List of Publication

Thesis submitted by Sri Modan Mohan Bora for the degree of Doctor of Philosophy (Chemistry) of the Gauhati University. The following papers based on this study have been published.

Journal Paper


Reactive Extraction of 6-Aminopenicillanic Acid with Aliquat-336: Equilibrium and Kinetics

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The reactive extraction of 6-aminopenicillanic acid (6-APA) from aqueous buffer solution has been studied using a liquid membrane technique with quaternary ammonium chloride (Aliquat-336) in n-butyl acetate as the solvent. The extraction equilibrium constant and partition coefficient increase with increase in pH up to a limiting value of pH 4.3 for buffer and hydrolytic decompensation of 6-APA. The experimental data could be predicted from an equilibrium model which takes into account the ideal behavior of the two liquid phases. The extraction of the buffer anion under low pH conditions was found to be negligible. The extra rate measured in a stirred-cell mass transfer of the two species as well as on the interfacial reaction through its dependence on the concentration of 6-APA in the aqueous and organic phase, respectively.

On a vaste l'étude de l'extraction réactive de l'anion 6-amino penicillamine d'une solution tampon aqueuse à l'aide d'un échangeur d'anions liquide. Le chlore d'ammonium tétraéthylmethyl (Aliquat-336) dans du n-butyl acétate comme solvant contient l'équilibre d'extraction et le coefficient de partage augmentent avec le pH jusqu'à une valeur limitante du pH 4.3. L'extraction va des deux phases liquides. L'extraction de l'anion tamponne dans le pH au-dessous de 4.3 est négligeable.

Keywords: 6-Aminopenicillanic acid, Aliquat-336, reactive extraction, interfacial reaction.

6-APA constitutes a beta-lactam nucleus, based upon which structures of various natural and semisynthetic penicillin antibiotics can be designed. A starting material for commercially important ampicillin and amoxicillin, 6-APA can be derived from either penicillin V or G via enzymatic hydrolysis, which also produces phenoxycetic acid as the side product (Biswal and Srivastava, 1989). Isolation of 6-APA from the reaction mixture generally involves solvent extraction of the side products with methyl isobutyl ketone at pH 2.5 and adjustment on an exchange resin, i.e. Lewatit MP500Na followed by adjustment of the aqueous 6-APA solution to pH 7.3, concentration under vacuum and precipitation at the isoelectric point (pI) of pH 4.3. The above method of 6-APA isolation is somewhat capital and energy intensive. Reactive extraction (extraction accompanied by chemical reaction) can provide an efficient method for separation and purification of beta-lactam antibiotics from low concentration aqueous solution. In view of reactive extraction, extensive studies have been conducted for penicillin G (Likhisaf et al., 1987; Lee and Lee, 1994; Reschke and Schlegel, 1984; 1985), cephalosporin C (Hano et al., 1992), chloramphenicol, penicillin G, and ampicillin in phenyl acetic acid (Haris et al., 1990), etc. Furthermore, the concept has been exploited to develop liquid membrane processes where facilitated transport could be realized to give high flux and selectivity (Hich et al., 1994; Lee et al., 1993, 1994; Likhisaf and Schlegel, 1997; Lee and Lee, 1992; Gillet et al., 1993; Sahrawi et al., 1994). Various aspects of extraction and purification of beta-lactam antibiotics in general and the liquid membrane process in particular have been recently reviewed by the authors (Gillet et al., 1996, 1997). 6-APA, a lipophilic quaternary ammonium salt known as an efficient liquid anion exchanger has been studied for reactive extraction of amino acids (Hano et al., 1993; Hanew et al., 1993; Reinsinger and Mar, 1994) and other anionic species like Cl, Br, CO32-, and OH- (Sahrawi et al., 1994), etc. In this paper, we present the results of an experimental investigation of the equilibrium and kinetics of 6-APA extraction with Aliquat-336, which will be useful for developing a liquid membrane or a non-dispersive extraction process.

Theoretical aspects

As a result of the presence of the carboxylic acid and amino groups in the molecule, 6-APA exists in the form of a zwitterion, depending on the pH of the medium as evident from the relationship shown in Figure 1. At pH below 2.3, the predominant form is cationic, at pH above 5.1 it is anionic, whereas in the range 2.3 < pH < 5.1, the zwitterion as a whole is predominant. It is decomposed by alkali, but is relatively stable to acid at its isoelectric point (pI = 4.3). The anionic form of 6-APA at pH > 5.1 is amenable for ion-exchange with an anion exchanger such as Aliquat-336 (a carrier hereafter termed OCl). The 6-APA anion, PO-, complexes with the carrier, OCl-, dissolved in an organic solvent as follows.

\[ P^- + OCl^- + OCl^- + Cl^- \] (1)

The anion exchange takes place in the interface of the aqueous-organic phase, the PO- ion being extracted as a complex OPO' into the organic phase liberating Cl into the aqueous phase.
6-APA (H₃P) dissociates in aqueous solution at pH > 5.1 to give the anion $P^-$ and a proton, $H^+$, as follows:

$$\text{H}_3\text{P} \leftrightarrow \text{H}_2\text{P}^- + \text{H}^+$$  

The dissociation equilibrium constant, $K_d$, is given by

$$K_d = \frac{[\text{H}_2\text{P}^-][\text{H}^+]}{[\text{H}_3\text{P}]}$$  

The dissociation equilibrium constant, $K_a$, is given by

$$K_a = \frac{[\text{H}_2\text{P}^-][\text{H}^+]}{[\text{H}_3\text{P}]}$$  

The co-extraction of the buffer anion, $A^-$, by QCI at the interface may take place according to

$$A^-_a + \text{QCI} \rightleftharpoons A^-_a + \text{QCI}^-$$  

The equilibrium constant, $K_{eq}$, of co-extraction is given by

$$K_{eq} = \frac{[\text{QCI}^-][A^-_a]}{[\text{QCl}][A^-_a]}$$  

The distribution coefficient ($m$) of H₃P is defined as

$$m = \frac{[\text{H}_3\text{P}]}{[\text{H}_2\text{P}^-] + [\text{H}^+] + [\text{H}_3\text{P}]}$$  

The following material balance equations hold for Aliquat-336/6-APA:

$$V_a[QCl]_a = V_a[QCl]_{int} - V_a[QCl]_p$$  

$$V_a[H₃P] = V_a[H₃P]_{int} - V_a[H₃P]_p$$  

The extraction equilibrium constant can be arranged as

$$K_i = \frac{V_a[H₃P]_{int} - V_a[H₃P]_p}{V_a[QCl]_{int} - V_a[QCl]_p}$$  

Experimental

6-APA procured from Sigma (St. Louis, MO) was of 99.94% purity. Aliquat-336 (Aldrich, Milwaukee, WI) had a mean molar mass of 404 kg/kmol and was used as received. Butyl acetate and other analytical grade reagents such as sodium mono and dihydrogen phosphate were procured from BDH (Bombay, India).

The extraction equilibrium experiments were carried out in a closed glass vessel by mixing 30 mL each of aqueous 6-APA solution and butyl acetate solution of Aliquat-336. The aqueous phase pH was maintained at 5 to 8 by using phosphate buffer. The time of equilibration was 3 hours. The 6-APA concentration was determined by a UV-visible spectrophotometer (Shimadzu Model, UV 160A) calibrated at 264 nm. In absence of the measurement of the $C^+$ concentration in the aqueous phase, $[C^+]$ was assumed to be equal to the exchanged 6-APA amount i.e. $[C^+] = [QCl]_p$. Thus $[C^+]$ can be eliminated from the $K_i$ expression which can be simplified to the following form

$$K_i = \frac{[QCl]_{int} - [QCl]_p}{[H₃P]_{int} - [H₃P]_p}$$  

By plotting $[QCl]_{int}$ vs $[H₃P]_{int}$, $K_i$ can be determined by a linear regression of the data.

The extraction kinetics was studied in a stirred cell of standard design reported in literature (Haenschel et al., 1986). 6-APA was transferred between the aqueous solution and organic phase of Aliquat-336 which has been equilibrated with the buffer used. Both the liquid phases were independently stirred, however without causing phase dispersing such that the geometric area (19.2 cm²) can be considered as the interfacial area. 6-APA concentration was measured at an interval of about 5 minutes.

Results and discussion

EXTRACTION EQUILIBRIUM

The extraction efficiency of 6-APA appears to be influenced by the buffer capacity of the aqueous phase through a dependence on aqueous phase pH. This pH dependence is advantageous because of the ease of extraction of the anion at high equilibrium constant. The values of the equilibrium constant of 6-APA were determined for various combinations of 6-APA and Aliquat-336 concentrations at different pH values. Figures 2 and 3 show the plots used to determine the value of $K_i$. As expected, $K_i$ increases with an increase of pH up to a pH of 8.0. However, our experiments at high pH (pH 9.5) showed reduced extraction or no extraction at all. This must be attributed to the probable decomposition of 6-APA and co-extraction of $OH^-$ and buffer anions at high pH condition. In as much as the high pH condition is favourable to keeping 6-APA in a highly dissociated form can otherwise be considered to provide a higher $K_i$ value. $C^+$ ion in excess of that required to be replaced by $P^-$ ion is considered as a general measure of the
Figure 2 — Evaluation of equilibrium constant for 6-APA-Aliquat-336 system. Initial 6-APA concentration 0.46 mol/m³; [QCl] = 50–200 mol/m³.

Figure 3 — Evaluation of equilibrium constant for 6-APA-Aliquat-336 system. Initial [QCl] = 200 mol/m³; 6-APA concentration: 0.23–0.93 mol/m³.

The values of $K_a$ were determined from linear regression of the data shown in Figures 2 and 3 and are listed in Tables 1 and 2, wherein the theoretically predicted values (Equations (10) and (11)) are also shown in order to assess the effect of buffer anion co-extraction. The values of the regression coefficient are reasonable to justify that the model satisfactorily describes the equilibrium of the system. Furthermore, since the equilibrium is weakly dependent on the concentration of the species in the two liquid phases, the equilibrium behaviour can be explained by considering the ideality of both the phases (Galán et al., 1994). Any probability of non-ideality of the organic phase may be ruled out because of the negligibly small aggregation of the lipophilic carrier in polar butyl acetate (Asai et al., 1991) used as the solvent. This fact perhaps is reflected also in the observation that at a constant pH, the value of $K_a$ is higher for the case of constant 6-APA concentration (varying carrier concentration) as compared to one for constant carrier concentration (varying 6-APA concentration). Comparable values of $K_a$ (expt.) and $K_a$ (calc.) particularly at low pH values indicate that the effect of co-extraction of the buffer anion is negligible under the experimental conditions used in this study. It may, however, be noted that the deviation of $K_a$ (expt.) from $K_a$ (calc.) at pH > 7.4 is higher than that observed at low pH conditions, which may be attributed to higher degree of co-extraction at high pH condition. As is evident from Figure 4, the highest degree of coextraction occurs at pH = 10. The $K_a$ value at pH = 10 considering coextraction is $18.4 \times 10^{-4}$ (Figure 4), which is lower than the one at pH = 8 ($K_a = 23 \times 10^{-4}$), indicating a relative decrease of the degree of coextraction at low pH. The estimated values of the coextraction constant ($K_a$) are $62 \times 10^{-4}$ and $80.5 \times 10^{-4}$ at pH values of 8.0 and 10.0, respectively. The $K_a$ value at pH = 10 without considering coextraction is $9.5 \times 10^{-4}$, whereas at pH = 8.0,
Partition coefficient vs pH

Initial 6-APA concentration: ______0.46 mol/m³; __________0.93 mol/m³.

Figure 7: The equilibrium concentration of 6-APA, in fact, decreases with decreasing pH. The lower range of 6-APA concentration.

Figure 7 shows the effect of pH on the partition coefficient for various carrier concentrations and two levels of initial 6-APA concentration. The value increases with an increase of pH (up to pH = 8.0), the extent of increase being higher for higher carrier concentrations. A similar effect of carrier concentration was also observed for the extraction of di-phenylamine (Houssel et al., 1986) using the same carrier. The partition coefficient for physical extraction \([QCI] = 0\) is also shown in Figure 7. The value of \(m\) for physical extraction is appreciably lower than that of reactive extraction and the pH variation of \(m\) is insignificant over the range of pH studied in this work.

Extraction kinetics

Since 6-APA is insoluble in the solvent phase and the 6-APA carrier complex has very low solubility in the aqueous phase, the reactive extraction is a case of interfacial reac-
The initial rate of extraction, $N$, was obtained from the following relation:

$$N = \frac{\frac{I}{A}}{\text{d}[P^+]_{a}/\text{d}t}$$

where $\text{d}[P^+]_{a}/\text{d}t$ represents the initial slope of the line obtained by plotting the amount of 6-APA transferred against contact time, $t$. Figures 8 and 9 show the effect of carrier concentration on $N$ for various $[QCI]_o$ and 6-APA concentration, $[P^+]_{a}$ in the aqueous phase. The logarithmic plot is a straight line with a slope of 1.15 at lower concentrations, $[QCI]_o$, whereas the slope is less than unity in the range of higher $[QCI]_o$. Similarly, the plot of $N$ vs $[P^+]_{a}$ (Figure 9) is a straight line with a slope of 1.1 in the range of $[P^+]_{a}$ up to $192 \times 10^{-2}$ mol/m$^3$ beyond which $N$ remains essentially constant. The effect of $pH$ is shown in Figure 10, which incorporates the data at various $[P^+]_{a}$ and $[QCI]_o$. The slopes of the lines representing various $pH$ conditions are almost the same, implying that a common extraction mechanism holds good for the system under study. However, the results shown in Figures 8 and 9 indicate that the mass transfer mechanism of the reactive extraction process is dependent on the ranges of $[QCI]_o$ and $[P^+]_{a}$. At low $[QCI]_o$ and $[P^+]_{a}$, simple two film theory may be used to describe the
mass flux at the interface based on the model shown in Figure 11. The concentration of each species near the interface is obtained in its vicinity such that the interfacial reaction rate is equal to the mass transfer rate of each species as given by

\[ N = k_{pg} ([QCl]_n - [P^n]_n) \]

\[ = k_{op} ([QCl]_n - [QCl']_n) \]

\[ = k_{pp} ([QP]_n - [QP']_n) \]

The flux equation can be coupled by the mass action equilibrium constant given by

\[ K_A = \frac{[QP']_n^2}{[QCl]_n [P^n]_n} \]

Elimination of the interracial concentration yields the following equation, as derived elsewhere (Haemmel et al., 1986).

\[ -N = \frac{V}{A} \frac{d([P^n]_n)}{dt} = \frac{k_p}{V} ([P^n]_n + 0.5B^2 \bar{g} 25B^2 - R) \]

where,

\[ B = k_{QCl} k_{QCl'} \frac{2-K_A([QCl]_n + 3k_p k_{QCl'} [P^n]_n)}{k_p k_{QCl'} K_A - k_p^2 k_{QCl} K_A} + \]

\[ 2k_p k_{QCl} k_{QCl'} [QCl']_n - k_p k_{QCl'}^2 K_A [P^n]_n \]

\[ k_p k_{QCl'}^2 K_A - k_p^2 k_{QCl}^2 \]

and

\[ R = \frac{k_{QCl} k_{QCl'} [QP]_n + k_p [P^n]_n^2}{k_p k_{QCl'}^2 K_A - k_p k_{QCl} K_A} \]

Numerical integration of Equation (16) was carried out to obtain the theoretical profiles of \([P^n]_n\) vs time in a few specific cases. Figure 12 shows the experimental dimensionless concentration profiles at two levels of \([QCl]_n\) and \([P^n]_n\). The calculated profiles are also shown in the figure by the dotted lines. For the calculation, the mass transfer coefficients have been approximated as follows. Referring to Figure 8, the initial rate can be expressed as

\[ N = k_{QCl} [QCl]_n \]

\[ k_{QCl} \text{ calculated from the slope is around } 3.3 \times 10^{-7} \text{ m/s.} \]

\[ k_{op} \text{ estimated from} \]

\[ k_{pp} \]

where the diffusivities, \(D_{QP}\) and \(D_{OP}\), were calculated from the well known Wilke-Chang (1955) correlation and the values were found to be 1.1 \(\times 10^{-9}\) and 1.3 \(\times 10^{-9}\) m/s, respectively. Similarly, the value of \(k_{pp}\) calculated from the plot of \(N\) vs \([P^n]_n\) (Figure 9) was found to be 4.6 \(\times 10^{-7}\) m/s. It may however be noted that the use of these mass transfer coefficients is quite conservative in as much as their values can be considered to change with time of extraction. As shown in Figure 12, the experimental and calculated concentration profiles agree fairly well when the concentration of the species are low. However, deviation could be observed for high concentrations of both the species. This may be attributed to probable error in the values of the mass transfer coefficient which appear to be rather low. Since we do not anticipate any enhancement in their values by further increasing the stirrer speed (leading to phase dispersion), we presume that some other physio-chemical processes occurring at the interface affect the extraction rate. It is known that Aliquat-336 is a surface acting agent and lowering of interfacial tension between the aqueous solution and the butyl acetate is quite significant at high \([QCl]_n\) as reported in our previous work (Ghosh et al., 1995). The extraction kinetics may be analysed from an interfacial reaction model incorporating the effect of carrier adsorption at the interface. Such a model has been used to interpret extraction kinetics for metal ions like Cu, Ni etc. using various chelating agents (Tumono et al., 1985; Yoshizuka et al., 1985). We intend to report a detail analysis of the extraction kinetics in a separate communication.

**Conclusion**

A liquid-liquid ion exchange mechanism can be exploited for reactive extraction of 6-Aminopenicillanic acid from aqueous solution using Aliquat-336 and butyl acetate as the carrier and the solvent, respectively. The equilibrium constant and partition coefficient are influenced by the aqueous phase pH. The reactive extraction is a typical interfacial reaction. The simple two-film model provides a satisfactory description of the mass transfer process, particularly at low 6-APA and carrier concentrations.

**Acknowledgement**

The authors gratefully acknowledge the financial support from DSI-New Delhi vide grant no III-4 (15)/94-ET.
Nomenclature

\[ A = \text{interstitial area, m}^2 \]
\[ [I] = \text{concentration of buffer anion, mol/m}^3 \]
\[ N = \text{transit vector defined in Equation (16), [I]} \]
\[ [Cl^-] = \text{concentration of chloride ion, mol/m}^3 \]
\[ [P^-] = \text{concentration of \(P^-\) anion, mol/m}^3 \]
\[ K = \text{equilibrium constant defined by Equation (14), [I]} \]
\[ K_a, K_b = \text{extraction equilibrium constants defined by Equations (4) and (5), respectively, [-]} \]
\[ k = \text{mass transfer coefficient, m/s} \]
\[ m = \text{distribution coefficient defined by Equation (7), [-]} \]
\[ N = \text{initial rate of extraction, mol/m}^2 . s \]
\[ [P^-] = \text{concentration of 6-APA anion, mol/m}^3 \]
\[ [S] = \text{concentration of substrate complex, mol/m}^3 \]
\[ [S] = \text{concentration of butyl anion - carrier complex, mol/m}^3 \]
\[ [\text{Cl}^-] = \text{concentration of the carrier, Alquat-336, mol/m}^3 \]
\[ r^2 = \text{coefficient of determination} \]
\[ R = \text{parameter defined in Equation (16), [-]} \]
\[ t = \text{time of extraction, s} \]
\[ V = \text{volume of solution, m}^3 \]

Subscript/Superscript

\[ v = \text{aqueous phase} \]
\[ o = \text{organic phase} \]
\[ t = \text{initial value} \]
\[ * = \text{interfacial concentration} \]

References


Extraction of 7-Aminocephalosporanic Acid with Secondary, Tertiary, and Quaternary Amines

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Extraction of 7-aminocephalosporanic acid (7-ACA) has been investigated using secondary and tertiary amine as a carrier and 7-ACA ammonium salt (Aliquat-336) as the activator. The values of the distribution coefficient have been reported in a range of aqueous-phase pH values and concentrations of 7-ACA using different carriers in n-butyl acetate as the diluent. As expected, ion-pair extraction with secondary and tertiary amine exhibits lowering of the distribution coefficient with increase of pH, whereas the ion exchange extraction with quaternary ammonium chloride (Aliquat-336) exhibits an opposite result but more pronounced pH dependence, providing at the same time higher values of distribution coefficient at high values of pH. Both the extraction principles can be exploited to achieve extraction and reextraction of 7-ACA in the pH range of 5 to 8 in which it is relatively stable.

Introduction

7-Aminocephalosporanic acid (7-ACA) is an important intermediate for semisynthetic cephalosporins such as cephalosporin C, ceftazidime, and cefotaxime, etc. It is usually made by chemical deacylation of cephalosporin-C (CPC), which is a fermentation product of Cephalosporium acremonium (Hach et al., 1972). In the classical process, 7-ACA is isolated from the fermentation broth by removal of the solvent under vacuum, dissolution of the residue in water, acidification, and precipitation at the isoelectric point (pH = 3.3) of 7-ACA. However, such methods involve Capital and energy intensive operations particularly when a dilute solution is dealt with. Since 7-ACA is an amphipatic molecule, physical (solvent) extraction is difficult. Various methods of extraction and purification of cephalosporin antibiotics have been discussed in our recent review (Ghosh et al., 1997). Reactive extraction has been thought to be a potential method for recovery of lactam antibiotics from complex culture broth (Ghosh et al., 1996, 1997; Hana et al., 1992). We have been trying to exploit the principle of reactive extraction for developing liquid membrane processes for various cephalosporin antibiotics (Ghosh et al., 1995; Sahoo et al., 1995, 1997). Data on equilibrium and kinetics of reactive extraction are useful for optimizing the operating condition of liquid membrane processes. We have recently reported the use of Aliquat-336 for reactive extraction of 6-aminopenicillanic acid (Bora et al., 1997). In this paper, we report complementary studies on reactive extraction of 7-ACA using secondary and tertiary amines as well as an Aliquat-336 with butyl acetate as the solvent.

Extraction Equilibrium

7-ACA is an amphipatic molecule, and in aqueous solution, it exists in some forms of different charges depending on the pH of the medium. It does, however, show unstable behavior at high pH (Yamane and Tsuji, 1976). At pH below 9.0, the predominant form is cationic, at pH above 4.42, it is amionic, and in the range 2.02 < pH < 4.42, the zwitterion as a whole is predominant as evident from the scheme shown below.

\[ H_3N - CH_2 - COO^- + H^+ \rightarrow H_2N - CH_2 - COOH \]

The pH at which the amino group ionizes keeps the carboxyl group in the COO\(^-\) form.

The reactive extraction of 7-ACA with aliphatic amines can proceed as follows. 7-ACA (HP) dissociates in the aqueous phase to give an anion, P, and a proton, H\(^+\), as

\[ HP \rightarrow H^+ + P^- \]

The dissociation constant, \(K_a\), is given by

\[ K_a = \frac{[H^+][P^-]}{[HP]} \]  

(1)

where \(C_{HP}\), \(C_{P^-}\), and \(C_{H^+}\) are concentrations of H\(^+\), P\(^-\), and HP, respectively.

The amine, A, dissolved in the organic phase reacts with 7-ACA anion, P\(^-\), and the proton, H\(^+\), in the aqueous phase. In a simple (1:1) stoichiometry, the reaction is given by
The equilibrium constant, $K_d$, and the distribution coefficient, $K_P$, are given by

$$K_d = \frac{P_A}{C_A}, \quad (1)$$

$$K_P = \frac{C_{P_H} - C_P}{C_{P_H} - C_{P_T}} \cdot (2)$$

where $C_{P_H}$ and $C_{P_T}$ are the overall concentrations of acid and amine, respectively.

Considering physical extraction of undissociated 7-ACA, the following equations (Fieschke and Schugorl, 1984) were used for theoretical prediction of $K_d$ and $K_P$

$$K_d = \frac{C_{P_{III}} - C_P}{C_{P_{III}} - C_{P_T}} \cdot (3)$$

The distribution coefficient, $K_d$, is defined as

$$K_d = \frac{C_{P_T}}{C_P} \cdot (4)$$

Experimental Section

Materials. Table 1 shows the pertinent properties of the carriers investigated in this work. 7-ACA was procured from Albright Chemical Co. (United States). Butyl acetate, acetic acid, sodium mono/diphosphato, citric acid, NaCl, etc., were procured from E. Merck (India) and were of analytical grade and were used without further purification.

Methods. 1. Measurement of Distribution Coefficient. The distribution coefficient $(K_d)$ was measured in a 50-mL stopped flask provided with magnetic stirrer. The experiments were carried out at 25 °C by mixing 10 mL each of the aqueous 7-ACA solution and butyl acetate solution of the carrier. The pH values of the aqueous phase maintained by using standard buffer solution ranged from 4 to 8. Carbonate-bicarbonate buffer was used to maintain the aqueous phase pH of 9.0, while phosphate buffer was used for pH values of 6, 7, and 8. Citrate-phosphate buffer was used to maintain the pH values at 2.5, 3.4, and 6. Carbonate-bicarbonate buffer was prepared by using 0.2 M solution of sodium carbonate and 0.2 M solution of sodium bicarbonate. 4.0 mL of 0.2 M sodium carbonate was mixed with 46.0 mL of sodium bicarbonate, and the resulting solution was diluted to a total volume of 200 mL each to obtain pH values of 9.0. For phosphate buffer, 5.3, 39.00, and 87.7 mL of 0.2 M monobasic sodium phosphate solutions were mixed with 94.7, 61, and 12.3 mL of 0.2 M dibasic sodium phosphate solutions, and the resulting solutions were diluted to a total volume of 200 mL each to obtain pH values of 8, 7, and 6, respectively. Similarly for citrate-phosphate buffer, 44.6, 39.6, 54.7, and 24.3 mL of 0.2 M citric acid solution were mixed with 5.4, 10.3, 10.3, and 25.7 mL of 0.2 M dibasic sodium phosphate solutions, and the resulting solutions were diluted to a total volume of 200 mL each to obtain pH values of 2.5, 3.4, and 6, respectively. The concentrations of 7-ACA in the aqueous phase and that of the carriers in the organic phases were varied over a wide range in order to assess the concentration effect. An equilibrium time of 2 h was provided in each experiment. After attainment of the equilibrium and phase separation, the concentration of 7-ACA and the pH change were determined. 7-ACA concentration was determined in a UV-visible recording spectrophotometer (model UV 160A Shimadzu), which was calibrated at 264 nm. Analysis was done in triplicate, and the reproducibility was within ±1%.
Figure 1. Effect of carrier on the pH dependence distribution coefficient ($K_p$). $C_t = C_p = 1.0 \times 10^{-3}$ mol/L; (O) diethylamine; (C) Amberlite LA-2; (A) Amberlite LA-2; (a) tri-n-octylamine; (A) Aliquat-336; (•) $C_A = 0$.

Results and Discussion

Effect of Carrier. For discussion on carrier effect and pH dependence if $K_p$, experimental data generated with butyl acetate were considered, as this being a proton-donating diluent leads in general to stability of the acid-carrier complex (owing to the formation of hydrogen bond with the diluent; Bieck et al., 1993).

Primary amines have not been used as they are amenable for formation of stable emulsion and are too soluble in water. The pH dependence of $K_p$ for various carriers is shown in Figure 1 for the pH range of 5-8 where 7-ACA is relatively stable. As shown in the figure, $K_p$ for ion-pair extraction with amino decreases with an increase of pH and ultimately drops to zero at certain values of pH. The most effective amine carrier appears to be Amberlite LA-2, which, however, exhibits weaker and opposite pH dependence of $K_p$ as compared to that achieved with Aliquat-336, which provides a liquid-liquid ion-exchange extraction. Table 2 shows the experimental equilibrium constant ($K_p$) values for the carriers, and it is apparent that, out of the secondary and tertiary amines studied, trioctylamine exhibits the highest $K_p$ values and stronger pH dependence, implying that smaller pH gradient may be adequate for extraction and reextraction. It may be inferred from Figure 1 that Aliquat-336 should be the preferred choice for the reactive extraction of 7-ACA as both extraction and back-extraction can be performed easily at pH values of 8 and 5, respectively, and it provides finite extraction at both low and high pH values, unlike the behavior exhibited by the secondary and tertiary amines. For extraction of carboxylic acids, Aliquat-336 was superior to triethylamine and butyl acetate as the solvent: $C_p = (1-1.6) \times 10^{-3}$ mol/L; $C_A = (1-10) \times 10^{-3}$ mol/L; pH = 4; (O) Amberlite LA-1; (a) diethylamine; (A) tri-n-octylamine.

Reactive Extraction with Secondary Amine $n$-Butyl Acetate Systems. The variation of $K_p$ with pH of 7-ACA solution for the diethylamine-$n$-butyl acetate system is shown in Figure 3a, from which the effect of carrier concentration, $C_t$, is quite apparent. At fixed pH and 7-ACA concentrations, $K_p$ increases for an increase of $C_t$, the effect being more pronounced at low pH, whereas at high pH $K_p$ tends to attain asymptotic values of similar orders of magnitude. As compared to diethylamine, Amberlite LA-1 exhibits relatively low pH dependence of $K_p$.
as shown in Figure 3b. For Amberlite LA-2, an increase in carrier concentration (Figure 3c) increases the pH dependence of $K_d$ and the magnitude of $K_d$ values are higher than those of diocetylamine and Amberlite LA-1 in the pH range of 5 to 8. Thus, the extraction can be carried out at pH 5 and reextraction at pH 8, and the extraction efficiency can be controlled by the concentration of the carrier. Stronger pH dependence of $K_d$ was reported also for the extraction of Penicillin-G by Amberlite LA-2 (Schugerl and Degener, 1992). This is advantageous as the extraction and reextraction can be achieved through a shift in the pH value of the aqueous phase. The other advantage is that for a fixed carrier concentration and aqueous-phase pH value, $K_d$ increases with a decrease in the 7-ACA concentration, and thus reactive extraction will be more effective at low concentration such as that encountered in fermentation broth.

In Figures 3a–c, the smoothed curves are calculated values whereas the experimental points are represented by the symbols. For all the secondary amines, the agreement between measured and calculated profiles of $K_d$ vs pH cannot be considered reasonable possibly because of the deviation from a 1:1 reaction stoichiometry.

For this extraction, a nonstoichiometric reaction model can be suggested as follows:

$$mA + n\text{II}^- + n\text{H}^+ \leftrightarrow A_n\text{(HP)}$$

(15)

for which the equilibrium constant, $K_v$, is given by (Reschke and Schugerl, 1984)

$$K_v = \frac{(1/n)C_{V,\text{II}} - C_{\text{II}})}{C_{\text{H}^+}C_P^nC_{\text{HP}} - (m/n)C_{\text{H}^+}C_{\text{II}}C_P^n}$$

(16)

By using the calculated $K_v$ vs pH curve to the measured curves, a formal complex composition can be established. In this way, the identified complex compositions are $A_1\text{II}$ (HP) for diocetylamine, $A_2\text{III}$ (HP) for Amberlite LA-1, and $A_2\text{III}$ (HP) for Amberlite LA-2. The calculated slopes of the lines in Figure 2 are also less than unity for all these amines implying the validity of a nonstoichiometric model in the reaction.

Reactive Extraction with Tertiary Amine. In this work, triocetylamine was used as the only tertiary amine for which the results on $K_d$ are shown in Figure 4. In this case also, the agreement between experimental data and the computed profiles based on simple stoichiometric eq 3 appears unsatisfactory. The formal complex composition computed by fitting the data on $K_d$ has been found to
Figure 6. Distribution coefficient \(k_d\) as a function of the pH of 7-ACA solution at various concentrations of Aliquat-336: \(C_P = 1 \times 10^{-5}\) mol-L\(^{-1}\); (tri) \(C_A = 5 \times 10^{-3}\) mol-L\(^{-1}\); (tri) \(C_A = 1 \times 10^{-3}\) mol-L\(^{-1}\); (mai) \(C_A = 1 \times 10^{-2}\) mol-L\(^{-1}\).

Figure 7. Variation of extraction equilibrium constant with relative permittivity \(\mu\) of solvents at pH = 8 for Aliquat-336 and pH = 4 for tri-\(n\)-octylamine: \(C_P = (1-1.5) \times 10^{-3}\) mol-L\(^{-1}\); \(C_A = (1-10) \times 10^{-3}\) mol-L\(^{-1}\); (tri) \(C_A = (1-10) \times 10^{-3}\) mol-L\(^{-1}\); (tri) \(C_A = 1 \times 10^{-3}\) mol-L\(^{-1}\); (tri) \(C_A = 1 \times 10^{-3}\) mol-L\(^{-1}\).

Table 3. Equilibrium Constants (\(K_p\)) for Extraction of 7-ACA in Different Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>(10^6K_P)</th>
<th>(10^6\mu_P(\text{tri-octylamine/}))</th>
<th>Relative Permittivity</th>
<th>(\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td>butyl acetate</td>
<td>20.00</td>
<td>3.08</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>1-octanol</td>
<td>11.00</td>
<td>3.7</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>dichloromethane</td>
<td>7.05</td>
<td>3.01</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>chloroform</td>
<td>3.7</td>
<td>2.94</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>cyclohexane</td>
<td>1.71</td>
<td>2.09</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>benzene</td>
<td>1.11</td>
<td>1.75</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

* \(C_P = 1 \times 10^{-3}\) mol-L\(^{-1}\), and \(C_A = (1-10) \times 10^{-3}\) mol-L\(^{-1}\).

shows also the effect of carrier concentration. An increase in pH and carrier concentration increases \(K_P\), the pH effect being attributable to the large fraction of dissociated 7-ACA anion at high pH values. Furthermore, the pH dependence of \(K_P\) is appreciably higher than that obtained with any of the amines within the pH range of interest. Thus, this extraction can be controlled by properly maintaining the pH value of the 7-ACA solution and the carrier concentration in the organic phase. The measured and calculated \(K_P\) values agree fairly well at low carrier concentrations, but deviation seems to occur at higher carrier concentration. The curves were calculated with a predetermined \(K_P\) value of 1.65 under the studied experimental conditions. This deviation may be attributed to the coextraction of buffer anion, an effect being inferred also from the observed pH shift of buffered 7-ACA solution. Indeed, the coextraction of anion such as OH\(^-\), CO\(_3\)^{2-}, SO\(_4\)^{2-}, and PO\(_4\)^{3-} by Aliquat-336 was found to be comparable to that of amino acid extraction (Hanno et al., 1989; Haenel et al., 1986) at the pH under which the amino acids exist in the anionic form.

**Solvent Effect.** Solvent effect was studied using n-butyl acetate, n-octanol, dichloromethane, cyclohexane, chloroform, and benzene with Aliquat-336 and tri-\(n\)-octylamine at pH 8 and pH 4, respectively. The observation of our study on solvent effect is dissimilar to that obtained for extraction of organic acid using tri-\(n\)-octylamine (Puttemans et al., 1985), in which the solvent found to be in the order: dichloromethane, pentanol, chloroform, methyl isobutyl > butyl acetate > hexane. We have attempted to correlate the \(K_P\) values with solvent relative permittivity \(\mu\) \(D^{-1}\), where \(\mu\) and \(D\) are the dipole moment and dielectric constant, respectively, and the relationship can be understood from Table 3 and Figure 7. It appears that for both ion-exchange and ion-pair extractions, the values of \(K_P\) increase with increase of dipole moment implying that extraction probably occurs via solvation of the complex based on dipole–dipole interaction. Similar solvent effect was observed for amino acid extraction of citric acid (Blieck et al., 1993).

**Loading Effect of Carriers.** Loading of the extractant, \(Z\), is defined as the ratio of the total concentration of acid, 7-ACA (all forms), in the organic phase to the total concentration of carrier (all forms) in the organic phase, at equilibrium. Thus for amines

\[
Z = \frac{C_{P,\text{org}}}{C_{A,\text{org}}} = \frac{C_{P,\text{org}}}{C_{A,\text{org}}} = \frac{C_{P,\text{org}}}{C_{A,\text{org}}} \tag{17}
\]

and for Aliquat-336

\[
Z = \frac{C_{P,\text{org}}}{C_{A,\text{org}}} = \frac{C_{P,\text{org}}}{C_{A,\text{org}}} \tag{18}
\]
The loading curve in a plot of $Z$ versus $C_{rav}$ as shown in Figure 8. For all the amines and Alquat-336 studied in this work, no overlapping ($Z < 1$) was observed, implying that complexes with not more than one acid molecule per amine molecule have been formed. One T-ACA molecule may complex with more than one amine molecule. It may be presumed that the systems are characterized by only one amine per complex, which is further evidenced by the observation of no effect of total amine concentration on the loading. These results also indicate that the systems do not exhibit aggregation even at the pH that provides a higher degree of saturation.

Stability of T-ACA. The stability of T-ACA in aqueous solution is affected by specific $H^+$ ion catalyzed, spontaneous, and specific hydroxide ion catalyzed degradation. The spontaneous degradation rate could be influenced by the dissociation of the ammonium group. However, the stability is not known under reactive extraction conditions. The effect of carrier and n-butyl acetate on the T-ACA stability was studied by first equilibration of T-ACA solution with equimolar ($10 \times 10^{-3}$ mol/L) carrier in n-butyl acetate and then mixing the organic solution with fresh T-ACA solution at two levels of pH 6.5 and 8 for an extended period of time, during which T-ACA concentrations were monitored periodically. The first-order decomposition rate constant, $k$, given by

$$
\ln(C_p/C_{p0}) = -kt
$$

was evaluated from the experimental profiles of $C_p/C_{p0}$ vs time, $t$, where $C_{p0}$ is the initial concentration of T-ACA.

Conclusions

Secondary, tertiary, and quaternary amines can be used as the carriers for reactive extraction of T-ACA in the pH range of 5 to 8 in which T-ACA is relatively stable. In case of extraction with secondary and tertiary amines, the distribution coefficient, $K_d$, decreases with an increase of pH of T-ACA solution. An opposite but more pronounced pH dependence of $K_d$ is obtained for extraction with Alquat-336, which also provides values of $K_d$ higher than those of secondary and tertiary amines in the above pH range. Unlike the secondary and tertiary amines, Alquat-336 complexes with T-ACA anion in a simple 1:1 stoichiometric ratio. Stability of T-ACA should be considered while performing reactive extraction with the carriers.

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Received for review September 9, 1997. Accepted January 22, 1998.

Financial support from the Department of Science and Technology, New Delhi, India, vide grant III-41394-ET is gratefully acknowledged.

JE9702192
Extraction Equilibria of Cephalosporin Antibiotics with Aliquat-336

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The extraction equilibria of Cephalosporin antibiotics with Aliquat-336 (tricaprylylmethylammonium chloride) in various solvents were studied at pH values above the pKₐ values of the antibiotics. The reaction extraction, which is essentially an anion-exchange reaction, takes place in a 1:1 stoichiometry. The extraction equilibrium constants (Kₑ) could be correlated well with the hydrophobicity scale of the antibiotics. The equilibrium constants for coextraction of the buffer ions were found to be lower than those of the cephaparin antibiotics, indicating the possibility of exploiting the reactive extraction technique for process application. The Kₑ values obtained for a specific solute were also found to correlate well with the dipole moment of the solvents.

Introduction

Reactive extraction in a liquid membrane can provide an effective method for separation and purification of cephaparin antibiotics from dilute solutions (Ghosh et al., 1996, 1997). Accordingly, extensive studies have been performed to demonstrate the feasibility of liquid membranes for their separation (Ghosh et al., 1996; Sahoo et al., 1996, 1997, 1999a,b). Complementary studies on reactive extraction with carriers such as secondary, tertiary, and quaternary amines have also been reported, and it was found that Aliquat-336 is the best choice of carrier for specific cases (Horn et al., 1997, 1998; Hano et al., 1992). The same carrier has also been found to be effective for reactive extraction of amino acids (Hano et al., 1991; Ibenvel et al., 1991), clavulanic acid (Harris et al., 1990), and other amine species (Galan et al., 1991).

In this paper, we report a comprehensive study on extraction equilibria of various cephaparin antibiotics with Aliquat-336 in butyl acetate as the solvent and the relationship of the equilibrium constant with the hydrophobicity of the antibiotic molecules. 6-APA, a beta-lactam with a five-membered thiazolidine ring which belongs to the penicillin group, is also used for the study for the purpose of comparison with the cephaparin. In addition, the extraction equilibrium constant for a specific solute obtained in various solvents was correlated with a molecular property of the solvents.

Theoretical Considerations

The structures of the cephaparin molecules considered for this investigation are shown in Figure 1. As a result of the presence of carboxylic acid and amine groups, all the molecules exist in an ionic form of different charges depending on the pH of the medium. At pH < pKₐ, the predominant form is cationic, at pH > pKₐ the predominant form is anionic, and in the pH range pKₐ < pH < pKₛ, the zwitterion as a whole is predominant.

The anionic form of the molecule is amenable for ion-exchange with Aliquat-336 dissolved in a solvent providing the reactive extraction. The cephaparin anion, P⁻, in the aqueous phase complexes with Aliquat-336 (hereafter termed as QC₁) according to

$$\text{P}^- + \text{QC} \rightleftharpoons \text{QP} + \text{Cl}^-$$

(1)

The anion-exchange reaction takes place at the interface of the organic phase, the P⁻ ion being extracted as a complex, QP, to the organic phase, liberating Cl⁻ into the aqueous phase.

The cephaparin molecule (HP) first dissociates in aqueous solution into the anion, P⁻, and a proton, H⁺, as follows

$$\text{HP} \rightleftharpoons \text{H}^+ + \text{P}^-$$

(2)

The dissociation equilibrium constant, K_d, is given by

$$K_d = \frac{C_{HP}}{C_H C_P}$$

(3)

where C stands for the concentration and the subscript represents the species. In eq 3 and subsequent expressions for equilibrium relationships, the charges of the ions are omitted for the sake of simplicity.

The extraction equilibrium constant, Kₑ, is given by

$$K_e = \frac{C_{QP} C_{Cl}}{C_{Q} C_{P}}$$

(4)

The coextraction of buffer ion, A⁻, by QC₁ at the interface may take place according to

$$\text{A}^- + \text{QC} \rightleftharpoons \text{QA} + \text{Cl}^-$$

(5)
The equilibrium constant, $K_A$, of cationization is given by

$$K_A = \frac{C_{eq1}}{C_{eq} C_A}$$  \hspace{1cm} (6)

The following material balance equation holds for Aliquat-336/Cl

$$V_e C_{eq1} = V_i C_{eq,1} - V_e C_i$$  \hspace{1cm} (7)

and for cephalosporin

$$V_e C_{eq} = V_i C_{eq,1} - C_{eq,1}$$  \hspace{1cm} (8)

where $V_i$ and $V_e$ represent the volumes of the aqueous and organic phases, respectively, and the subscripts $i$ and $e$ stand for the initial and equilibrium values, respectively.

Defining $pK_a$ as $-\log K_a$ and considering the Hencelbach–Henderson equation (Hencel et al., 1996)

$$C_p = C_{eq,1} \left[1 - \frac{1}{1 + 10^{pK_a}}\right]$$  \hspace{1cm} (9)

The extraction equilibrium constant can be arranged as

$$K_p = \frac{V_e C_{eq,1}}{V_i C_{eq,1}}$$  \hspace{1cm} (10)

Equation 10 follows from eq 4, and by plotting $C_{eq,1}/C_i$ versus $C_{eq,1}/C_p$, the equilibrium constant ($K_p$) value can be determined. In the absence of measurement of the $C_i$ concentration in the aqueous phase, $C_{eq,1}$ may be assumed to be the exchanged cephalosporin, as may be determined from $V_i C_{eq,1} = V_e C_{eq}$. Thus, $C_{eq,1}$ can be eliminated from the $K_p$ expression, which, for the case of $V_i = V_e$, can be simplified to the following form

$$K_p = \frac{C_{eq}^2}{C_{eq} C_p}$$  \hspace{1cm} (11)

The extraction effect can be considered implicitly by the excess amount of chloride ion present in the aqueous phase after equilibration

$$V_e C_{eq,1} = V_i C_{eq,1} - V_e C_{eq}$$  \hspace{1cm} (12)

By plotting $C_{eq,1}/C_i$ versus $C_{eq,1}/C_p$ on an ordinary graph, the equilibrium constant $K_p$ can be determined from the slope of the line. The slope of the logarithmic plot of the above functions gives an inference of the reaction stoichiometry, and the intercept would also give the $K_p$ value.

**Experimental Section**

**Reagent.** The cephalosporin antibiotics shown in Figure 1 were procured from Sigma Chemical Co. (St. Louis, Missouri), and all were of 99.9% purity. Aliquat-336 (Aldrich, Milwaukee, WI) has a mean molecular weight of 404 and was used as received. The solvent and other analytical grade buffer reagents were procured from BDH (Mumbai India).

**Extraction Equilibria**

The extraction equilibrium experiments were conducted by placing 20 mL each of buffered aqueous cephalosporin solution and the organic solvent of Aliquat-336 in a 100 mL capacity round-bottom flask and mixing the contents with a magnetic stirrer. The temperature was maintained at 30 ± 0.5 °C by putting the flask in a thermostatically controlled water bath. The aqueous-phase pH was maintained at 8 by using phosphate buffer for 6-APA and was used as received. The solvent and other analytical grade buffer reagents were procured from BDH (Mumbai India). The methods for preparation of the buffer solution are the same as those reported in our previous papers (Bora et al., 1997, 1998, 1999). The time of equilibration for the extraction was 4–5 h, as established from the equilibrium concentration measurement. After attainment of the equilibrium and phase separation, the aqueous-phase concentration of cephalosporin was deter-
Table 1. Values of Equilibrium Constants for Extraction ($K_v$) and Coextraction ($K_{Av}$) of Various Antibiotics

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$K_v \times 10^2$</th>
<th>$K_{Av} \times 10^2$</th>
<th>Standard Deviation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cephalosporin-c</td>
<td>89.0</td>
<td>38.2</td>
<td>3.17</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>75.0</td>
<td>25.0</td>
<td>4.74</td>
</tr>
<tr>
<td>7-ADCA</td>
<td>30.0</td>
<td>12.5</td>
<td>1.8</td>
</tr>
<tr>
<td>6-APA</td>
<td>23.0</td>
<td>11.8</td>
<td>5.1</td>
</tr>
<tr>
<td>7-ACA</td>
<td>20.0</td>
<td>9.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>9.0</td>
<td>3.35</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Figure 2 shows the logarithmic plots of $C_{CoL}/C_0$ versus $C_{CoC}/C_0$, from which the slopes of the lines are evaluated to be 0.945, 1.0, 0.95, 0.912, 0.95, and 0.95 for cephalosporin-c, cephalexin, 7-ADCA, 6-APA, 7-ACA, and cephaloridine, respectively. It may, therefore, be inferred that almost all these beta-lactams complex with Aliquat-336 in a 1:1 ratio. The extraction equilibrium is weakly dependent on the concentration of the species in the two liquid phases; the equilibrium behavior can be explained by considering the ideality of both the phases (Ghosh et al., 1995). Any probability of nonideality of the organic phase can be ruled out because of the negligibly small aggregation of the lipophilic carrier in the polar butyl acetate used as the solvent (Assi et al., 1991).

The equilibrium behavior of the reactive extraction with Aliquat-336 may be expected to be different for different solvents depending on their polarity. Accordingly, various other solvents such as 1-octanol, dichloromethane, chloroform, cyclohexane, and benzene have been investigated in order to understand their effect on the extraction equilibrium. Figure 3 shows typical equilibrium plots for the extraction of cephalosporin-c in various solvents. From this figure, it is evident that the $K_v$ values are affected by the nature of the solvent, but the solute-carrier complexation ratio remains unaltered for all the solvents, as may be inferred from the slopes of the lines. Our observation appears to contradict that reported for extraction of carbonylic acid with trietylamine (Tomnides et al., 1990; Bizek et al., 1993; Poposa et al., 1997), in which case the stoichiometry of the complexation reaction was found to be diluent (solvent) dependent and the strength of the complex solvation decreased in the order alcohol > nitrobenzene > halogenated hydrocarbons > ketones > halogenated aromatics > alkyl aromatics > aliphatic hydrocarbons. The decrease in solvation is reflected in the extraction capability of the carrier. For amino extraction of organic acids, the efficiency of the solvent was found to be in the following order: dichloromethane > pentanol > chloroform > methyl isobutyl ketone > butyl acetate > hexane (Putteman et al., 1985). The $K_v$ values determined from linear regression of the data for various other solvents are shown in Table 2. However, the values of the coextraction constants for other solvents have not been estimated.

Results and Discussion

The equilibrium experiments were conducted at different Aliquat-336 and cephalosporin concentrations and at pH values above the higher $pK_a$ value such that only the anionic form of the cephalosporin exists in the aqueous phase. Figure 2 is a plot of $C_{CoC}/C_0$ versus $C_{CoL}/C_0$ used to determine the values of $K_v$ for various cephalosporins when n-butyl acetate was used as the solvent. In the same figure, plots of $C_{CoL}/C_0$ versus $C_{CoC}/C_0$ are also shown in order to assess and quantify the coextraction effect in terms of $K_{Av}$ values.

The $K_v$ values determined from linear regression of the data of Figure 2 have been listed in Table 1. It is apparent that the $K_v$ values obtained without considering coextraction are higher than those obtained by considering coextraction. The difference in the values is assigned to the coextraction constant, $K_{Av}$, which is also shown in Table 1. Such a coextraction effect was observed also in case of extraction of DL-phenylalanine using the same carrier (Hansen et al., 1986).
To provide a semiquantitative relation between extraction efficiency and solvent property, we have attempted to correlate the values of $K_F$ with the polarity as well as the relative permittivity ($\mu/D$) of the solvents, where $\mu$ and $D$ are the dipole moment and debye units, respectively. While no reasonable correlation with polarity could be obtained, the $K_F$ value could be correlated well with $\mu/D$, as shown in Figure 5 for all the betalactam molecules studied. It is apparent that $K_F$ increases with an increase of the dipole moment, implying that the reactive extraction occurs probably via solvation of the complex based on dipole–dipole interaction.

The effect of the chemical nature of the solute on the extraction efficiency of Alquit-336 was analyzed from the data generated in butyl acetate as the solvent. It appears that the $K_F$ value increases with the increase of the carbon number of the beta-lactams, suggesting that hydrophobicity affects the degree of extraction. Figure 6 is a plot of $\log K_F$ versus absolute hydrophobicity (expressed as $J$-mot $^{-1}$), the value of which was measured according to a method reported in the literature (Nozaki and Tanford, 1971). The observed relation between $K_F$ and hydrophobicity is akin to that obtained for amino acid extraction by the same carrier (Iino et al., 1991). From the data, such a relation appears to hold true for all other solvents. However, since the $K_F$ values are relatively low, the relations for other solvents have not been given in Figure 6. A linear correlation was obtained for the hydrophobicity and the initial extraction flux of amino acids also in an emulsion liquid membrane extraction system wherein the principle of reactive extraction with Alquit-336 was exploited (Thien et al., 1988). In our recent communication (Sahoo et al., 1999b), we have also reported a similar correlation with the initial permeation flux for facilitated transport of cephalosporin antibiotics in a bulk liquid membrane system.
Conclusion

The extraction equilibria of various zwitterionic cephalosporin antibiotics with Aliquat-336 have been studied at pH values above the pK_a value for a specific solute, the values of the extraction equilibrium constant obtained in various solvents appear to correlate well with the relative permittivity of the solvents, implying that the extraction occurs in solution of the complex based on dipole–dipole interaction. Furthermore, the extraction equilibrium constant obtained in a specific solvent was found to correlate with the relative values of the exit action equilibrium constant obtained in pH values above the pK_a value. A specific solute will interact with Aliquat-336 have limited kinetic at high pH.

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Received for review September 1, 1999 Accepted January 5, 2000.
REACTIVE EXTRACTION
OF BETA-LACTAM ANTIBIOTICS:
IR SPECTROSCOPIC STUDIES

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1. INTRODUCTION

Reactive extraction in liquid-liquid system can provide effective method for separation of amphoteric beta-lactam antibiotics from dilute solution (Ghosh et al., 1997). This principle can be effectively exploited to develop liquid membrane system which can provide enhanced separation potential (Ghosh et al., 1996). We have reported studies on reactive extraction of
6-APA and 7-aminoccephalosporanic acid with extractants such as secondary, tertiary and quaternary amines and found that Aliquat-336 is the best choice of carrier for specific cases (Bora et al., 1997, 1998, 1999a). The extraction equilibrium constant of various beta-lactams has been correlated with hydrophobicity of the molecules indicating that the solute hydrophobicity plays a dominant role in the extraction equilibrium in a specific solvent (Bora et al., 1999b).

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The stoichiometries of the complexes were analysed from experimental extraction equilibrium data. The mass action law analysis reveals patterns of extraction behaviour, but it is limited to examining the net results of complicated nature of interactions. In this paper, we present a comprehensive spectroscopic study, the results of which have been combined with the mass action law analysis to elucidate the structures of the complexes at a molecular level and develop an understanding of the nature of the chemical interactions involved in the reactive extraction of 6-APA and CPC with Amberlite LA-2, Trioclyamine and Aliquat-336.

1.1. Equilibrium Relationship: Theoretical Background

As a result of the presence of carboxylic acids and amino groups in the molecule, both 6-APA and CPC exist in the form of zwitterion in aqueous solution depending on the pH of the medium. The values of the dissociation constant for 6-APA are $pK_{a_1} = 2.3$ and $pK_{a_2} = 5.1$ whereas for CPC, $pK_{a_1} = 2.6$, $pK_{a_2} = 3.1$ and $pK_{a_3} = 9.3$.

1.2. Reactive Extraction with Aliphatic Secondary and Tertiary Amines (Ion-pair Extraction)

In a simple stoichiometry, the extractant (A) dissolved in the organic phase and reacts with beta-lactam anion (P) and a proton (H+) in the aqueous phase according to

$$\text{A} + \text{P}^- + \text{H}^+ \rightleftharpoons \text{AHP}$$

(1)

The transport of the beta-lactam anion from one phase to the other requires the co-transport of a proton. The reaction may be assumed to be instantaneous and the extraction rate is controlled by the mass transfer at the interface. The amine needs protonation for the complexation reaction which is favoured at lower pH range.

The equilibrium constant $K_F$ of the reaction is defined as
REACTIVE EXTRACTION OF BETA-LACTAM ANTIBIOTICS

\[ K_F = \frac{[\text{AHP}]}{[\text{A}][\text{P}][\text{H}]} \]  \hspace{1cm} (2)

where the charge of the ion is omitted for simplicity.

The overall material balance of beta-lactam and amine can be expressed as

\[ [\text{P}] - [\text{P}_0] = [\text{AHP}] \]  \hspace{1cm} (3)

\[ [\text{A}] = [\text{A}_0] - [\text{AHP}] \]  \hspace{1cm} (4)

The value of [AHP] in Eq. (2) was estimated from Eqs. (3) and (4) for deducing the equilibrium relationship.

1.3. Reactive Extraction with Quaternary Onium Salt
   (Ion-exchange Extraction)

The mechanism known as liquid-liquid ion exchange, involves water insoluble extractant and counter transport of a second anion occurs in order to maintain the electroneutrality. The removal of a beta-lactam anion \( (P^-) \) from the aqueous phase by an ion-exchange with the anion \( (\text{Cl}^-) \) of the extractant, Aliquat-336 (QC1) dissolved in the organic phase takes place according to the reaction in a simple stoichiometry

\[ P^- + QC1 \rightleftharpoons QP + Cl^- \]  \hspace{1cm} (5)

The extraction efficiency depends on the type of beta-lactam molecule (dissociation constant) and the solvent. For the ion-exchange extraction to be efficient, it is necessary that the beta-lactam molecule be present in the anionic form \( \text{P}^- \), at pH above the upper \( pK_a \) value.

The equilibrium constant, \( K_F \) of this reaction is given by

\[ K_F = \frac{[QP][Cl^-]}{[P^-][QC1]} \]  \hspace{1cm} (6)

where, the charge of the ion is omitted for simplicity.

In absence of the measurement of Cl' concentration and on the assumption of \([QP] = [Cl^-] \), \( K_F \) may be expressed as

\[ K_F = \frac{[QP]}{[P^-][QC1]} \]  \hspace{1cm} (7)
The logarithmic plots of the experimental values of the numerator and denominator of Eqs. (2) and (7) give inference of the stoichiometric ratios of the complexation reaction. Such inference may be substantiated from the IR spectroscopic studies as discussed in the section to follow.

2. EXPERIMENTAL

2.1. Reagents

Cephalosporin-C and 6-APA used as the beta-lactams were procured from Sigma Chemical Co. (St. Louis, Missouri, USA) and are of 99.9% purity. Aliquat-336 (Aldrich, Milwaukee, USA), Amberlite LA-2 and Trimethylamine (Fluka) were used as received. Butyl acetate and other analytical grade buffer reagents were procured from BDH (Mumbai, India).

2.2. Method

The equilibrium experiments were carried out at 25°C by mixing 10 ml each of buffered aqueous cephalosporin solution and organic solution of butyl acetate with the extractant in a stoppered glass flask of 100 ml capacity. The aqueous phase pH of 6-APA and CPC solutions was maintained at a value of 4 by using citrate buffer for extraction with secondary and tertiary amines (ion-pair extraction). In case of extraction with the quaternary amine salt the aqueous phase pH values were maintained at 8 and 10 for 6-APA and CPC, respectively, i.e., above the upper pKa value at which the beta-lactam exists in the anionic form. In case of Cephalosporin-C, the pH was maintained at 10.0 (above pKa, value 9.3) by using a carbonate-bicarbonate buffer for extraction with quaternary amine salt (Aliquat-336). The method for preparation of the buffer solution are the same as reported in our previous papers (Bora et al., 1997, 1998). The time of equilibration for the extraction was 2 to 3 hours. After attainment of equilibrium, the aqueous and organic phases were separated and the aqueous phase concentrations were determined by UV-Visible Spectrophotometer (Shimadzu, Model 160 A) which was calibrated at 262 nm and 204 nm for CPC and 6-APA, respectively. Analysis was done in triplicate and the reproducibility was within ± 5%.

IR spectra were recorded in a Perkin-Elmer Model 237B ratio-recording double beam IR spectrometer with a 0.02 cm NaCl window. Chloroform and KBr were used as the neat materials and all spectra were recorded at room temperature. Nitrogen gas was passed through the organic phase to
remove the moisture from the organic phase before taking the spectra of the solute-carrier complex.

3. RESULTS AND DISCUSSION

Infrared spectroscopic studies reported in the literature support the mass action law of carboxylic acid with amine extractants (Tamada et al., 1989). It was found that complexes with more than one acid per amine molecule are formed (Barrow and Yerger, 1954). The acid first interacts directly with the amine to form an ion-pair and the OH of the second acid forms a hydrogen bond with the conjugated CO\(^{-}\) of the carboxylate of the first acid to form a (2:1) complex. The findings of Smith and Vitoria (1968); Detar and Novak (1970); Duda and Szafrań (1978) and Chibizov and Komissarova (1984) for complexation of a variety of carboxylic acids with aliphatic amines in various solvents also supported this type of bonding. This was in agreement with the stable complex observed to be formed in their batch extraction experiments.

The IR spectra obtained in the present systems appear to exhibit nearly identical behaviour as discussed below:

3.1. Cephalosporin-C with Amberlite LA-2

The IR spectra of pure CPC (Fig. 1) show peaks at 3300 cm\(^{-1}\) (N—H stretching vibration of primary amine group), 2920 cm\(^{-1}\) (C—H stretching of \(-\text{CH}_2\) or \(-\text{CH}_3\) group), 2350 cm\(^{-1}\) (sulphur in the thiazolidine ring), 1730 cm\(^{-1}\) (>\(\text{C} = \text{O}\) stretching of carboxylic group), 1650 cm\(^{-1}\) (>\(\text{C} = \text{O}\) stretching of carboxylic group).
stretching of secondary amine group), 1550 cm$^{-1}$ (>N—H deformation of amine group) and 1250 cm$^{-1}$ (C—O stretching of carboxylic group). Similarly for Amberlite LA-2, the peaks are observed at 3400 cm$^{-1}$ (N—H stretching of amine group), 2900 cm$^{-1}$ (C—H stretching of $\text{CH}_2$ or $\text{CH}_3$ group), 1745 cm$^{-1}$ (>C=O stretching of carboxylic group) and 1550 cm$^{-1}$ (N—H deformation of amine group). The peak 3400 cm$^{-1}$ in case of Amberlite LA-2 is broad due to the secondary amine group present in the compound. As it is evidenced from the Figure 2 and also Table 1 at (1:1) solute-amine ratio, the sharp band due to the primary amine group retains its property and is dominant over the weak peak of secondary amine. However, the band almost disappears at a ratio of 1:10. The methyl or methylene group peaks due to C—H stretching vibration do not show any remarkable change either in case of the pure compounds (2920 cm$^{-1}$ for CPC and 2900 cm$^{-1}$ for Amberlite LA-2) nor in the complex at both the solute-amine ratios. A distinguishable change has been observed in case of the $\text{C} = \text{O}$ group peak, which appears at 1730 cm$^{-1}$ for pure CPC and has shifted to 1720 cm$^{-1}$ for (1:1) solute-amine ratio and 1725 cm$^{-1}$ for (1:10) solute-amine ratio. This may be attributed to the formation of carboxylate ion in CPC. The generation of a negative charge on oxygen atom in the carboxylate ion extended over the carboxyl group gives a positive effect to the group and thereby shifts the adsorption to lower frequency range. The peak at 1650 cm$^{-1}$ is due to the secondary amine (—C—NH—) present in CPC which does not show any shifting in frequency indicating no change in chemical environment of this group during complexation. Only the intensity of the peak increases which may be due to change in concentration of new amide groups formed during the chemical change. At solute-amine ratios of
TABLE I Peak assignment (cm⁻¹) of the IR spectra of Cephalosporin complexes with various extractants

<table>
<thead>
<tr>
<th>CPC</th>
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<th>CPC: LA-2</th>
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<td></td>
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<td>Amberlite LA-2</td>
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<tr>
<td>3300 (s)</td>
<td>3300 (s)</td>
<td>3350</td>
<td></td>
<td>— NH₂ Amine group — N—H — stretching</td>
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<td>2920 (s)</td>
<td>2900 (w, w)</td>
<td>2900 (m)</td>
<td>2900 (m)</td>
<td>— CH₂ — or — CH₃ group C—H stretching</td>
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<tr>
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<td>-</td>
<td>2350 (m)</td>
<td>2350 (m)</td>
<td>Cephalosporin sulphur</td>
</tr>
<tr>
<td>1745 (w)</td>
<td>1720 (v, s)</td>
<td>1720 (s)</td>
<td>1725 (s)</td>
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<td>-</td>
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<td>1550 (s)</td>
<td>— C = O stretching of secondary amide group</td>
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<tr>
<td>1250 (w)</td>
<td>-</td>
<td>1250 (m)</td>
<td>1250 (s)</td>
<td>— N—H deformation ammonium salt/amide</td>
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<td>2900 (s)</td>
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<td>2350 (m)</td>
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<td>2350 (m)</td>
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<tr>
<td>1740 (s)</td>
<td>1720 (w)</td>
<td>1720 (s)</td>
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<tr>
<td>1550 (w)</td>
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<td>1550 (w)</td>
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<tr>
<td>1250 (w)</td>
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<table>
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<th>QCI</th>
<th>CPC: QCI</th>
<th>CPC: QCI</th>
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<td></td>
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<td>Aliquat-336 (QCI)</td>
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<td>1300 (s)</td>
<td>3300 (s)</td>
<td>3300 (v, w)</td>
<td>3350 (m)</td>
<td>— N — H — stretching amine group</td>
</tr>
<tr>
<td>2920 (s)</td>
<td>2900 (v, w)</td>
<td>2900 (v, w)</td>
<td>2900 (m)</td>
<td>— CH₂ — or — CH₃ group C—H stretching</td>
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<tr>
<td>2350 (m)</td>
<td>-</td>
<td>2350 (w)</td>
<td>2350 (w)</td>
<td>Cephalosporin sulphur</td>
</tr>
<tr>
<td>$C^+$</td>
<td>$Q$C7</td>
<td>$C^+$ QCI 1.1</td>
<td>$C^+$ QCI 1.10</td>
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</tr>
<tr>
<td>1640 (s)</td>
<td>1715 (s)</td>
<td>1720 (m)</td>
<td>1720 (s)</td>
<td>--- H-V stretching (carboxyl group) --- N-H ---</td>
</tr>
<tr>
<td>1460 (s)</td>
<td>1650 (s)</td>
<td>1650 (m)</td>
<td>1650 (s)</td>
<td>--- C---H deformation ---</td>
</tr>
<tr>
<td>1250 (s)</td>
<td>1250 (m)</td>
<td>1250 (s)</td>
<td>1250 (s)</td>
<td>--- C---O stretching (carboxyl group) ---</td>
</tr>
<tr>
<td>1075 (s)</td>
<td>1075 (s)</td>
<td>1075 (s)</td>
<td>1075 (s)</td>
<td>--- C---N (ammonium salt/amine) ---</td>
</tr>
</tbody>
</table>

* s, v, m, w = strong, very, medium, weak band intensity, respectively
1.1 and 1.10, the peak at 1550 cm⁻¹ of the complex is more prominent in comparison to pure CPC and Amberlite LA-2 which confirms the formation of an ammonium salt during the process. In other words, it can be said that during the process, an ionic complex is formed between the carboxylate ion of CPC and secondary ammonium ion of Amberlite LA-2. Two Amberlite LA-2 molecules may combine at one carboxyl group and with one amine group to form a new amide group giving the complex having CPC to Amberlite LA-2 ratio of 1:2 as shown by the Structure I.

\[ \text{CPC} \rightarrow \text{Amberlite LA-2} \]

3.2. Cephalosporin-C with Trioctylamine

The IR spectra of Trioctylamine (Fig. 3) give the peaks at 2900 cm⁻¹ which is due to the C—H stretching vibration of —CH₂ — or —CH₃ group. Besides this, another broad peak is observed at 1740 cm⁻¹ which may be due to >C = O stretching vibration of the ester group present in butyl acetate. The sharp peak at 3300 cm⁻¹ of CPC which is due to the N—H stretching vibration of primary amine group of the compound does not show any shifting in its position when complexed with Trioctylamine (Fig. 3), the peaks only broadens which may be due to decrease in the number of the —NH₂ group during complex formation. There is no change in the peak position and intensity at 2900 cm⁻¹ (C—H stretching of —CH₂ — or —CH₃ group) indicating no change in the chemical environment surrounding the group. At the solute to Trioctylamine ratio of 1.1 and 1.10,
all other peaks of CPC show a similar trend as discussed earlier in case of CPC and Amberlite LA-2 confirming the formation of 1:2 complex shown by the Structure II.

3.3. Cephalosporin-C with Aliquat-336

In case of Aliquat-336, the IR spectra (Fig. 4) show peaks at 2900 cm⁻¹ for 11 stretching of CH₂ or CH₃ group, 1730 cm⁻¹ (C=O).
strecthing of carbonyl group present in butyl acetate), 1460 cm$^{-1}$ (C—H deformation of —CH$_2$— or —CH$_3$ group) 1250 cm$^{-1}$ (C—O stretching of carboxylate from butyl acetate) and 1075 cm$^{-1}$ (C—N stretching). The peaks at 3300 cm$^{-1}$, 1730 cm$^{-1}$ and 1250 cm$^{-1}$ may be due to the presence of butyl acetate. The peak positions at 3300 cm$^{-1}$ and 2900 cm$^{-1}$ in case of CPC and 2900 cm$^{-1}$ in case of Alquat-336 do not change significantly indicating no change in these groups during complex formation. However, the peak at 1730 cm$^{-1}$ ($>\text{C}==\text{O}$ stretching) of CPC (Fig. 4), shifts to 1720 cm$^{-1}$ when complexed with Alquat-336 for solute-amine ratios of 1:1 and 1:10 which may be due to the formation of the carboxylate ion. One interesting observation is that the peak at 1650 cm$^{-1}$ ($>\text{C}==\text{O}$ stretching of amide group) does not change its position and intensity, unlike the previous case indicating that a 1:1 molecular complex may be formed. This is reasonable as Alquat-336 is a liquid anion exchanger capable of imparting instantaneous ion-exchange reaction with CPC. The probable structure of the complex is shown in III

3.4. 6-APA with Amberlite LA-2

The IR spectra of pure 6-APA (Fig. 5) shown peaks at 3300 cm$^{-1}$ (N—H stretching), 2920 cm$^{-1}$ (C—H stretching), 2350 cm$^{-1}$ (sulphur in the thiazolidine ring), 1760 cm$^{-1}$ ($>\text{C}==\text{O}$ stretching), 1625 cm$^{-1}$ ($>\text{C}==\text{O}$ stretching of ring containing nitrogen adjacent to it), 1420 cm$^{-1}$ (C—H
deformation) and 1250 cm$^{-1}$ (C--O-- stretching), 1030 cm$^{-1}$ (C--N stretching) and 800 cm$^{-1}$ (due to tertiary carbon atom in ring). The peaks for pure Amberlite LA-2 are the same as discussed earlier. The peak positions (Fig. 6) at 3300 cm$^{-1}$ and 2900 cm$^{-1}$ of 6-APA remain unchanged after complexing with Amberlite LA-2 in the ratios of 1:1 and 1:10. However, notable changes take place in the peak positions at 1760 cm$^{-1}$ and 1625 cm$^{-1}$. The peak due to $\gt$C$\equiv$O stretching of the carbonyl group of pure 6-APA shifts to 1730 cm$^{-1}$ for both the solute to amine ratios of (1:1) and (1:10) indicating the formation of carboxylate ion. Similarly, the N--H deformation peak of 6-APA at 1625 cm$^{-1}$ changes to 1600 cm$^{-1}$ indicating the formation of an amide group and a complex having solute to amine ratio of approximately 1:2 as shown by the Structure IV.
3.5. 6-APA with Triocylamine

In this case, the peak positions at 3330 cm\(^{-1}\) and 2900 cm\(^{-1}\) (N—H and C—H stretching respectively) of 6-APA remain unchanged after complexation with Triocylamine in the ratios of 1:1 and 1:10 as shown in Figure 7 and Table II. The peak at 1760 cm\(^{-1}\) which is due to the \(>\text{C}=\text{O}\) stretching of carboxyl group of 6-APA has changed to 1725 cm\(^{-1}\) due to formation of a carboxylate ion. Similarly the N—H deformation peak of 6-APA at 1625 cm\(^{-1}\) has changed to 1600 cm\(^{-1}\) which may be due to the formation of an amide group as discussed in case of 6-APA and Amberlite LA-2. Hence, a similar type of complex having solute to amine ratio 1:2 may be formed as shown by the Structure V.
3.6. 6-APA with Aliquat-336

In this case also, the peaks (Fig. 8) at 3330 cm\(^{-1}\) and 2900 cm\(^{-1}\) of 6-APA remain intact after complexation with Aliquat-336. All other peaks also remain unchanged except the peak at 1760 cm\(^{-1}\) (\(\gt C=O\) stretching) which is changed to 1730 cm\(^{-1}\) after complexation with Aliquat-336 indicating the formation of carboxylate ion giving the ionic complex with Aliquat-336. As Aliquat-336 is a quaternary salt, it reacts directly with the carboxylate ion without any recourse to the formation of amide group and thereby leading to the formation of 1:1 molecular complex as shown by the Structure VI.
TABLE II Peak assignments (cm⁻¹) of the IR spectra of 6-Aminopenicillanic acid with various extractants

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<tr>
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<tr>
<td>Amberlite LA-2</td>
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</tr>
<tr>
<td>3300 (w)</td>
<td>3400 (w)</td>
<td>3300 (w)</td>
<td>3350 (v*)</td>
<td>— NH₂ Amine group — N—H — stretching</td>
</tr>
<tr>
<td>2920 (m*)</td>
<td>2900 (v*, w)</td>
<td>2900 (w)</td>
<td>2900 (w)</td>
<td>— CH₂ or — CH₃ group C—H stretching</td>
</tr>
<tr>
<td>2350 (m)</td>
<td>2350 (m)</td>
<td>2350 (m)</td>
<td>2350 (m)</td>
<td>Cephalosporin sulphur</td>
</tr>
<tr>
<td>1760 (v s)</td>
<td>1745 (w)</td>
<td>1730 (m)</td>
<td>1725 (v s)</td>
<td>&gt; C = O stretching (carboxylic group)</td>
</tr>
<tr>
<td>1620 (m)</td>
<td>—</td>
<td>1600 (w)</td>
<td>1600 (s)</td>
<td>&gt; C = O stretching of secondary amide group</td>
</tr>
<tr>
<td>1450</td>
<td>1450</td>
<td>—</td>
<td>1420</td>
<td>— CH₃ group</td>
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<td>1390 (s)</td>
<td>1375 (m)</td>
<td>1375 (m)</td>
<td>1375 (m)</td>
<td>C—H deformation</td>
</tr>
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<td>1250 (m)</td>
<td>1250 (m)</td>
<td>1250 (v s)</td>
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<td>2900 (v, w)</td>
<td>2900 (w)</td>
<td>2900 (w)</td>
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</table>

* v, s, m: strong, very, medium, weak band intensity, respectively.
4. COMPARISON WITH EQUILIBRIUM DATA OF BATCH EXPERIMENTS

The experimentally evaluated extraction equilibrium relationships of CPC and 6-APA are shown in Figures 9 and 10, respectively. It is apparent from the slopes of the lines of Figures 9 and 10 that both CPC and 6-APA complex with Amberlite LA-2 and Trioctylamine in a non-stoichiometric ratio, whereas complexation with Aliquat-336 is stoichiometric (1:1) in nature. A non-stoichiometric reaction model given by

\[ mA + nH^+ + nP^+ = \Lambda_m(HP)_n \]  

was suggested for reactive extraction of 7-aminocephalosporanic acid with various amines in our previous publication (Bora et al., 1998). By fitting
Figure 9: Evaluation of equilibrium relationship of Cephalosporin C in n-butyl acetate. 
[P] 10 mM, [Cl] 10 mM, Amoxicillin 1x2, [*]. Triazolamine, *A. Alquadrat 336.

Figure 10: Evaluation of equilibrium relationship of 6-Aminopenicillanic acid in n-butyl acetate [P] 10 mM, [Cl] 10 mM, Amoxicillin 1x2, [*]. Triazolamine, *A. Alquadrat 336.
experimental data to the theoretical prediction of $K_P$ value, the identified solute-amine complexation ratios were found to be 1:1.4, 1:1.5 and 1:1.8 for dioctryamine, Amberlite LA-1 and Amberlite LA-2, respectively. In the present study, CPC was found to complex with both Amberlite LA-2 and trioctylamine in a 1:1.8 ratio whereas 6-APA complexes with the respective amines in 1:2 and 1:1.8 ratios. The present spectroscopic study appears to provide a better understanding of the chemical interaction involved in the reactive extraction system.

5. CONCLUSION

IR spectroscopic studies indicate that the reactive extraction of zwitterionic Cephalosporin-C and 6-aminopenicillanlic acid with secondary and tertiary amines in $n$-butyl acetate as the solvent take place via the formation of complex with acid-amine ratio of 1:2. This is indicative of the formation of ion-pair or hydrogen bond with the secondary and tertiary amines or hydrogen bond with the carboxylate of the acidic group present in the beta-lactam molecules. This is supported by the data on equilibrium relationship deduced from extraction equilibrium experiment. On the other hand, both the beta-lactams complex with quaternary amine in a 1:1 ratio via a liquid anion exchange reaction.

Acknowledgments

The authors are grateful to the Director, Regional Research Laboratory, Jorhat, India, to grant permission to publish this paper.

NOMENCLATURE

- $A$: Amine (carrier)
- $A_{\text{HIP}}$: Secondary, tertiary amine complex with beta-lactam
- $6\text{-APA}$: 6-Aminopenicillanic acid
- $\text{Cl}^-$: Chloride ion (counter-ion of anion carrier)
- $\text{CPC}$: Cephalosporin-C
- $\text{H}^+$: proton
- $K_P$: extraction equilibrium constant $[\text{-}]$
- LA-2: Amberlite LA-2
- $P$: negatively charged beta-lactams
QCI  Aliquat-336
QP  Quaternary amine-beta-lactam complex
TOA  Trioctylamine

Subscripts/superscripts
aq  aqueous phase
O  overall
org  organic phase (solvent)

References
REACTIVE EXTRACTION OF 7-AMINOCEPHALOSPORANIC ACID WITH ALIQUAT-336: EQUILIBRIUM AND KINETICS

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(Received 3 February 1993; In final form 31 August 1999)

The equilibrium and kinetics of 7-aminocephalosporanic acid (7-ACA) extraction with Aliquat-336 (tricaprylylmethyl ammonium chloride) dissolved in n-butyl acetate as the solvent have been studied over a pH range of 5 to 8. It was found that Aliquat-336 reacts with the dissociated anion of 7-ACA in a stoichiometric ratio of 1:1 and the apparent extraction equilibrium constant, K, increases with an increase of the aqueous phase pH. The extraction kinetics has been analysed based on the assumption that the interfacial reaction between the 7-ACA anion and the interfacially adsorbed carrier complex controls the extraction rate and the mass transfer resistances of the aqueous and organic phases are unimportant. The role of the interfacial chemical reaction was semiquantitatively assessed from certain measured data on interfacial tension between the aqueous 7-ACA solution and the organic solution of Aliquat-336.

Keywords: Reactive extraction, 7-aminocephalosporanic acid; liquid-liquid ion exchange, Aliquat-336, co-extraction; interfacial reaction

INTRODUCTION

7-ACA is an important intermediate for semisynthetic cephalosporins such as cephaloglycin, cephalothin, cephapirin etc. It is usually made by chemical deacylation of cephalosporin-C (CPC), however utilising expensive and
highly reactive reagents (Huber et al., 1972). In view of this, enzymatic process has been extensively studied in the last decade for reasons of simplicity and the environmentally safe conditions involved in the enzymatic process. At least, a two step process has been developed wherein CPC is chemically oxidized to glutaryl 7-ACA which is converted to 7-ACA by an acylase enzyme (Tsuzuki, et al., 1989). Isolation of pure 7-ACA from an enzymatically produced reaction mixture is complicated due to the complex nature of the culture broth, the low concentration at which 7-ACA is present in the broth and the presence of α-aminoadipic acid as by-product cum impurity.

Since 7-ACA is an amphoteric molecule, physical solvent extraction is difficult. Various methods of extraction and purification of cephalosporin antibiotics have been discussed in our recent review (Ghosh et al., 1997). Reactive extraction in liquid membrane has been thought to be a potential method for recovery of beta-lactam antibiotics from a complex culture broth (Ghosh et al., 1996). We have been studying the liquid membrane technique for extraction of cephalosporin antibiotics. By suitably manipulating the aqueous phase environment in the feed and stripping phases and selecting the extracting agent (carrier) for the membrane phase, facilitated transport in the membrane could be demonstrated at least for CPC, 7-ACA and cephalixin (Ghosh et al., 1995; Sahoo et al., 1996, 1997, 1999). Complimentary studies on equilibrium and kinetics of the reactive extraction of these compounds are being made separately in our laboratory as these data are useful for optimising the operating conditions of liquid membrane processes. Our investigating on reactive extraction of 7-ACA with various carriers revealed that Aliquat-336 is the best choice of carrier for 7-ACA (Bora et al., 1998) and butyl acetate as the solvent provides the highest extraction efficiency. Aliquat-336 has also been studied for reactive extraction of amino acids (Ilano et al., 1991; Haensel et al., 1986; Reisinger and Marr, 1993); cephalosporin-C, (Ilano et al., 1992); lactic acid (Lazarova and Peeva, 1992), other anionic species (Galan et al., 1994) etc.

In this paper, the extraction equilibrium and kinetic have been reported for the 7-ACA-Aliquat-336 system. The kinetics analysis is based on the assumption that interfacial reaction between the 7-ACA anion and the interfacially adsorbed carrier complex plays a dominant role on the overall extraction rate.

THEORETICAL DEVELOPMENTS

Extraction Equilibrium

7-ACA is a zwitterionic molecule the pKa1 and pKa2 values being 2.02 and 4.42 respectively. In the pH range between 2.02 and 4.42, the zwitterion
as a whole is predominant as is evident from the dissociation behaviour shown below

\[
\begin{align*}
\text{PK}\alpha_1 &= \text{pH} \\
\text{PK}\alpha_2 &= 4.42
\end{align*}
\]

The pH at which the amino group ionises keeps the carboxyl group in the COO⁻ form

The anionic form of 7-ACA at pH > 4.42 is amenable for ion-exchange with anionic exchanger such as Aliquat-336 (a carrier hereafter termed as QC1). The 7-ACA anion, P⁻ complexes with the carrier, QC1, dissolved in the organic solvent according to the following reaction

\[
P_{aq}^- + QC_{org} \rightleftharpoons QP_{org}^- + Cl_{aq}^-
\]  (1)

The anion exchange takes place at the interface of the organic phase, the P⁻ ion being extracted as a complex QP to the organic phase liberating Cl⁻ into the aqueous phase.

The extraction equilibrium constant, \( K_P \) is given by

\[
K_P = \frac{[QP]_{org}^- [Cl^-]_{aq}}{[QC]_{org}^- [P^-]_{aq}}
\]  (2)

The following material balance equation holds for Aliquat-336/Cl⁻

\[
V_{org}[QC]_{org} = V_{org}[QC]_{org, i} - V_{aq}[Cl^-]_{aq}
\]  (3)

and for 7-ACA

\[
V_{org}[QP]_{org} = V_{aq}([HP]_{aq, i} - [HP]_{aq, e})
\]  (4)

where, \( i \) and \( e \) stand for the initial and equilibrium values, respectively.
Defining pKa as $-\log_{10}K_d$ and in consideration of the Hasselbach-Hendersson equation (Haensel et al., 1986)

$$[P^{-}]_{aq} = [HP]_{aq,r} \left[ 1 - \frac{1}{1 + 10^{pH - pK_a}} \right]$$

(5)

The extraction equilibrium constant can be arranged as

$$K_P = \frac{V_{aq}( [HP]_{aq,t} - [HP]_{aq,r} ) \ V_{org}[Cl^-]_{aq}}{[V_{org}[Cl^-]_{org,t} - V_{aq}[Cl^-]_{aq}] \ V_{org}[P^-]_{aq}}$$

(6)

Equation (6) follows from Eq. (2) and by plotting $[QP]_{org} \ [Cl^-]_{aq}$ versus $[Cl^-]_{org} \ [P^-]_{aq}$, the equilibrium constant can be determined. In absence of the measurement of Cl$^-$ concentration in the aqueous phase, $[Cl^-]$ may be assumed to be equal to the exchanged 7-ACA amount i.e., $V_{aq}[Cl^-]_{aq} = V_{org}[QP]_{org}$. Thus, $[Cl^-]_{aq}$ can be eliminated from the $K_P$ expression which can be simplified to the following form:

$$K_P = \frac{V_{org}[QP]_{org}^2}{[Cl^-]_{org} \ V_{aq}[P^-]}$$

(7)

The co-extraction of buffer anion, $A^-$ by QC1 at the interface may take place according to

$$A^-_{aq} + QC1_{org} \rightleftharpoons QA_{org} + Cl^-_{aq}$$

(8)

The equilibrium constant, $K_A$ of co-extraction is given by

$$K_A = \frac{[QA]_{org} \ [Cl^-]_{aq}}{[Cl^-]_{org} \ [A^-]_{aq}}$$

(9)

The co-extraction effect can be considered implicitly by the excess chloride moles present in the aqueous phase after equilibration

$$V_{org}[QA]_{org} = V_{aq}[Cl^-]_{aq} - V_{org}[QP]_{org}$$

(10)

Extraction Kinetics

Disassociation Equilibrium

Due to zwitterionic nature, 7-ACA can exist in three forms with different charges depending on the pH ($P^+, P, P^-$) according to the following
dissociation equilibrium

\[ P^+ \xrightarrow{K_{d1}} P + H^+ \quad K_{d1} = \frac{[P][H^+]}{[P^+]} \quad (11) \]

\[ P^+ \xrightarrow{K_{d2}} P^- + H^+ \quad K_{d2} = \frac{[P^-][H^+]}{[P]} \quad (12) \]

At 25°C, \( K_{d1} = 9.5 \times 10^{-3} \) and \( K_{d2} = 3.8 \times 10^{-5} \). Total concentration of 7-ACA in the aqueous solution is given by

\[
[HP]_{aq} = [P^+]_{aq} + [P^-]_{aq}
\]

\[
= [P^-]_{aq} \left(\frac{[H^+]_{aq}^2}{K_{d1} K_{d2}} + \frac{[H^+]_{aq}}{K_{d2}} + 1\right)
\]

where,

\[
\gamma_\gamma = \frac{[H^+]_{aq}^2}{K_{d1} K_{d2}} + \frac{[H^+]_{aq}}{K_{d2}} + 1
\]

By using Eq. (13), the concentration of \( P^- \) can be estimated from the values of \([HP]\) and pH.

**Kinetic Model**

Assuming that the interfacial chemical reaction controls the extraction rate, the following steps may be considered for 7-ACA (\( P^- \)) extraction rate

1. Reactants, \( P^- \) and \( QC1 \) diffuse from the bulk phase to the interface
2. \( QC1 \) adsorbs at the interface according to Langmuir model
3. \( QC1 \) reacts with \( P^- \) at the interface to form \( QP \) and \( Cl^- \)
4. \( QP \) desorbs from interface
5. Products \( Cl^- \) and \( QP \) diffuse back to the bulk phases

The following interfacial reaction mechanism may be proposed

**Adsorption of QC1**

\[ QC1 + \Phi \xrightleftharpoons[k_{-1}]{k_1} QC1^S \]

\[ r_{-1} = k_1 \theta_{QC1} [QC1]_{org} - k_{-1} \theta_{QC1} \quad (15) \]

\[ K_I = k_1/k_{-1} \]
Interfacial reaction between $P^-$ and QCl$^-$

$$P^- + QCl^- \xrightleftharpoons[k_1']{k_2} QP + Cl^-$$

$$r_2 = k_2 [P^-]_{aq,in} \theta_{QCl} - k_2 [Cl^-]_{aq,in} \theta_{QP}$$

$$K_2 = k_2 / k_2'$$

Desorption of QP$^-$

$$QP \xrightleftharpoons[k_1']{k_3} Q + Cl^-$$

$$r_3 = k_3 \theta_{QP} - k_3 \theta_0 [QP]_{org,in}$$

$$K_3 = k_3 / k_3'$$

By assuming a Langmuir type adsorption and desorption isotherm, the interfacial extraction rate, $r$ of $P^-$ can be expressed as

$$r = r_2 = \frac{k_2 k_1}{K_F} \times \frac{[P^-]_{aq.in} [QCl]_{org,in} - [Cl^-]_{aq.in} [QP]_{org,in}}{(1 + K_1 [QCl]_{org,in} + K_3 [QP]_{org,in})}$$

(18)

where,

$$K_F = \frac{K_1 K_2}{K_3}$$

$$\theta_0 + \theta_{QCl} + \theta_{QP} = 1$$

Considering a pseudo-steady state assumption, the extraction flux may be expressed as

$$J_1 = -\frac{V_{aq}}{S} \frac{d[QP]_{aq}}{dt} = J_1 = r = J_2 = \frac{V_{org}}{S} \frac{d[QP]_{org}}{dt}$$

(20)

where,

$$J_1 = k_L ([P^-]_{aq} - [P^-]_{aq,m})$$

$$J_2 = k_o ([QCl]_{aq} - [QCl]_{aq,m})$$

$$J_2 = k_c ([Cl^-]_{aq} - [Cl^-]_{aq,m})$$
The following mass balance holds

\[ V_{aq}(\{\text{HP}\}_{aq,i} - \{\text{HP}\}_{aq,f}) = V_{aq}(\{\text{QP}\}_{org} - \{\text{QP}\}_{aq}) \quad (23) \]

\[ [\text{QCl}]_{org,i} + [\text{QP}]_{aq,i} = [\text{QCl}]_{org} + [\text{QP}]_{org} \quad (24) \]

\[ V_{aq}(\{\text{Cl}^-\}_{aq,i} - \{\text{Cl}^-\}_{aq,f}) = V_{org}(\{\text{QCl}\}_{org,i} - \{\text{QCl}\}_{org}) \quad (25) \]

It is possible to eliminate the interfacial concentration in the flux Eqs. (18)–(22) with the help of the above mass balance equations. For the limiting case of interfacial reaction controlled extraction and considering negligible mass transfer resistance's (high \(k_2\) and \(k_{a}\) values) in the aqueous and organic phases, the overall flux may be equivalent to the interfacial reaction rate (Eq. (18)) and the flux can be obtained according to a reported procedure (Chan and Wang, 1993) as given below

\[ J = \frac{k_2 K_1 K_{2e}}{K_F} \frac{\{\text{HP}\}_{aq,i} [\text{QCl}]_{org} - \{\text{Cl}^-\}_{aq,i} [\text{QP}]_{org}}{1 + K_1 [\text{QCl}]_{org} + K_2 [\text{QP}]_{org}} \quad (26) \]

which also can be used for initial flux.

For \([\text{QP}]_{org,i} = \{\text{Cl}^-\}_{aq,i} = 0\)

\[ J_i = \frac{k_2 K_1 [\text{HP}]_{aq,i} [\text{QCl}]_{org,i}}{\gamma - \frac{1}{1 + K_1 [\text{QCl}]_{org,i}}} \quad (27) \]

Rearranging Eq. (27)

\[ \frac{1}{J_i} = \frac{\gamma}{K_1 k_2 [\text{HP}]_{aq,i} [\text{QCl}]_{org,i}} + \frac{\gamma}{k_2 [\text{HP}]_{aq,i}} \quad (28) \]

and plotting \(1/J_i\) vs. \(1/[\text{QCl}]_{org,\text{i}}\), the values of \(K_1\) and \(k_2\) can be determined. The value of \(K_1\) can be obtained by fitting Eq. (26) with known value of \(K_F\), and \(K_2\) can consequently be calculated from \(K_F = K_1 K_2 / K_3\).

**EXPERIMENTAL METHODS**

**Reagents**

7-ACA procured from Sigma (St. Louis, Missouri, USA) was of 99.9% purity, Aliquat-336 (Aldrich, Milwaukee, USA) had a mean molar mass of
404 Kg/Kmol and was used as received. Butyl acetate and other analytical grade buffer reagents were procured from BDH (Bombay, India).

Procedure

The extraction equilibrium experiments were carried out in a closed flask by mixing 10 ml each of aqueous 7-ACA solution and butyl acetate solution of Aliquat-336. The aqueous phase pH was maintained at 5 to 8 by using suitable buffer solution. Phosphate buffer was used to maintain values pH of 6, 7 and 8, while citrate—phosphate buffer was used to maintain a pH value of 5. For the phosphate buffer, 5.3, 39.0 and 87.8 ml of 0.2 M monobasic sodium phosphate solutions were mixed with 94.7, 61.0 and 12.3 ml of 0.2 M dibasic sodium phosphate solutions and the resulting solutions were diluted to a total volume of 200 ml each to obtain a pH values of 8, 7 and 6, respectively. Similarly for citrate—phosphate buffer 24.3 ml of 0.2 M citric acid solution was mixed with 25.7 ml of 0.2 M dibasic sodium phosphate solution and the resulting solution was diluted to a total volume of 100 ml to obtain pH of 5. The time of equilibration was 2–3 hours. After attainment of equilibrium and phase separation, the aqueous phase concentration of 7-ACA, [HP] was determined by UV-visible Spectrophotometer (Shimadzu, Model 160 A) calibrated at 264 nm. The value of [P−] has been estimated from Eq. (5) and \( [QP]_{org} \) value has been taken as equivalent to \( [P−] \) that has been extracted at equilibrium i.e., difference of initial and equilibrium values of \( [P−] \). The chloride ion concentration in the aqueous phase was estimated by the well-known Volhardt method (Vogel, 1962). The concentration of the buffer ion i.e., phosphate was estimated by standard gravimetric method (Vogel, 1962).

The extraction kinetic was studied in a stirred transfer cell of standard design reported in literature (Chan and Wang, 1993; Bora et al., 1996) and a schematic diagram of the cell is shown in Figure 1. The cell was a glass cylinder with inside diameter of 5 cm and a height of 10 cm which was divided into two halves by an acrylic circular disc placed right at the interface in order to reduce the disturbance of the interface. The interfacial area of the annular gap was varied by changing the size of the disc. 7-ACA was transferred between the aqueous phase and organic phase of Aliquat-336 solution which has been equilibrated with the buffer used. Both the liquid phases were independently stirred, however without causing phase dispersion such that the geometric area can be considered as the interfacial contact area. The stirring speed was measured with a digital tachometer. The volume of the upper organic phase as well as the lower aqueous phase were
75 ml each. The temperature was maintained at 25 ± 0.5°C by circulating water from a constant temperature bath through a jacket provided in the cell.

The aqueous phase was first introduced to the cell and then the organic solution was poured carefully without disturbing the interface. Samples of aqueous phase were drawn with a glass syringe at regular interval of time and the 7-ACA concentration was determined spectrophotometrically.

Interfacial tension was measured at 25 ± 0.5°C by the well known drop weight method. The apparatus and the procedure followed are the same as reported in our previous communication (Dutta and Patil, 1993). Before measurement of the interfacial tension between the two phases, the organic phase was saturated with the aqueous 7-ACA solution. The apparatus was calibrated using water/toluene, water/heptane and water/n-pentanol system whose interfacial tension values were 36.15, 50.0 and 2.0 mN/m, respectively.
RESULT AND DISCUSSION

Extraction Equilibrium

The efficiency of 7-ACA anion extraction appears to be influenced by buffer capacity of the aqueous phase through a dependence on the aqueous phase pH. The values of the equilibrium constant were determined for various combinations of 7-ACA and Aliquat-336 concentrations at different pH values. Figure 2 shows the plots of $[QP]_{org} [Cl^-]_{aq}$ versus $[QCl]_{org} [P^-]_{aq}$ used to determine the values of $K_P$. In the same figure, plot of $[QP]_{org}$ vs. $[QCl]_{org} [P^-]_{aq}$ is also shown to assess the co-extraction effect. As expected, $K_P$ increases with an increase of pH within the range of pH studied obviously due to larger fraction of 7-ACA anion that exists at high pH conditions. It is apparent that at high pH, co-extraction becomes significant whereas at lower pH, co-extraction is negligible. The Cl$^-$ ion in excess of that required to be replaced by P$^-$ ion is considered to be the general measure of the degree of co-extraction. Indeed, analysis of the Cl$^-$...
concentration in the aqueous phase indicated appreciable degree of co-extraction particularly at a pH values of 8.

The $K_P$ values determined from linear regression of the data of Figure 2 have been listed in Table 1. $K_P$ values p17 and 8 without considering coextraction are $11.7 \times 10^{-2}$ and $20.0 \times 10^{-2}$, respectively whereas the apparent values obtained by considering coextraction are $14.0 \times 10^{-2}$ and $29.0 \times 10^{-2}$, respectively. The equilibrium constants for coextraction have been estimated to be $2.85 \times 10^{-2}$ and $10.87 \times 10^{-2}$ at the respective pH values. Thus it may be inferred that the coextraction increases with increase of pH, an observation similar to that reported for extraction of 6-aminopenicillanic acid (Bora et al., 1997) and d-l-phenylalanine (Hansen et al., 1986) with Aliquat-336. The coextraction may also be inferred from the observation of slight reduction of the aqueous phase pH.

It appears that the equilibrium is weakly dependent on the concentration of the species in the two liquid phase and therefore, the equilibrium behaviour can be explained by considering the ideality of both the phases (Galan et al., 1994). Any probability of the non-ideality of the organic phase can be ruled out because of the negligibly small aggregation of the lipophilic carrier in polar butyl acetate as the solvent (Asai et al., 1991).

A logarithmic plot of $[QP]_{org} [Cl^-]_{aq}/[P^-]_{aq}$ vs. $[QCl]_{org}$ is shown in Figure 3 from which the slope of all the lines is calculated to be 1. It is therefore inferred that 7-ACA anion complexes with Aliquat-336 in a 1:1 ratio.

### Extraction Kinetics

Under various experimental conditions, the initial rate of extraction, $J_i$ (mol/cm²s) was obtained from the following relation

$$J_i = \frac{V_{aq}}{S} \left( \frac{d[P^-]_{aq}}{dt} \right)_{t=0} \text{ mole/cm}^2\text{s}$$

where, $(d[P^-]_{aq}/dt)_{t=0}$ represents the initial slope of the curve of $[P^-]$ vs. $t$.

<table>
<thead>
<tr>
<th>pH</th>
<th>$K_P \times 10^{-2}$</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>20.0</td>
<td>0.91</td>
</tr>
<tr>
<td>7</td>
<td>11.7</td>
<td>0.91</td>
</tr>
<tr>
<td>6</td>
<td>6.63</td>
<td>0.92</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>0.93</td>
</tr>
</tbody>
</table>
FIGURE 4 Effect of stirring speed on the initial extraction flux. \([\text{QCI}]_\text{org} = 2 \text{ mol/m}^3; [\text{H}$\text{P}]_\text{aq} = 1.5 \text{ mol/m}^3; \text{pH} = 8\)

Effect of Stirring Speed

The effect of stirring speed on the initial extraction flux can provide the information on the regime of the transport process. The dependence of \(J_t\) on stirring speed, \(N\) (rpm) is shown in Figure 4 from which it is apparent that
Extraction kinetics is controlled by film diffusion below a stirring speed of 110 rpm. In the range of 100–150 rpm, the extraction rate is independent of the stirring speed implying that the extraction occurs in the kinetic regime. Since the interface becomes unstable above 150 rpm, further studies on the extraction rate have been performed at 120 rpm i.e., under the kinetic regime.

Studied under the kinetic regime, the effect of the interfacial area, $S$, on the initial extraction rate, $N_i$, is shown in Figure 5 which exhibits a linear relationship between $S$ and $N_i$. This observation along with the result of Figure 4 imply that interfacial chemical reaction rather than homogeneous phase chemical reaction controls the overall extraction rate such an interfacial reaction controlled mechanism was also inferred for the extraction of penicillin-G by Amberlite LA-2 [Lee and Wang, 1995].

**Effect of Initial 7-ACA Concentration and pH**

The relation between $J_i$ and initial 7-ACA concentration, $[P_-]_{aq}$ is shown in Figure 6 where from the effect of pH can also be assessed. It is apparent that $J_i$ increases with $[P_-]_{aq}$ and pH with a linear relationship between $J_i$ and $[P_-]_{aq}$.
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FIGURE 6  Effect of initial 7-ACA concentration on the initial extraction flux. $[\text{QCl}]_{\text{org}} = 2 \text{ mol/m}^3$.

Effect of Carrier Concentration

The effect of carrier concentration in the organic phase, ([QCl]$_{\text{org}}$) on the initial flux is shown in Figure 7. An increase of [QCl]$_{\text{org}}$ increases $J_i$ in an exponential way as represented by Eq. (27), a result that probably implies the hypothesis of an interfacial reaction control mechanism for the extraction of 7-ACA with Aliquat-336 which has good surfactant properties. Indeed significant lowering of the interfacial tension between aqueous cephalosporin solution and butyl acetate solution of Aliquat-336 could be demonstrated as an important factor for stability of an immobilized liquid membrane system (Ghosh et al., 1995) wherein the principle of reactive extraction was exploited.

In order to provide some evidence on interfacial chemical reaction as the rate controlling step, the measured data on interfacial tension were considered. As shown in Figures 8 and 9, the interfacial tension, $\sigma$ decreases with increase of [QCl]$_{\text{org}}$ and the organic phase containing QCl saturated with 7-ACA solution exhibits lower interfacial tension than that of the unsaturated solution. Figure 9 also shows the effect of 7-ACA loading ratio on the interfacial tension behaviour in terms of $[\text{QP}]_{\text{org}}/[\text{QCl}]_{\text{org}}$ vs. $\Delta \sigma$. $[\text{QP}]_{\text{org}}$
FIGURE 7 Effect of carrier concentration on the initial extraction flux. \([\text{HP}]_{\text{org}} = 1 \text{ mol/m}^3\).

FIGURE 8 Interfacial tension between aqueous 7-ACA and organic phase of Aliquat-336 \([\text{HP}]_{\text{org}} = 1.5 \text{ mol/m}^3\); open symbol: organic phase saturated with 7-ACA solution; closed symbol: organic phase not saturated with 7-ACA solution.

was changed by adding 7-ACA saturated QCI solution. The interfacial tension difference between saturated and fresh unsaturated QCI solution is defined as \(\Delta \sigma = \sigma_s - \sigma\), where, \(\sigma_s\) is the value for unsaturated solution. The
data on \([QP]_{\text{org}}/[QCl]_{\text{org}}\) vs. \(\Delta\sigma\) pertain to various initial QCI concentrations in the organic phase. It is apparent that increase of 7-ACA loading ratio exhibits increased lowering of interfacial tension between the phases. The change in \(\Delta\sigma\) may be attributed to the change in interfacial concentration of the solute-carrier complex by the reaction and adsorption at the interface.

Under the assumption that interfacial chemical reaction is the rate controlling step, the mass transfer resistances of aqueous and organic phase can be neglected, so that the bulk concentrations of the solute carrier complex, \([QP]\) and the carrier, \([QCl]\), are equal to the interfacial concentrations. Therefore, the values of \([QP]\) and \([QCl]\) at the interface may be obtained. However, the complex concentration cannot be determined directly, but \(\Delta\sigma\) may be used to represent its value indirectly with the help of Figure 9 following an approach reported for the study of interracial reaction kinetics of Penicillin-G extraction with Amberlite LA-2 (Lee and Wang, 1995). Our analysis using Figure 9 revealed that the interfacial concentration of QP is proportional to that of QCI in conformity with the relationship for chemical equilibrium. It may therefore, be reasonable to assume that interfacial chemical reaction controls the extraction rate.

Figure 10 shows the plot of \(1/I_1\) vs. \(1/[QCl]_{\text{org}}\) for the present system. At a pH of 7.0 \((K_p = 11.7 \times 10^{-2})\) the estimated parameters values are \(K_1 = 605.07 \text{l/mol}\), \(K_2 = 3.654 \times 10^{-4} \text{l/mol}\), \(K_3 = 1.892 \text{l/mol}\), \(k_2 = 1.05 \times 10^{2}\).
FIGURE 10 Plot of $\frac{1}{J}$ vs. $\frac{1}{[\text{Cl}]_{\text{org}} \cdot \text{mol/m}^3 \cdot \text{s}^{-1}}$ for $\text{pH} = 5-8$, $[\text{H}^{+}]_{\text{aq}} = 1.5 \text{ mol/m}^3$.

FIGURE 11 Comparison of experimental and predicted concentration profile. $[\text{H}^{+}]_{\text{aq},i} = 1 \text{ mol/m}^3$; $[\text{QCl}]_{\text{org},i} = 2 \text{ mol/m}^3$, $\text{pH} = 8$.

$10^{-3} \text{ cm/s}$, $k_{-2} = 2.875 \text{ cm/s}$. Based on the above kinetic model and the assumption that co-extraction does not affect the solute extraction kinetics, a theoretical concentration profile has been evaluated at $\text{pH}$ of 7 as shown in Figure 11. Apparently, the agreement between experimental and theoretical profile is reasonable.
CONCLUSION

The reactive extraction of 7-ACA anion with Aliquat-336 takes place in a 1:1 stoichiometric ratio. The extraction kinetics could be represented by a model based on the mechanism of interfacial reaction wherein the interfacial reaction between the 7-ACA anion and adsorbed solute-carrier complex play significant role in the extraction kinetics over the pH and concentration ranges studied in this work.

Acknowledgment

Financial support from DST-New Delhi vide grant No. III 4(15) 94-ET has been gratefully acknowledge.

NOMENCLATURE

\( A^- \)  
buffer anion

\( Cl^- \)  
Chloride ion (counter-ion of anion exchange carrier)

\( H^+ \)  
proton

\( Hp \)  
7-ACA

\( j \)  
extraction flux, \((\text{mol/m}^2\text{-sec})\)

\( k_o \)  
oil phase interfacial mass transfer coefficient of \([QP]\) or \([QCl]\), \((\text{m/s})\)

\( k_{Cl} \)  
aqueous phase interfacial mass transfer coefficient of \(Cl^-\), \((\text{m/s})\)

\( k_{L} \)  
aqueous phase interfacial mass transfer coefficient of \(P^-\), \((\text{m/s})\)

\( k_{1}, k_{2}, k_{3} \)  
forward rate constant defined by Eqs. (15), (16) and (17)

\( k_{11}, k_{22}, k_{33} \)  
reversed rate constant defined by Eqs. (15), (16) and (17)

\( K_{A}, K_{2}, K_{3} \)  
equilibrium constant defined in Eqs. (15), (16) and (17)

\( K_{d1}, K_{d2} \)  
dissociation equilibrium constant defined by Eqs. (11) and (12), [-]

\( K_{pr} \)  
equilibrium constant, [-]

\( N_j \)  
specific initial extraction rate, \((\text{mol/sec})\)

\( N \)  
stirring speed, \((\text{rpm})\)

\( P^+ \)  
positively charged 7-ACA

\( P^- \)  
negatively charged 7-ACA

\( QA \)  
buffer anion-carrier complex
QC1  Aliquat-336
QP  quaternary amine-7-ACA complex
\( r \)  interfacial chemical reaction rate, (mol/m²·sec)
\( S \)  interfacial area, m²
\( t \)  time of extraction, (sec)
\( V \)  volume of solution, (m³)
\( \sigma \)  interfacial tension between aqueous 7-ACA solution and organic phase, (mN/m)
\( \Delta \sigma \)  difference of interfacial tension between organic phase saturated and unsaturated solution, (mN/m)
\( \theta \)  fraction of interfacial area unoccupied, [-]
\( \theta_{QC1} \)  fraction of interfacial area occupied by QC1, [-]
\( \theta_{QP} \)  fraction of interfacial area occupied by QP, [-]
\( \gamma \)  constant defined in Eq. (14)
\( \varphi \)  interfacial vacant site
\( [J \)  concentration, (mol/m³)

Subscript/Superscript

O  overall
aq  aqueous phase
e  equilibrium
i  initial
in  interface
org  organic (solvent) phase
u  unsaturated

References


