pH on Distribution Ratio

The distribution behavior of PABC and OABC is illustrated in Fig. 2. At a constant extractant concentration (B_0) the distribution ratio presents a peak with the increased pH. An increase of the initial extractant concentration (B_0) corresponds to an increase in the distribution ratio. The initial feed concentration of PABC has little effect on its distribution ratio in the whole pH range. The extraction phenomena are similar to those in the extraction of PABC and OABC with D2EHPA,[12] but the peak shifts toward the higher pH value, because the basicity of TOA is higher than that of D2EHPA.

From the similarity between Fig. 1 and Fig. 2, one can infer that the neutral molecules of aminobenzoic acid were extracted with TOA.

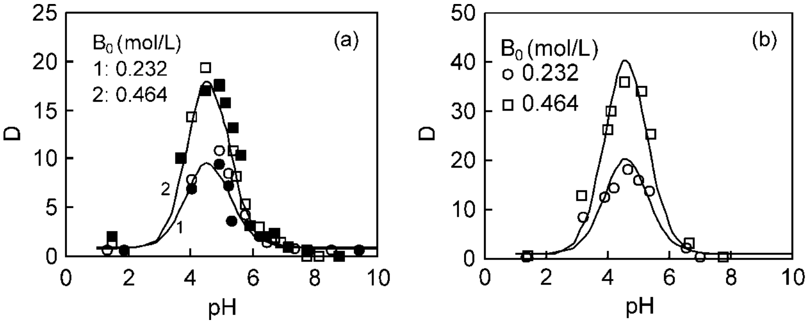

Figure 2. (a) Effect of the initial concentration of TOA (B_0) and PABC (C_0) and pH value on distribution ratio. C_0(mmol l^{-1}): ○, □: 7.29; ●, ■: 13.19. (b) Effect of the initial concentration of TOA (B_0) and pH value on distribution ratio. Initial concentration of OABC is 7.29 mmol l^{-1}.

Additionally, the peak of D should be at the point of pH where the molar fraction of neutral molecules of aminobenzoic acid is maximum. This pH value is marked as pH_{max}. However, the value of pH_{max} would be affected by the apparent acidity or basicity. If the extractant is neutral (e.g., n-octanol), the pH_{max} value is nearly equal to pH_{equiv}, as illustrated in Fig. 1. In the extraction of PABC and OABC with D2EHPA,[12] the pH_{max} value is lower than pH_{equiv}. In the extraction with TOA, the pH_{max} value is higher than pH_{equiv}.

As shown in Fig. 3, the initial extractant concentration, equilibrium pH value, and the polarity of bulk solvent have little effect on *D* of L-phe, L-trp, and L-ile between the organic and aqueous phases. Additionally, almost all of *D* is less than 0.1, which indicates the complexation capability of TOA with these α-amino acids is rather weak.

From the comparison between the extraction of L-phe (2), L-trp (3), and L-ile (4) with D2EHPA and that with TOA (shown in Fig. 3), one can conclude that the complexation capability of D2EHPA with these α-amino acids is much stronger than that of TOA with them.

As shown in Eqs. 5 and 6, in the $pH < pH_{equiv}$ range, the main dissociated species of α-amino acid is the cation $H_3^+N{-}R{-}COOH$. TOA does not combine with the cation because it is very easy to combine with H^+. The equilibrium constant of the combination of TOA with H^+ is 16906 L mol^{-1}.[13] In the $pH > pH_{equiv}$ range, the main dissociated species of α-amino acid is the anion $H_2N{-}R{-}COO^-$. TOA does not react with it to form complex. Consequently, the extraction capacity of TOA with α-amino acid is very poor. However, TOA can react with aminobenzoic acid

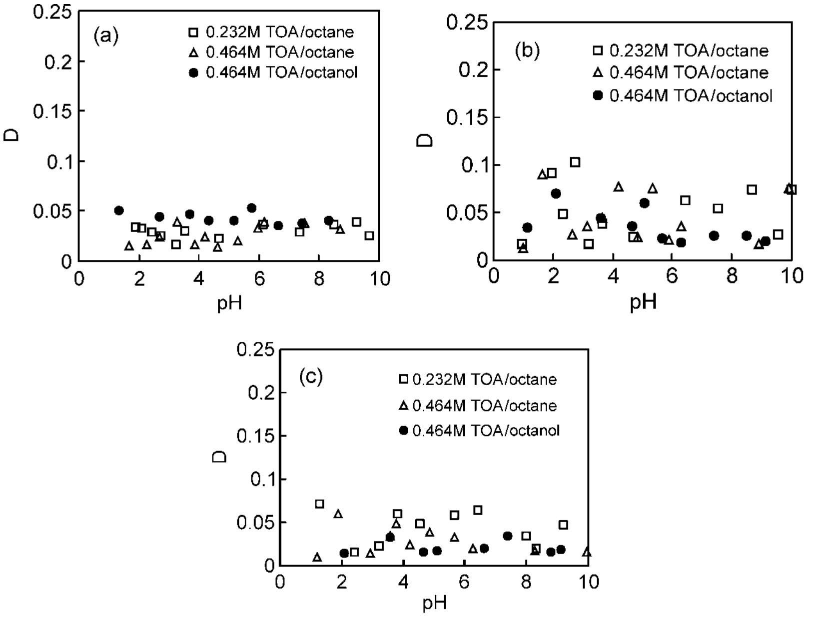

Figure 3. (a) Effect of the initial concentration of TOA (B_0) and pH value on distribution ratio L-phe, $c_0 = 6.06\,\mathrm{mmol/L}$. (b) Effect of the initial concentration of TOA (B_0) and pH value on distribution ratio L-trp, $c_0 = 4.90\,\mathrm{mmol/L}$. (c) Effect of the initial concentration of TOA (B_0) and pH value on distribution ratio L-ile, $c_0 = 7.63\,\mathrm{mmol/L}$.

effectively to form a complex because of the presence of neutral molecular $H_2NC_6H_4COOH$. The infrared spectrogram of an organic-phase-loaded solute demonstrated that TOA combines with the $-COOH$ group of $H_2NC_6H_4COOH$ and the group $-NH^+$ forms.[13]

Determination of the Extraction Model for PABC and OABC

The extraction equilibria with TOA can be described as

$$R_3N + H^+ \overset{K_1}{\rightleftharpoons} R_3NH^+ \tag{11}$$

$$R_3N + NH_2PhCOOH \overset{K_2}{\rightleftharpoons} (R_3NH^+)COO^-PhNH_2 \tag{12}$$

where R_3N is TOA. Aside from the two equilibria, two dissociation equilibria of aminobenzoic acid, Eqs. 1 and 2, and a physical extraction equilibrium can be determined.

The equilibrium constants in Eqs. 11 and 12 can be expressed as

$$K_1 = \frac{[\overline{R_3NH^+}]}{[H^+]\cdot[\overline{R_3N}]} \tag{13}$$

$$K_2 = \frac{[\overline{(R_3NH^+)COO^-PhNH_2}]}{[\overline{R_3N}]\cdot[NH_2PhCOOH]} \tag{14}$$

$$B_0 = [\overline{R_3N}] + [\overline{R_3NH^+}] + [\overline{(R_3NH^+)COO^-PhNH_2}] \tag{15}$$

According to the mass balance of extractant and solute, the following equations can be obtained

$$C_0 = (1+D)[A](1+10^{pK_{a1}-pH}+10^{pH-pK_{a2}}) \tag{16}$$

After a series of mathematical calculations for an equation group that consists of Eqs. 3, 4, 13, 14, 15, and 16, the expression for D can be obtained:

$$D = \frac{\phi D_m\chi + K_2B_0 - K_2C_0 - \chi}{2\chi} + \frac{\sqrt{\alpha^2+4\beta}}{2\chi} \tag{17}$$

where

$$\alpha = K_2C_0 - K_2B_0 + \chi - \phi D_m\chi \tag{18}$$

$$\beta = (K_2B_0 + K_2\phi D_mC_0 + \phi D_m\chi)\chi \tag{19}$$

$$\chi = (1+K_1 10^{-pH})(1+10^{pK_{a1}-pH}+10^{pH-pK_{a2}}) \tag{20}$$

By using the computer programming to optimize the following object function, we kept $E(K_1, K_2)$ at a minimum

$$E(K_1,K_2) = \sum_{i=1}^{i=n}(D_{\exp,i} - D_{cal,i})^2 \tag{21}$$

where $D_{\mathrm{exp,i}}$ is the experimental value and $D_{\mathrm{cal,i}}$ is the value calculated from Eq. 17. K_1 and K_2 can be found to be 17,000 L/mol and 81.0 L/mol for PABC, and 17,000 L/mol and 154.0 L/mol for OABC, respectively.

When the volume ratio of the organic phase to aqueous phase is unity, the distribution ratio (D) of aminobenzoic acid between the organic phase and the aqueous phase can be calculated by using the following equations and Eq. 16:

$$B_0 = [\overline{R_3N}] + K_1 \cdot 10^{-pH} \cdot [\overline{R_3N}] + K_2[\overline{R_3N}] \cdot [A] \tag{22}$$

$$D = \frac{\phi D_m \chi + K_2 B_0 - K_2 C_0 - \chi}{2\chi} + \frac{\sqrt{\alpha^2 + 4\beta}}{2\chi} \tag{23}$$

where the expressions of α, β, and χ are shown in Eqs. 18, 19, and 20. The solid lines in Fig. 2 are as predicted by the model.

Discussion

D2EHPA and TOA can react with aminobenzoic acid and form the respective complex. However, their extraction mechanisms are different. TOA combines with the —COOH group of $H_2NC_6H_4COOH$ and the complex is $(R_3NH^+)COO^-PhNH_2$. In the Ref.[12], the conclusion is that D2EHPA reacts with the $—NH_2$ group of $H_2NC_6H_4COOH$, and one aminobenzoic acid molecule is extracted by forming a complex with two dimeric D2EHPA molecules. So the complex is $(HP)_2 \cdot (HP_2^- \cdot NH_3^+)PhCOOH$, where HP is D2EHPA and $(HP)_2$ is the dimeric D2EHPA.

CONCLUSIONS

In this paper, the extraction equilibria of L-phe, L-trp, L-ile, PABC, and OABC with TOA dissolved in n-octane and n-octanol were presented in detail. The initial extractant concentration, equilibrium pH value, and polarity of bulk solvent have little effect on *D* of L-phe, L-trp, and L-ile between the organic and aqueous phases. The complexation capability of TOA with these α-amino acids is rather weak. At a constant extractant concentration (B_0), the distribution ratio of PABC and OABC between the organic and aqueous phases presents a peak with an increased pH value. An increase of B_0 is associated with an increase in *D*. Expressions for the equilibrium distribution ratio of PABC and OABC between the organic and aqueous phases were proposed. The experimental data present good agreement to the obtained models, as can be seen from Fig. 2.

NOMENCLATURE

A^+	cation of L-phe, L-trp, L-ile, PABC, and OABC
A^-	anion of L-phe, L-trp, L-ile, PABC, and OABC
A	neutral molecule of aminobenzoic acid
$A^\pm$	zwitterion of L-phe, L-trp, L-ile
$[A]_{(o)}$	concentration of L-phe, L-trp, L-ile, PABC, and OABC in the organic phase (L/mol)
$[A]_{(w)}$	concentration of L-phe, L-trp, L-ile, PABC, and OABC in the aqueous phase (L/mol)
B_0	initial extractant (TOA) concentration in the organic
C_0	initial aqueous concentration of L-phe, L-trp, L-ile, PABC, and OABC (mol/L)
D	distribution ratio (dimensionless)
K_1	equilibrium constant in Eq. 11 (L/mol)
K_2	equilibrium constant in Eq. 12 (L/mol)
$\mathrm{K_{a1}}$, $\mathrm{K_{a2}}$	dissociation constant of L-phe, L-trp, L-ile, PABC, and OABC (L/mol)
ϕ	volume fraction of n-octanol in bulk solvent
φ	molar fraction of neutral molecule in Eq. 10
α,β,χ	symbols equivalent to the right-hand terms of Eqs. 18–20

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