Extraction Equilibria of Cephalosporin Antibiotics with Aliquat-33

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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 pK_{a2} values of the antibiotics. The reactive extraction, which is essentially an anion-exchange reaction, takes place in a 1:1 stoichiometry. The extraction equilibrium constants (K_P) 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 cephalosporin antibiotics, indicating the possibility of exploiting the reactive extraction technique for process application. The K_P 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 cephalosporin 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., 1995; Sahoo et al., 1996, 1997, 1998, 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 (Bora 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; Haensel et al., 1991), clavulanic acid (Harris et al., 1990), and other anionic species (Galan et al., 1994).

In this paper, we report a comprehensive study on extraction equilibria of various cephalosporin 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 thiozolidine ring which belongs to the penicillin group, is also used for the study for the purpose of comparison with the cephalosporin. 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 cephalosporin molecules considered for this investigation are shown in Figure 1. As a result of the presence of carboxylic acid and amino groups, all the molecules exist in an ionic form of different charges depending on the pH of the media. At pH < pK_{a1}, the predominant form is cationic, at pH > pK_{a2} (pK_{a3} for cephalosporin-c), the predominant form is anionic, and in the pH range pK_{a1} < pH < pK_{a2}, 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 cephalosporin anion, P^-, in the aqueous phase complexes with Aliquat-336 (hereafter termed as QCl) according to

$$P^- + QCl \rightleftharpoons QP + Cl^- \quad (1)$$

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 cephalosporin molecule (HP) first dissociates in aqueous solution into the anion, P^-, and a proton, H^+, as follows

$$HP \rightleftharpoons H^+ + P^- \quad (2)$$

The dissociation equilibrium constant, K_d, is given by

$$K_d = \frac{C_H C_P}{C_{HP}} \quad (3)$$

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, K_P, is given by

$$K_P = \frac{C_{QP} C_{Cl}}{C_{QCl} C_P} \quad (4)$$

The coextraction of buffer anion, A^-, by QCl at the interface may take place according to

$$A^- + QCl \rightleftharpoons QA + Cl^- \quad (5)$$

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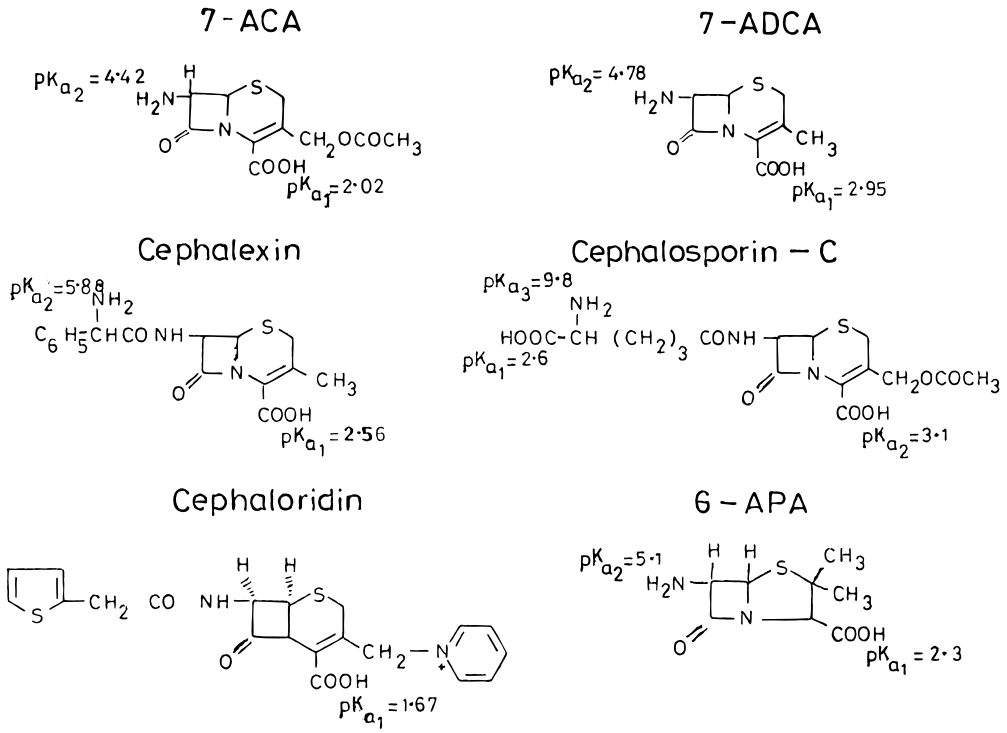

Figure 1. Structure of the cephalosporin antibiotics.

The equilibrium constant, K_A, of coextraction is given by

$$K_A = \frac{C_{QA}C_{Cl}}{C_{QCl}C_A} \tag{6}$$

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

$$V_oC_{QCl} = V_oC_{QCl(i)} - V_aC_{Cl} \tag{7}$$

and for cephalosporin

$$V_oC_{QP} = V_a(C_{HP(i)} - C_{HP(e)}) \tag{8}$$

where V_a and V_o 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 $-\log K_d$ and considering of the Hasselbach−Henderson equation (Haensel et al., 1986)

$$C_P = C_{HP(e)}\left[1 - \frac{1}{1 + 10^{pH-pK_a}}\right] \tag{9}$$

The extraction equilibrium constant can be arranged as

$$K_P = \frac{V_a(C_{HP(i)} - C_{HP(e)})V_aC_{Cl}}{(V_oC_{QCl(i)} - V_aC_{Cl})V_oC_P} \tag{10}$$

Equation 10 follows from eq 4, and by plotting $C_{QP}C_{Cl}$ verus $C_{QCl}C_P$, the equilibrium constant (K_P) value can be determined. In the absence of measurement of the Cl concentration in the aqueous phase, C_{Cl} may be assumed to be the exchanged cephalosporin anion, as may be determined from $V_aC_{Cl} = V_oC_{QP}$. Thus, C_{Cl} can be eliminated from the K_P expression, which, for the case of $V_a = V_o$, can be simplified to the following form

$$K_P = \frac{C_{QP}^2}{C_{QCl}C_P} \tag{11}$$

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

$$V_oC_{QA} = V_aC_{Cl} - V_oC_{QP} \tag{12}$$

By plotting $C_{QP}C_{Cl}/C_P$ versus C_{QCl} 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, Milwakee, 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 all the cephalosporins except cephalosporin-c, in the case of which the pH was 9.8, maintained by using carbonate−bicarbonate buffer. 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-

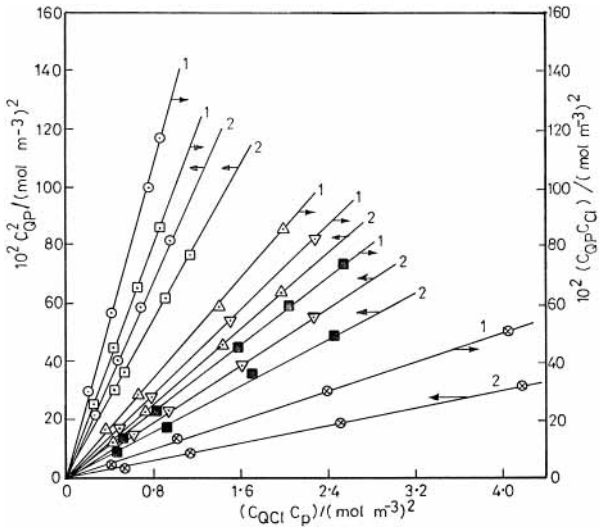

Figure 2. Evaluation of equilibrium constant for various cephalosporins in *n*-butyl acetate (1) considering coextraction and (2) without coextraction. $C_p = 0.5-1.5$ mol·m^{-3}; $C_{QCl} = 1-10$ mol·m^{-3}; ○, cephalosporin-c; △, 7-ADCA; □, cephalexin; ▽, 6-APA; ■, 7-ACA; ⊗, cephaloridine.

Table 1. Values of Equilibrium Constants for Extraction (K_P) and Coextraction (K_A) of Various Antibiotics

solute	K_P ($\times 10^2$)	K_A ($\times 10^2$)	standard deviation (%)
cephalosporin-c	89.0	38.2	3.17
cephalexin	75.0	25.0	4.74
7-ADCA	30.0	12.5	1.8
6-APA	23.0	11.8	5.1
7-ACA	20.0	9.0	1.41
cephaloridine	9.0	3.35	1.09

mined with a UV−vis spectrophotometer (Shimadzu, Model 160 A) calibrated at the wavelengths 264, 265, 262, 260, 240, and 204 nm for 7-ACA, 7-ADCA, cephalexin, cephalosporin-c, Cephaloridin, and 6-APA, respectively. The concentration of chloride ion in the aqueous phase was estimated by the well-known Volhardt method involving precipitation with silver nitrate, and those of the buffer ions, that is, phosphate and carbonate, were estimated by a standard gravimetric method using molybdate blue and $BaCl_2$, respectively (Vogel, 1962). The extraction equilibrium experiments were carried out in duplicate, and reproducibility was found to be ±5%.

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 medium. Figure 2 is a plot of C_{QP}^2 versus $C_{QCl}C_P$ used to determine the values of K_P for various cephalosporins when *n*-butyl acetate was used as the solvent. In the same figure, plots of $C_{QP}C_{Cl}$ versus $C_{QCl}C_P$ are also shown in order to assess and quantify the coextraction effect in terms of K_A values.

The K_p values determined from linear regression of the data of Figure 2 have been listed in Table 1. It is apparent that the K_p 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_A, which is also shown in Table 1. Such a coextraction effect was observed also in case of extraction of D,L-phenylalanine using the same carrier (Haensel et al., 1986).

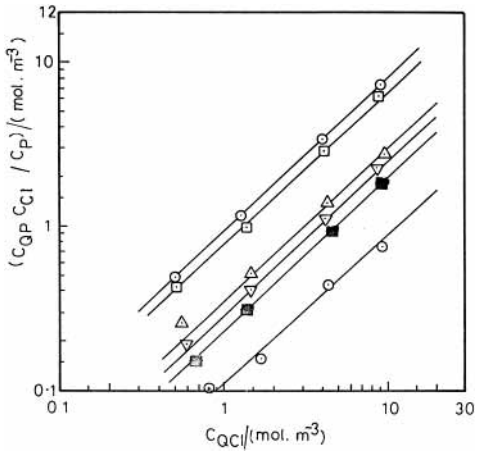

Figure 3. Extraction equilibria of various beta-lactams. Conditions and symbols are the same as those in Figure 2.

Figure 3 shows the logarithmic plots of $C_{QP}C_{Cl}/C_P$ versus C_{QCl}, 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. It may be noted that Figure 3 pertains to data generated in *n*-butyl acetate as the solvent. In fact, this was found to be the ideal choice of solvent for reactive extraction of 7-ACA with Aliquat-336 (Bora et al., 1998). Since 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 (Ghsoh 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 (Asai 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 4 shows typical equilibrium plots for the extraction of cephalosporin-c in various solvents. From this figure, it is evident that the K_P 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 carboxylic acid with trioctylamine (Tamada et al., 1990; Bizek et al., 1993; Poposka 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 amine 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 (Puttemans et al., 1985). The K_P 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.

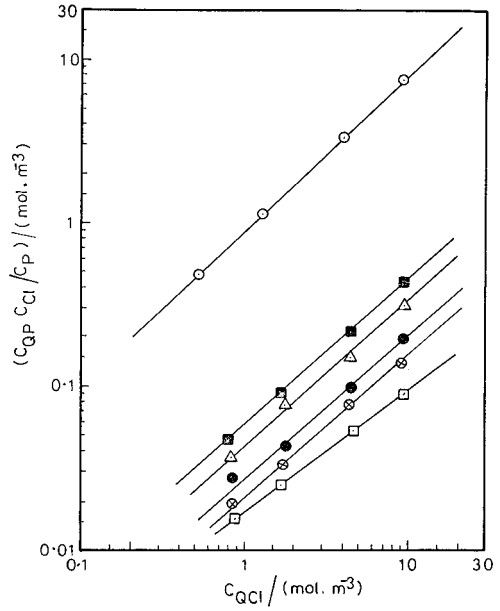

Figure 4. Extraction equilibria of cephalosporin-c in various solvents. $C_{QCl} = 1-10$ mol·m^{-3}; $C_P = 1.0$ mol·m^{-3}; pH = 9.8; ○, butyl acetate; △, dichloromethane; □, benzene; ●, chloroform; ■, 1-octanol; ⊗, cyclohexane.

Table 2. Values of K_P of Various Antibiotics in Various Other Solvents

solvent	solute	K_P ($\times 10^2$)	standard deviation (%)
octanol	cephalosporin-c	47.0	4.8
	cephalexin	30.0	2.76
	7-ADCA	18.9	3.79
	6-APA	15.0	1.78
	7-ACA	11.0	2.04
dichloromethane	cephalosporin-c	35.8	4.9
	cephalexin	23.8	5.4
	7-ADCA	15.2	1.5
	6-APA	11.0	2.8
	7-ACA	7.05	1.19
chloroform	cephalosporin-c	21.8	1.45
	cephalexin	10.1	3.7
	7-ADCA	6.0	1.37
	6-APA	3.8	1.32
	7-ACA	3.7	0.57
cyclohexane	cephalosporin-c	8.0	1.63
	cephalexin	6.0	0.33
	7-ADCA	4.0	0.94
	6-APA	2.5	2.32
	7-ACA	1.71	0.75
benzene	cephalosporin-c	4.0	0.31
	cephalexin	3.5	0.85
	7-ADCA	2.5	0.67
	6-APA	1.33	1.37
	7-ACA	1.11	0.55

To provide a semiquantitative relation between extraction efficiency and solvent property, we have attempted to correlate the values of K_P with the polarity as well as the relative permittivity (μ/D) of the solvents, where μ and D are the dipole moment and debye units, respectively. While no reasonable correlation with polarity could be obtained, the K_P value could be correlated well with μ/D, as shown in Figure 5 for all the beta-lactam molecules studied. It is apparent that K_P 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 Aliquat-336 was analyzed from the data generated in butyl acetate as the solvent. It appears

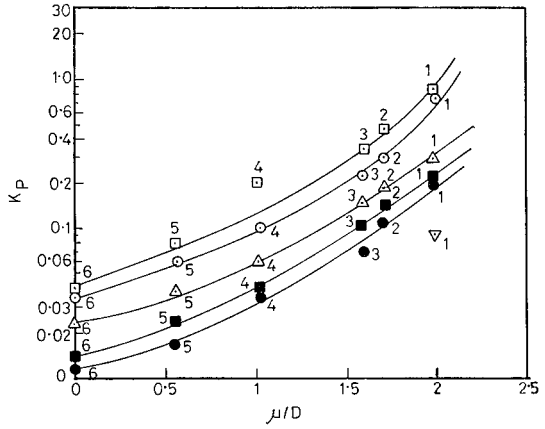

Figure 5. Relation of extraction equilibrium constant to the relative permittivity (μ/D) of solvents: ○, cephalexin; △, 7-ADCA; □, cephalosporin-c; ▽, cephaloridine; ●, 7-ACA; ■, 6-APA. Solvent: curve 1, *n*-butyl acetate; curve 2, 1-octanol; curve 3, dichloromethane; curve 4, chloroform; curve 5, cyclohexane; curve 6, benzene.

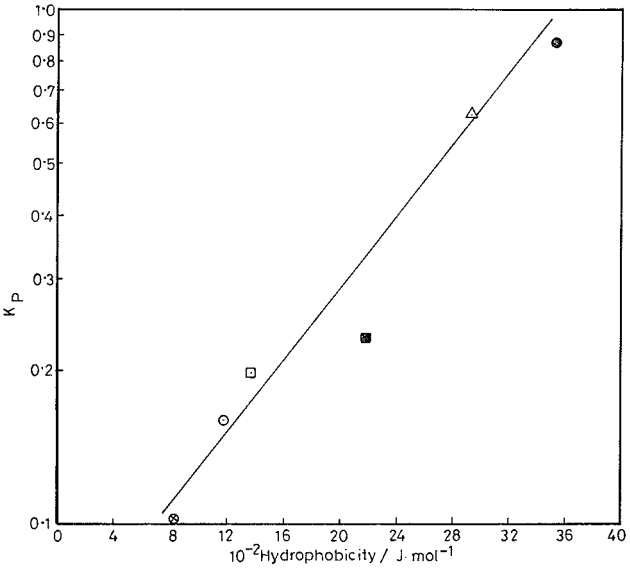

Figure 6. Relation of equilibrium constant and hydrophobicity scale: ○, 7-ADCA; △, cephalexin; □, 6-APA; ●, cephalosporin-c; ■, 7-ACA; ⊗, cephaloridine.

that the K_P 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_P versus absolute hydrophobicity (expressed as J·mol^{-1}), the value of which was measured according to a method reported in the literature (Nozaki and Tanford, 1971). The observed relation between K_P and hydrophobicity is akin to that obtained for amino acid extraction by the same carrier (Hano et al., 1991). From the date, such a relation appears to hold true for all other solvents. However, since the K_P 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 Aliquat-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_{a2} 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 via solvation of the complex based on dipole−dipole interaction. Furthermore, the extraction equilibrium constant obtained in a specific solvent was found to correlation well with the hydrophobicity of cephalosporins, implying that the extraction equlibria are dependent on the chemical nature of the solute. The beta-lactams complex with Aliquat-336 in a 1:1 ratio.

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