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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2010, 2(4):257-265

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Selective Extraction of 7-aminodeacetoxycephalosporanic acid and Cephalexin antibiotics with Aliquat-336

M M Bora

North East Institute of Science and Technology, Jorhat -785006, Assam India (Council of Scientific & Industrial Research)

ABSTRACT

The reactive extraction of 7-ADCA (aminodeacetoxycephalosporanic acid) and Cephalexin with Aliquat-336 (tricaprylmethyl ammonium chloride) dissolved in n-butyl acetate as solvent has been studied over a pH range of 5 to 8. The effect of such parameters as Aliquat-336 concentration, pH and 7-ADCA and Cephalexin concentrations on distribution coefficient in the reactive extraction system has been evaluated. It was found that distribution coefficient (K_d) increases with increases of the aqueous phase pH implying that the anionic forms of the dissociated 7-ADCA and cephalexin are amendable for reactive extraction. Furthermore inference of practical relevance that could be drawn from the present study is that 7-ADCA and cephalexin can be selectively extracted from an aqueous solution typical of that encountered in biosynthesis of 7-ADCA and cephelexin. Study on reextraction of 7-ADCA and cephalexin from the extracted phase was carried out using an aqueous solution of acetate was found to be pH dependent which also attribute to ionization behaviour of the beta-lactams. Such observation is considered important as re-extraction at an appropriate pH value is possible.

Keywords: Distribution coefficient, 7-ADCA and Cephalexin antibiotics, Ion exchange, re-extraction, Aliquat-336.

INTRODUCTION

Reactive extraction in liquid membrane can provide an effective method for separation and purification of cephalosporin antibiotics from dilute solution [1, 2] such as that encountered in

fermentation broth. Accordingly, extensive studies have been performed to demonstrate the feasibility of liquid membrane system, which can provide enhanced separation potential [1, 3, 4]. We had reported in our previous papers the complementary studies on reactive extraction of 6-APA and 7-ACA with extractants such as secondary, tertiary and quaternary amines and it was found that Aliquat-336 was the best choice of carrier for specific cases [4-9]. The same carrier has been found to be effective for reactive extraction of amino acids, lactic acid [11-12] and other anionic species [14]. However, a systematic study on the equilibrium of reactive extraction has not been reported earlier in the literature.

Our investigation on reactive extraction of cephalosporins with Aliquat-336 revealed that butyl acetate as the ideal solvent provides the highest extraction efficiency of 7-ACA [4]. Any probability of the non-identity 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.

In this paper, we report a comprehensive study on distribution coefficient of 7-ADCA and Cephalexin with Aliquat-336 in butyl acetate as the solvent system and try to assess the selectivity from results obtained from single component extraction experiment. Biosynthesis of antibiotics has drawn special interest from the researchers throughout the world. In the bioconversion of 7-ADCA to cephalexin, purification and separation of cephalexin from the mixer of unconverted 7-ADCA is essential. Reactive extraction as such and in liquid membrane in particular, may be an effective method for this practically useful separation

Theoretical considerations

7-ADCA and cephalexin are Zwitterionic molecules the pK_{a1} and pK_{a2} values being 2.95 and 4.78 for 7-ADCA and 2.56 and 5.88 for cephalexin. In the pH range between 2.95 and 4.78 for 7-ADCA and 2.56 and 5.88 for cephalexin, the zwitterions as a whole are predominant as evident from the dissociation behaviour shown in Figure 1

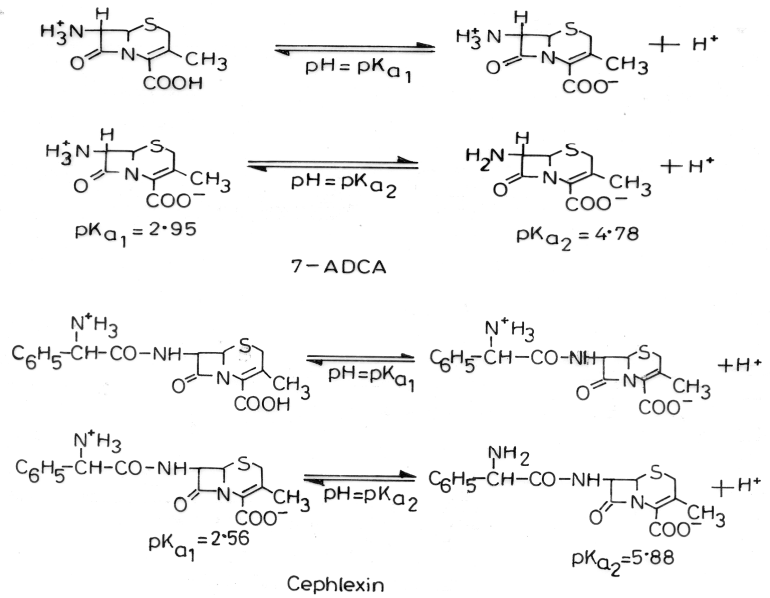

Figure 1 Dissociation behaviour of 7-ADCA and Cephalexin

The pH at which the amino group ionises keeps the carboxyl group in the COO^- form. The anionic forms 7-ADCA and cephalexin at pH> pK_{a2} are amenable for ion-exchange with anionic exchanger such as Aliquat-336 (a carrier hereafter termed as QCl). Beta-lactam anion (P^-) complexes with the carrier, QCl, dissolved in the organic solvent according to the following reaction.

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

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

7-ADCA (HP) and cephalexin (HP) molecule first dissociates in aqueous solution at pH > pK_{a2} to give the anion P^- and a proton, H^+ as follows

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

The dissociation equilibrium constant, K of this reaction is given by

$$Kp = \frac{C_H \, C_P^-}{C_{HP}} \quad (3)$$

The distribution coefficient (K_d) of HP is defined as

$$K_d = \frac{C_{QP} \, V_o}{C_{HP} \, V_a} \quad (4)$$

Co-extraction of inorganic anion is equally important because is the biosynthesis media contains buffer anions such as phosphate, sulphate etc. Thus the co-extraction would effect the extraction of primary beta-lactam anion and the extent of the co-extraction should be quantified from process design, point of view.

Since the co-extraction is relativity low at high pH value [4], it is not considered for analysis of extraction selectivity in the present work.

EXPERIMENTAL SECTION

Chemicals and reagents

7-ADCA and cephalexin the beta-lactam antibiotics used in the work were procured from Sigma Chemical Co (St Louis, Missouri, USA) and are of 99.9% purity. Aliquat-336 (Aldrich Milwakee, USA) were used as received Butyl acetate and other analytical grade buffer reagents were procured from BDH (Mumbai, India)

Procudure

Equilibrium experiments

The distribution coefficient experiments were conducted by using 10 ml each of aqueous solution of 7-ADCA and cephelexin and the organic solution of Aliquat-336 dissolved in butyl acetate in a stoppered 50 ml round- bottom flask which was placed over a magnetic plate and the

solution was agitated with a magnetic needle maintaining the temperature at 25^0C. The aqueous phase pH was maintained in the range of 5 to 8 by using. The method for preparation of the buffer solution is the same as reported in our previous papers [4, 5]. The time of equilibration for the extraction was 2 hours after optimization. After attainment of equilibrium, the two phases were allowed to settle, aqueous and organic phases were separated and aqueous of 0.1 ml aqueous solution was taken for analysis of beta-lactam content by using a UV-Visible Spectrophotometer (Shimadzu, Model 160 A) calibrated at the wave lengths of 265 and 262 nm for 7-ADCA and cephalexin, respectively. The distribution coefficient experiments were carried out in triplicate and the reproducibility was found to be ± 5%. The distribution coefficient was calculated as the ratio of beta-lactam content in organic to that in aqueous phase. Organic phase concentration was estimated from mass balance in the phase.

For stripping, the aqueous solution taken was an acetate buffer of pH 4 made by using 10 $MolL^{-1}$ acetate ion. In the organic phase, the initial concentration of solute-carrier complex (QP) and free carrier (QCl) was varied from 0.7 to 0.9 mM and 0.52 to 9.8 mM, respectively. The organic phase was loaded with beta-lactam ion by equilibrating equal volumes of the aqueous beta-lactam solution at appropriate pH and n-butyl acetate solution of Aliquat-336 (organic phase) under intense stirring in a glass vessel for two hours. The concentration, of the complex (QP) was varied by taking different concentrations of QCl during loading experiment and determine from a material balance.

The stripping experiments were carried out at 25^0C by mixing 10 ml each of buffered aqueous solution and loaded organic solutions in equal volumes in a stopper glass flask of 50 ml capacity round- bottom flask placed over a magnetic plate with magnetic capsule. The aqueous phase pH of 7-ADCA and cephalexin was maintained at a value of 3 to 4 by using acetate buffer respectively. Samples of the aqueous phase were collected at equal interval of time and the concentration of the solute was determined by a UV-Visible Spectrophotometer calibrated at appropriate wave length [4].

RESULTS AND DISCUSSION

The reactive extraction behaviour expressed in terms of distribution coefficients (K_d) of 7-ADCA and cephalexin as a function of aqueous phase pH and carrier concentrations are shows in Figures 2 and 3. The value of K_d increase with an increase of pH (upto pH 8), the extent of increase being higher for high range of carrier concentration such as observation of strong pH dependence of K_d at high carrier concentration was obtained also for the extraction of dl-phenylamine with Aliquat-336 [6,10] and other beta-lactam reported by us elsewhere [4]. Different K_d values at identical values of pH for different beta-lactams provide further evidence on the effect of chemical nature of the beta-lactams on extraction efficiency of Aliquat-336 [4].

Furthermore, finite and significant difference in distribution coefficients at high and low pH values as is evident from Figure 2 and 3 implies that extraction can be carried out at high pH while stripping will take place at lower pH which is advantageous from practical point of view.

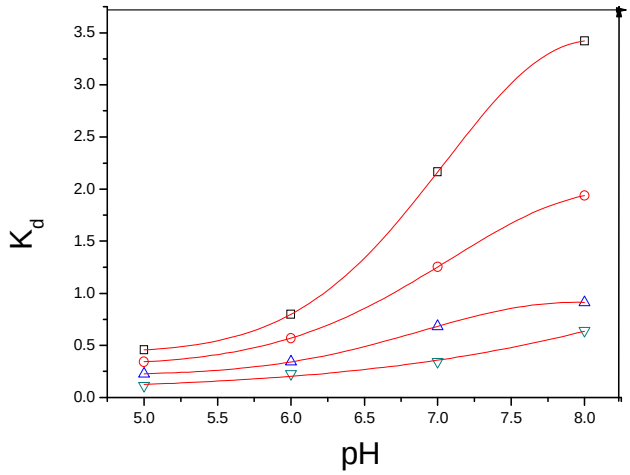

Figure 2 Effect of pH on K_d at various carrier concentrations for extraction of 7-ADCA with Aliquat-336.

C_P = 1 mM ; Symbol, Aliquat –336 = , 10mM ; O ,5 mM ; Δ, 2 mM ; ∇,1 mM.

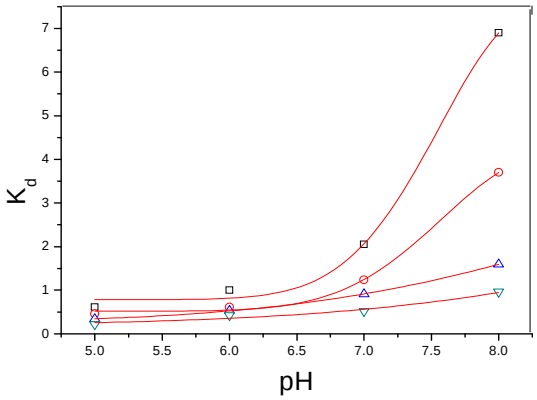

Figure 3 Effect of pH on K_d at various carrier concentrations for extraction of cephalexin with Aliquat-336. C_P = 1 mM ; Symbol,

Aliquat –336 = , 10mM; O , 5 mM ; Δ, 2 mM ; ∇,1 mM

The effect of beta-lactam (solute) concentration (at constant pH) of aqueous phase on K_d was studied in the solute concentration range of 0.5 to 1.5 mM at a carrier concentration of 1 mM. As is evident from Figures 4 and 5, K_d decreases with equilibrium solute concentration. This dependence is identical for various carriers studied in our previous works [3], the highest K_d value under otherwise identical experimental conditions was observed with Aliquat-336 from which it may be inferred that the ion-exchange extraction with such lipophilic carrier will perhaps be the best choice for reactive extraction of cephalosporin antibiotics. The Figures 4 and 5 also show that K_d value decrease with an increase of solute concentration in general, which may be attributed to a shift (decrease) in the equilibrium pH value probably as a result of the reduction in the mole fraction of the extractable anion. This probability is further supported by the observation of a lower K_d value at low pH, particularly at low equilibrium concentration of

the solute. This observed effect of solute concentration and pH on K_d for extraction of all the beta-lactams with Aliquat-336 appears to be identical. However, the lowering of K_d values with decreasing pH is more pronounced in the lower range of solute concentration.

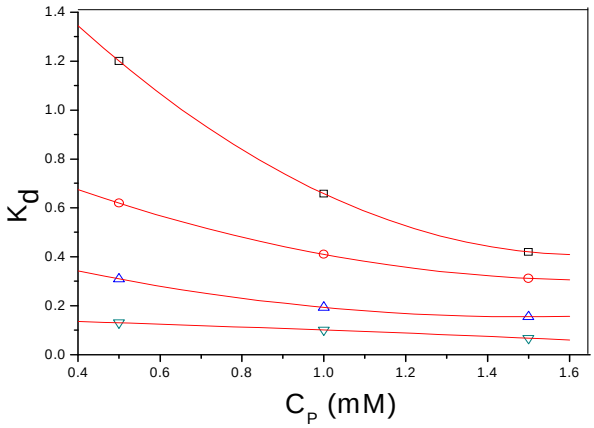

Figure 4 Effect of solute concentration on K_d for extraction of 7-ADCA with Aliquat-336 at various aqueous phase pH.

C_{QCl} = 1 mM ; Symbol, pH= , 8 ; O, 7 ; Δ , 6 ; ∇, 5

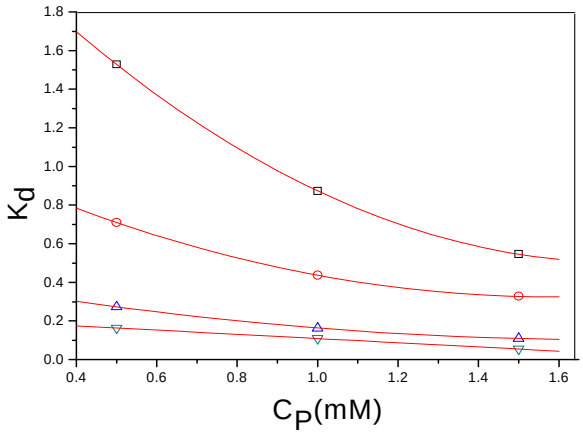

Figure 5 Effect of solute concentration on K_d for extraction of cephalexin with Aliquat-336 at various aqueous phase pH.

C_{QCl} = 1 mM ; Symbol, pH= 8 ; O, 7 ; Δ , 6 ; ∇, 5.

It is thought worth while to assess the selectivity of extraction as expressed be the following relation

$$\text{Selectivity (s)} = \frac{K_{d\ \text{Cephelexin}}}{K_{d\ 7\text{-ADCA}}} \qquad (5)$$

Where $K_{d\ \text{Cephelexin}}$ and $K_{d\ 7\text{-ADCA}}$ represent as distribution coefficient of Cephelexin and 7-ADCA respectively and were determined in dependant by using symmetric sample. Figure 6 is plot of

Selectivity (s) vs pH for various experimental conditions. From Figure 6, it is apparent that the reactive extraction exhibits selectivity for cephelexin by an order of magnitude. This implies that from a binary mixture of Cephalexin and 7-ADCA like that encountered in the biosynthetic media in cephelexin production, selective separation of cephelexin is possible at a pH value above the pK_{a2} of cephelexin. Thus reactive extraction using Aliquat-336 can be exploited in a liquid membrane system for practical purpose.

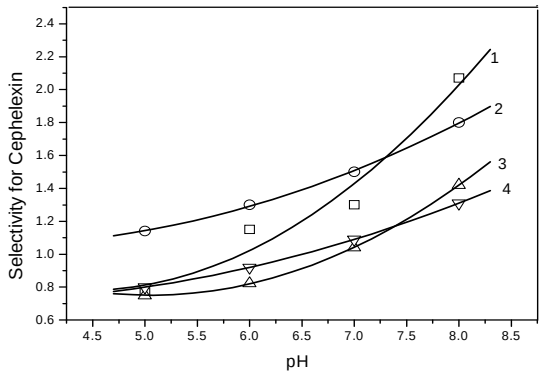

Figure 6 Selectivity for cephelexin vs pH at various carrier concentrations. Aliquat –336 concentration in plot

, 1 = , 10mM; 2 = O ,5 mM ; 3 = Δ, 2 mM ; 4= ∇,1 mM.

It appears that the experimental Cp_s versus time (t) profile is fairly well for 7-ADCA and cephalexin shown in figure 7. The loaded organic phase contains appreciable proportion of free QCl which has certain surface active properties and is likely to take part in another anion exchange reaction with buffer anion of the stripping aqueous solution. The re-extraction of cephalexin and 7-ADCA are 81% and 65.5% respectively.

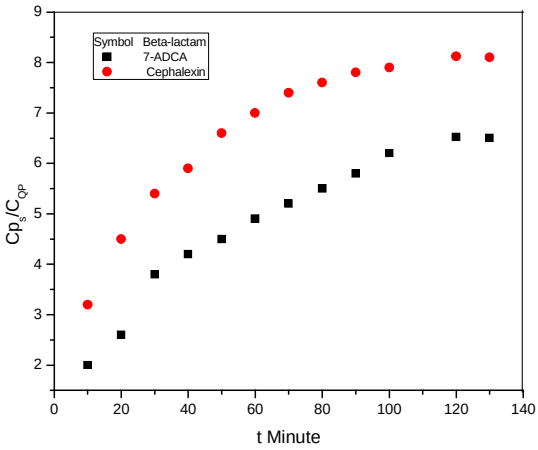

Figure 7 Re-extraction of 7-ADCA and Cephalexin

CONCLUSION

A liquid-liquid ion exchange a mechanism can be exploited for reactive extraction of 7-ADCA and cephalexin from aqueous solution using Aliquat-336 and butyl acetate as the carrier and solvent, respectively. The partition coefficients are influenced by the aqueous phase pH, carrier and solute concentration. The reactive extraction system can provide an attractive method for selectivity separation of cephelexin from a binary mixture of cephelexin and 7-ADCA. An additional ion-exchange reaction between free QCl and buffer anion is expected to facilitate the stripping process and the role of additional Cl^- concentration seems to be insignificant.

Acknowledgement:
Authors would like to Dr N N Dutta, Head, and Department of Chemical Engineering, NEIST, Jorhat and Dr P G Rao, Director, NEIST, Jorhat.

Nomenclature

6-APA	6-aminopenicillinic acid
7-ACA	7-aminocephalosporinic acid
7-ADCA	7-aminodeacetoxycephalosporanic acid
C	concentration
H^+	proton, mol/m^3
HP	7-ADCA and cephalexin anion, mM
K	dissociation equilibrium constant
K_d	distribution coefficient
$K_{d\ Cephelexin}$	distribution coefficient of Cephelexin
$K_{d\ 7\text{-}ADCA}$	distribution coefficient of 7-ADCA
P^-	7-ADCA and cephalexin anion, mM
QCl	carrier, Aliquat-336, mM
QP	solute-carrier complex, mM
S	Selectivity
V	Volume

Subscript/superscript

a	aqueous phase
o	organic phase

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