CHAPTER 1

INTRODUCTION
1.1 An Overview of beta-lactam antibiotics and their processes

The beta-lactam antibiotics are therapeutically important and advantageous for their broad antibacterial activity. The beta-lactam family has developed into an array of chemical structures with six basic nuclei (Figure 1.1). Out of the total worldwide antibiotic production of around $5 \times 10^7$ Kg/annum, the beta-lactam group constitutes an amount of the order of $3 \times 10^7$ Kg/annum. Penicillins and cephalosporins are the main constituents of commercially important beta-lactam as shown in Figure 1.2. Since the discovery of cephalosporin-C (CPC) by Newton and Abraham (1955), the processes for production of natural and semisynthetic cephalosporins have undergone various modifications (Abraham and Loder, 1972). Penicillins and cephalosporins are mainly produced by biosynthetic routes and the mechanism pathways for their biosynthesis are now fairly well understood (Martin and Liras, 1989). Penicillins are produced mainly by a class of fungi, particularly the *penicillium* and *Aspergillus* species. Penicillin G, F, K, X and N, dihydropenicillium and isopenicillin N have been isolated from *penicillium notatum* or *penicillium chrysogenum*. Semisynthetic penicillins and cephalosporins can be prepared by either enzymatic or chemical processes.

The growing interest in the various beta-lactam antibiotics over the last decade has resulted in improvement of their production methods by modification of either the basic process and/ or microbial strain or the downstream processing techniques, i.e., extraction and purification of the product from fermentation broth. Effective extraction and purification methods are necessary for both analytical purpose and large-scale application. Beta-lactams produced by the fermentation route are generally recovered via a series of downstream processing operations depending on the antibiotic properties and product distribution as well as their processing requirements. The fermentation broth usually represents around 75% of the final cost of the antibiotics and the cost of product recovery is often greater than 50% of the production cost. Even 1% increase in the overall yield of isolation in relation to a non-optimized situation for a plant producing $5 \times 10^5$ Kg/annum of CPC at a selling price of Rs. 3500 / Kg will represent an annual saving of around Rs. $1.75 \times 10^7$. Thus, production recovery is inherently an important
Figure 1.1: Six distinct classes of naturally occurring beta-lactam antibiotics.
Beta-lactams

Penicillins

Natural

Penicillin K, F, N, X (Penicillium chrysogenum).

Cephalosporins

Natural

Semisynthetic

6-APA

Ampicillin, Amoxicillin, Dicloxacillin, Flucloxacillin, Methicillin, Phenthi-cillin etc.

Cephalosporini - C (CPC)
Deacetyl - CPC, Deacetoxy - CPC (C. Acremonium)

7-ADCA

Cephaloridine, Cephazolin, Cephamandole, Cephaloglycine, Cefadroxyl,
Cephatholin, Cefradine, Cefalexin.

Semisynthetic

Cephemycin A, B, C
Clavulonic Acid (Strep. clavuligerus)

7-ACA

Cefoxitin, Cefmetazole, Cefotetan

Figure 1.2: Commercially important beta-lactam antibiotics.
component of the technology of beta-lactam antibiotics. It appears that almost all the known processes for commercial scale extraction are based on low yielding operations because of unfavorable physical properties of the beta-lactam antibiotics, particularly the cephalosporin group. Search of an alternative and improved method for extraction and purification is therefore highly warranted in technological perspectives. Liquid membrane extraction as discussed in our recent review (Ghosh et al., 1996) provides an alternative method for commercial application. In this technique, the principle of reactive extraction is utilized to facilitate transport rate and data equilibrium. The kinetics of the reactive extraction are highly useful for practical application. In view of this, the same has been included under the theme of the present thesis.

1.2 Method for extraction and purification of beta-lactam antibiotics:

1.2.1 Conventional methods

Traditionally, recovery of beta-lactams produced by fermentation method involves extraction of the filtered broth and its purification and crystallisation of the antibiotics at the isoelectric points. Parallel to traditional methods for extraction of filtered broth, direct broth extraction has been attempted in plant scale. Though the later process has unfavorable side effects, this can lead to several advantages from the point of the whole broth technology (Vandamme, 1984; Brunner, 1985). A brief discussion on conventional methods of extraction and purification is given in the following sections.

Adsorption /Chromatographic Method

The increasing application of liquid chromatography in all its forms to the analysis of beta-lactam indicates the versatility of the chromatographic technique for separation in a larger scale perspective too. The partitioning of beta-lactam between the solvent and a solid matrix is the basis for chromatographic separation (Margosis, 1989). The partitioning can be derived from such phenomena as adsorption, molecular sizing (gel
filtration chromatography), enzyme specificity (affinity chromatography) and molecular recognition (immunosorbent chromatography), etc. These methods are advantageous in that they are gentle in nature (no heat generation or shear), simple to scale-up and capable of giving a high degree of product quality and recovery.

Chromatographic media like octadecyl silane chemically bonded to silica particles, strong cation exchangers bonded to silica, etc., which are known for analytical purpose can also be used for preparative scale chromatography. C₈ or C₁₈ sorbents with ion pairing agents such as tetrabutyl ammonium hydroxide and acetyltrimethyl ammonium bromide have been used for resolution of penicillin broth (Margosis, 1989). Active carbon and macroreticular porous resins were the early generation of adsorbents used for primary isolation of CPC, cephalaxin, clavulanic acid, nocardicin, etc., from culture broth (Belter, 1985). A number of neutral polymeric sorbents such as polyaromatics (Amberlite XAD-4, 16, 1180, Diaion HP20), aliphatic esters (Amberlite- XAD-7) and nitrated aromatics (nitrated Amberlite XAD-16) proved effective for penicillins and cephalosporins, but the aromatic sorbents provide the highest sorption capacity for both the antibiotics (Chaubel et al., 1995). The macroporous resin adsorbents are well suited for transforming salts of organic acids or bases into their respective free acids or bases. Thus, these resins are more useful than ion exchange resins especially when amphoteric molecules are involved. The selectivity and capacity can be influenced by the nature of the inorganic ion if neutral feed solution is involved. Thus, the separation of CPC from deacetyl CPC in neutral solution using non-polar resin was found to be effective only in the presence of an alkaline earth metal (Voser, 1982). Typical of many large scale adsorption operations, CPC is recovered using on-off chromatography such that a large amount of fermentation broth is passed through the bed until breakthrough is observed after which the column is eluted (Wildfenur, 1985). Halogenated crosslinked polymer of aromatic polyvinyl moiety, such as brominated Diaion-HP20 can provide an effective method for recovery of cephalosporins (Lee et al., 1992). Amberchrome reverse phase resin can also be effective for adsorptive separation of CPC (Firoujtal et al., 1994). Ion-exchange adsorption of phenyl acetic acid onto Lewatit MP-500A can be a strategy for
isolation of zwitterionic 6-aminopenicillanic acid (6-APA) from its mixture (Shewale and Sivaraman, 1989).

For primary isolation of clavulanic acid from filtered broth, adsorption on activated carbon or polyaromatic resin such as Diaion PA306, Zeolite FFIP5RA61, etc., have been recommended (O'Sullivan and Sykes, 1986). Adsorption on activated carbon is followed by elution with 90% acetone whereas the eluant for the resin is NaCl solution, which needs subsequent desalting on a suitable ion exchange resin. The crude clavulanate obtained above can be further purified by chromatography of the compounds or its benzyl ester (obtained by reacting sodium clavulanate with benzyl bromide in dimethyl formamide as solvent) on dextran based lipophilic adsorbent (Sephadex LH20) using cyclohexane : chloroform (1:1 v/v) as the solvent followed by silica gel chromatography using cyclohexane and ethylacetate (1:1 v/v). The pure benzyl product can be converted to sodium clavulanate tetrahydrate by hydrogenolysis of the ester over 10% Palladium Charcoal in aqueous ethanol in the presence of NaHCO₃, and recovered by crystallization. An alternative method is to isolate as lithium clavulanate. In this case, the filtered broth adjusted to pH 5.45 is applied to a carbon column, elution being done with aqueous acetone. The eluate is concentrated and applied to a column of weakly basic polystyrene based anion exchange resin in the chloride form (Amberlite IRA68) and subsequently eluted with 5% aqueous lithium chloride yielding lithium clavulanate which can be obtained as pure crystal.

Aqueous extract of monobactams after desalting on a dextran based size exclusion medium (Sephadex G-10) can be purified by the adsorption on a cellulose based anion exchanger (diethyl amino ethyl cellulose) followed by reverse-phase chromatography on a polystyrene based resin such as Diaion HP20 AG (O'Sullivan and Sykes, 1986). The antibiotic is then converted to potassium salt on a polystyrene based strong cation exchange resin (Dowex 50WX2) and crystallized from aqueous methanol to give a pure product. Carbapenam antibiotics like thioenamycin can be isolated by ion-exchange adsorption and purified by combination of gel filtration and ion-exchange chromatography and reverse osmosis (O'Sullivan and Sykes, 1986).
The technology of large scale liquid chromatography, including column design, system scale-up, process control and instrumentation, has been developed and reported for a few cases only. Shih et al., (1983) had reported studies of large-scale chromatography in columns of two sizes (1 cm x 40 cm and 15 cm x 40 cm) using various reverse phase C_{18} packing for recovery of a semisynthetic antibiotic (Mevinolinic acid MK819). While the performance of various materials varies from supplier to supplier, the resolution achieved in the larger column packed with identical material was marginally better and the calculated number of theoretical plates for the larger column was 150 in comparison to 115 for the smaller one. This successful scale-up has been attributed to a unique proprietary design of the column distributor and collector in a column. Feasibility of producing purified cefonicid by preparative scale high performance liquid chromatography (HPLC) was studied in a series of Waters (USA) columns of 20 cm x 60 cm size using reverse phase microparticulate silica packing and water as the mobile phase (Cantwell et al., 1984). Since the preparative chromatography is performed at sample-column ratio much higher than used in analytical columns, it is important to select chromatographic systems under load conditions similar to those to be used for the process scale-up rather than those used in the analytical systems. The important parameter affecting the process throughput and product purity was found to be the column load (g sample/g HPLC support). In fact, many adsorbents are known, but the knowledge on their elution and regeneration properties, selectivities and hydraulic behaviour is far from being complete.

Liquid-Liquid Extraction

Schugerl (1994) has recently furnished an account of the technological status on liquid-liquid extraction of β-lactam antibiotics. The extraction is usually practised in filtered fermentation broth. Solvents useful for less hydrophilic penicillins are dimethylcyclohexanol, cyclohexylacetate, butyl acetate, amyl acetate, methyl-isobutylketone, methyl cyclohexanone, 2-ethyl hexanol, etc. In order to prevent decomposition of penicillins and maintain high distribution ratio in the solvent phase,
extraction is carried out at low pH (2-2.5) in a short contact time using centrifugal extractor. Phenyl and Phenoxyacetic acids (by-products of penicillins) can be extracted with acetone and methylene chloride. Direct extraction of penicillin from broth has the potential for increasing the extraction efficiency by about 10% as the penicillins adsorbed into the mycelium solids can be extracted. However, the inherent problems of emulsion formation during direct extraction lead to additional cost of product recovery because of the necessity of a suitable demulsifier (Szabo, 1992).

Though the processes for solvent extraction of penicillins are fairly well developed (Schugerl, 1994), it is difficult to extract highly hydrophilic cephalosporins using solvent extraction method as multiple ionisable groups are present in their structures. However, it is possible to chemically modify CPC into its lipophilic form, which is amenable for solvent extraction (Andrisano et al., 1976). Thus the reaction of CPC with sulphonyl chloride gives N-tosyl-N-p-cumyl sulphonyl derivatives (esters) which can be subsequently extracted by ethyl acetate or by n-butanol. The derivatives can further be transformed into 7-aminocephalosporanic acid (7-ACA) which is the key intermediate for a large number of semisynthetic cephalosporins (Figure 1.2).

For extraction of CPC and deacetyl CPC (DCPC) from fermentation broth, a method called “extractive esterification” has been suggested (Elks, 1997). This method involves blocking the basic group in the side chain with an acylating agent in slightly basic medium followed by treatment with a diazoalkane at low pH in a water immiscible solvent such as methylene dichloride. A mixture of the biesters of the N-blocked cephalosporins thus, accumulates in the organic phase, which can be processed to obtained a wide range of semisynthetic antibiotics. The use of solvent with high dielectric permitivities facilitates extraction.

The primary isolation of clavulanic acid from filtered broth can be carried out by solvent extraction at pH 2.0 with n-butanol and back extraction into water at pH 7.0 (Schugerl, 1994). Further purification involves chromatography of clavulanate or its benzyl ester or by crystallization.
1.2.2 Emerging methods

Aqueous two-phase partitioning (ATPP)

The partitioning behaviour of small biological micromolecules in aqueous two phase system provides an effective tool for their recovery from fermentation broth (Walter et al., 1989). The partition coefficient (K) for penicillin-G in polyethylene glycol (PEG)/phosphate two phase system can be as high as 200 (Yang and Chu, 1990) which offers a possibility of ATPP technique for practical application. CPC can be extracted from dilute or whole broth containing deacetyl DCPC by using PEG 6000/ammonium sulphate or phosphate aqueous two-phase system (Yang et al., 1994). The experimentally observed K values (K>1) for CPC and K<1 for deacetyl DCPC) in such a system can provide the basis for development of a technically feasible scheme for separation of CPC from DCPC which is rather a difficult task from the practical point of view. ATPP would provide easier isolation of products from a large amount of cell mass and viscous broth without significant dilution. However, before any commercial application can be established, it is necessary to address the problems of phase separation, PEG and salt recovery for recycling, developing an effective method for recovering the cell mass etc. It is also necessary to understand the design parameters of extractors for ATPP system.

Membrane (Synthetic) Separation: Ultrafiltration (UF)/microfiltration (MF)/reverseosmosis (RO)/electrodialysis (ED)

The early use of membrane in beta-lactam separation has been reported for enzymation of penicillin-G in an immobilized membrane reactor (Greco et al., 1983). In the ultra filtration membrane reactor studied by the above authors, purified penicillin acylase and stabilizing polymer injected into the system are drawn by the substrate permeating steam towards the membrane surface where accumulation occurs within the polarization layer. The substrate transfer occurred by a convective mechanism, thus
minimizing the mass transfer resistance. The product, 6-APA, is obtained in the permeate form in a concentrated and relatively pure form. Concentration of the reaction mixture by RO can significantly reduce solvent cost and increase precipitation efficiency of the product (Shewale and Sivaraman, 1989). Electrodialysis can be highly effective for in situ removal of phenylacetic acid, an inhibitory byproduct in enzymatic hydrolysis of penicillin-G giving 6-APA (Ishimura and Suga, 1992). Such a strategy can enhance the reaction rate by around 65% but at the cost of overall yield which can, however, be compensated by increasing the penicillin-G conversion using a high concentration of penicillin-G in the feed. Identical method can also be used for the enzymatic production of 7-Aminodeacetoxy cephalosporanic acid (7-ADCA). Due to the complicated nature of the fermentation broth of Cephalosporium acremonium, the whole broth processing very often requires a combination of membrane and chromatographic separation techniques. In a typical process (Figure 1.3) cells are first removed by a polyvinylidene fluoride 0.2 μm MF membrane and then proteins and polysaccharides by UF/diafiltration using a polysulfone 10 KMWCO membrane (Kalyanpur et al., 1985). The UF permeate is concentrated by RO and the antibiotic is finally purified by high performance liquid chromatography upto a recovery of 98.5%. In another method (Lee et al., 1992), a combination of UF and RO is suggested as shown (Figure 1.4). The yield could be as high as 90% through column chromatography using weakly basic anion exchanger (Diaion WA 30), neutral polyaromatic adsorbents (Diaion HP 20 and Amberlite XAD 200) and strongly acidic cation exchanger (Diaion SK 1B) in a sequential manner. In the tandem operation of column, the recovery yield could be increased upto 96%. The final product was prepared by spray drying of a feed with 85.5% of CPC, 6.3% water, 4.63% free sodium ion and traces of metal ions.

(a) Liquid membrane extraction

Since discovery two decades ago, liquid membrane (LM) has been extensively studied for the separation of metal ions, amino acids, etc., from aqueous solution (Ho and Li, 1992). There are two general forms of LM, i.e. emulsion liquid membrane (ELM) and
Permeate
(low dissolved solid)

Purified antibiotics

Chromatographic separation

MWCO : Molecular weight cut-off

Figure 1.3: A combined membrane process for isolation of cephalosporin-C from fermentation broth.
Figure 1.4: A combination of adsorption-membrane process for purification of cephalosporin-C.
supported liquid membrane (SLM). The applications of recent developments of LM technique for separation of various beta-lactam antibiotics are discussed below.

**Emulsion liquid membrane:**

In a fermentation broth, natural penicillins and cephalosporins usually exist in ionic form at considerably low concentration. In such a situation, ELM of the type Water/Oil/Water (Figure 1.5) via the so-called “facilitated transport” mechanism (Figure 1.6) can serve the basis for an effective separation method. The solute present in the outer aqueous phase (W) reacts reversibly with a carrier to form a solute-carrier complex which diffuses through the membrane (O) phase and reacts with the inner aqueous stripping phase (W) where the solute is released. The separation is effected by simultaneous extraction-stripping process occurring in a single step. Because of the reactive extraction, high separation selectivity and transport flux can be achieved. The formulation of a stable emulsion is of paramount importance for the ELM system. Surfactants used should be of proper hydration characteristics and chemical inertness. Solvent should be compatible to the solute and should provide an optimal viscosity of W/O emulsion. In addition, toxicity of the carrier should be carefully considered while designing an ELM system.

Though the reported studies on ELM separation of beta-lactam antibiotics are limited, there will be great incentives for further development in this technique. In a typical ELM system with a non-ionic polymeric ECA-4360J as the surfactant and di-n-octylamine as the extractant or carrier, complete extraction and re-extraction of penicillin-G can be achieved by an optimal adjustment of the pH of both the internal and external aqueous phases (Hano et al., 1990). The most effective carrier for penicillin extraction from simulated feed stream has been found to be tri-n-octyl amine because it exhibits lowest activity towards solvents (Tsikas et al., 1989). Tertiary amines require protonation in the extractive reaction and the protonated solute-carrier complex undergoes stripping reaction and the protonated solute-carrier complex undergoes stripping reaction in carbonate buffer according to

\[ \text{Protonated Complex} \rightarrow \text{Stripped Complex} \]
Figure 1.5: Schematic diagram of emulsion liquid membrane.
Feed (External phase)

Membrane

Strip (Internal phase)

— QH

P + H⁺ → A = a = A PH

P⁻ Penicillin or Cephalosporin anion

(a) Co-transport: Aliphatic amine as carrier
(via Amine protonation).

P⁻⁺ H⁺⁺ A ↔ APH

(b) Counter-transport: Tricaprylyl methyl ammonium chloride as carrier.

P⁻⁺ QCI ↔ QP⁺⁺Cl⁻

Figure 1.6: Mechanism of facilitated transport of beta-lactam in liquid membrane process.
Thus, by maintaining a pH difference in the two aqueous phases, the separation can be effected. At the early stages of extraction, penicillin-G is subjected to those pH values at which it is extremely unstable. It is necessary to have faster extraction rate and less membrane swelling so that the degree of decomposition may be low. The initial $\text{Na}_2\text{CO}_3$ concentration (in the stripping phase) determining the pH is dependent mainly on the initial concentration of penicillin-G, degree of extraction and the extraction time (Lee et al., 1994 a). The degree of decomposition was found to be less than 1% in 40 minutes of extraction time, which apparently proved the applicability of the ELM process, provided that initial $\text{Na}_2\text{CO}_3$ concentration could be properly determined and the extraction conditions optimised. The optimum extraction condition was found to be 20% (V/V) of span-80 in ECA-4360J as a surfactant, kerosene as a dilutent and Amberlite LA-2 as a carrier (Lee et al., 1994 b). Simultaneous removal and derivatization of penicillin-G from cell-free culture broth has been examined in an enzyme (immobilised)-ELM system (Scheper et al., 1987). In this system (Figure 1.7) penicillin-G is transported from an outer aqueous phase across the membrane via the formation of an ion-pair complex. In the inner phase, penicillin-G is hydrolysed by the encapsulated enzyme (penicillin-G acylase) forming the products, 6-APA and phenyl acetate acid (PhA) as shown in Figure 1.8, PhA is transported across the membrane by the carrier and is released into the external phase. The internal phase after breaking the emulsion can be reacted with D-phenyl glycine methyl ester to form ampicillin. It is thus, possible to extract penicillin-G, split enzymatically into 6-APA and enrich it in one step. The enriched phase can then directly be used for the synthesis of semisynthetic penicillins through enzymatic acylation. This combined ELM extraction and enzymatic conversion was studied in a continuously operated 10.1 Kuhni column with 75% span-80 and 1.5% Amberlite LA-2 in kerosene as the emulsion liquid membrane phase encapsulating the enzyme in the inner aqueous phase (Barenschee et al., 1992). The outer aqueous phase of penicillin-G

\[ 2\text{AHP}_{(\text{org})} + 2\text{Na}^+_{(\text{aq})} + \text{CO}_3^{2-}_{(\text{aq})} \overset{\text{A}}{\longrightarrow} (\text{A})_2\text{CO}_3^{2-}_{(\text{org})} + 2\text{Na}^+_{(\text{aq})} + 2\text{F}^{(\text{aq})} + 2\text{H}^+_{(\text{aq})} \]

\[ (1.1) \]
Figure 1.7: Liquid emulsion membrane based production of 6-APA from penicillin-G.

Membrane phase: Carrier (C) - Lauryl trialkyl amine
Surfactant - Span 80
Solvent - Butylacetate
Figure 1.8: Reaction scheme for hydrolysis of penicillin-G by acylase enzyme.

6-APA: aminopenicillanic acid
PhA: phenylacetic acid
solution, after the extraction cycle contains re-extracted PhA, which can be recycled to the penicillin fermentor. The membrane phase obtained by demulsification in an electrostatic coalescence was recycled and reused for emulsion formation. The 6-APA was converted into ampicillin at pH 6.0 by the same enzyme. The enzyme was recovered with an ultrafiltration membrane, recycled, and immobilised in the liquid membrane again. The advantages of the combined extraction-reaction process are that in this process, the penicillin-G concentration can be kept low, the penicillin losses, PhA consumption and the number of process stages required for ampicillin production can be reduced, etc. The major disadvantage is however the reduced stability of the ELM at high penicillin-G concentration. This problem can probably be overcome by carrying out the extraction in a hollow fiber membrane contactor, a strategy recently developed for extraction of heavy metal ions from aqueous stream (Raghuraman and Wiencek, 1993). The ELM extractor/reactor can perhaps be exploited for various other penicillins using the other side chain moieties shown in Table1.1.

If production of pure 6-APA is required, the reaction product should be free from PhA. In a classical downstream processing, PhA and residual penicillin-G are extracted at pH 2.5 with methyl isobutylketone. The aqueous phase, after extraction is adjusted to a pH of 7-8, concentrated by vacuum evaporation and the product is precipitated at 2-5°C by adjusting pH to 4.3, the isoelectric point of 6-APA. Alternately, reactive extraction in an ELM may be suggested for efficient recovery of 6-APA from the reaction product from the reaction mixture. Lipophilic quaternary salts (i.e. Aliquat-336) or other extractant such as bis-(2 ethylhexyl) phosphoric acid, 2-hydroxy-5-nonyl benzophenone or their analogues may be used in an ELM formulation.

Almost all the natural cephalosporins and their semisynthetic analogues such as 7-ACA, cephalaxin, cefatoxime, etc. are amphoteric in nature. These compounds are amenable for reactive extraction via liquid-liquid ion exchange mechanism. As mentioned earlier, anion exchange extraction of CPC with Aliquat-336 can provide a method for recovery of CPC from fermentation broth. Since the extraction equilibrium constant for CPC is higher than the other anions like phosphate, sulphate and carbonate,
<table>
<thead>
<tr>
<th>Side chain moiety</th>
<th>Products</th>
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<tbody>
<tr>
<td>P-Hydroxphenyl glycine methyl ester</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>P-Hydroxphenyl hydantion</td>
<td>Dicloxacillin</td>
</tr>
<tr>
<td>3-(2',6'-dichlorophenyl)</td>
<td></td>
</tr>
<tr>
<td>5-methyl isoxazole-4-carboxylic acid</td>
<td>Penethicillin</td>
</tr>
<tr>
<td>3-o-phenyl-5-methyl isoxazole-4-carboxylic acid</td>
<td>Oxacilin</td>
</tr>
<tr>
<td>3-o-chlorophenyl-5-methyl isoxazole-4-carboxylic acid</td>
<td>Cloxacillin</td>
</tr>
<tr>
<td>α-carboxyphenylacetic acid methyl ester</td>
<td>Carbenicillin</td>
</tr>
</tbody>
</table>
which exist in a fermentation broth, selective separation of CPC is possible with a suitable ELM system.

However, since the fermentation broth contains DCPC as the by-product, which is structurally similar to CPC, separation becomes complicated from practical point of view (Wildfenur, 1985). There is however scope to alter the extraction equilibrium constant of the two compounds by suitably manipulating the aqueous environment (such as pH) and selecting the solvent and carrier types to provide reactive extraction. 7-ACA, a versatile material for various semisynthetic cephalosporins is usually obtained from CPC by chemical deacylation using iminoether and nitrosyl chloride (Abraham and Loder, 1972). Aliquat-336 mediated facilitated transport of 7-ACA in bulk liquid membrane (BLM) has been demonstrated recently by us (Sahoo et al., 1995) and it revealed a scope for applying the liquid membrane technique for extraction for 7-ACA from bioreactor medium. ELM reactor described previously for 6-APA production can also be suggested for improved production of 7-ADCA. The membrane formulation should be such that the substrate and by-product can form an ion-pair complex with amine type of carrier whereas 7-ADCA being zwitterionic in nature will remain uncomplexed and concentrated in the internal aqueous phase which can subsequently be used for direct acylation giving various products (Table 1.2).

Clavulanic acid and cephamycin-C are produced simultaneously in fermentation broth of “Streptomycin cleavuligerus”, but the selectivity towards any of the compounds can be increased by adjusting the media composition (O’Sullivan and Sykes, 1986). Cephamycin-C being more hydrophilic due to presence of multiple ionisable groups in its structure is not amenable for solvent extraction, but clavulanic acid can be solvent extracted with n-butanol. Reactive extraction of cephamycin-C via liquid-liquid ion exchange mechanism in an ELM system can be explored for its recovery from fermentation broth.
### Table 1.2 Acylation of 7-ACA and 7-ADCA Acylase enzyme

<table>
<thead>
<tr>
<th>Side chain moiety</th>
<th>Nucleus</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Thiophene acetic acid</td>
<td>7-ACA</td>
<td>Cephalothin</td>
</tr>
<tr>
<td>Phenylglycine methyl ester</td>
<td>7-ACA</td>
<td>Cephaloglycin</td>
</tr>
<tr>
<td>1-Acetyl 1 H-tetrazol</td>
<td>7-ACA</td>
<td>Cephazoline</td>
</tr>
<tr>
<td>Mendalic acid metylester</td>
<td>7-ACA</td>
<td>Cephamandole</td>
</tr>
<tr>
<td>Phenyl glycin methyl ester</td>
<td>7-ADCA</td>
<td>Cephalexin</td>
</tr>
<tr>
<td>P-Hydroxy phenyl glycine methyl ester</td>
<td>7-ADCA</td>
<td>Cephadroxyl</td>
</tr>
<tr>
<td>Dihydroxy phenyl glycine</td>
<td>7-ADCA</td>
<td>Cepharadine</td>
</tr>
</tbody>
</table>
Liquid membrane involving microporous polymeric membrane, the so-called supported liquid membrane (SLM) offers better feasibility for scale-up and adaptability for continuous operation. The liquid phase containing the extraction agent (in reactive extraction) is immobilized in the pores via capillary forces. SLM in a planner flat sheet configuration is more suited for fundamental studies in mass transfer mechanism. However, the availability, now-a-day of high surface area modular membrane units like hollow fiber (HF) and spiral would open up wide avenue for practical application of the SLM technique. Marchese et al., (1989) had studied first the facilitated transport of penicillin in a SLM using tetrabutylammonium hydrogen sulphate and various amines as the carriers and dichloromethane, butyl acetate etc. as the solvent. Facilitated transport of penicillin-G using Amberlite LA-2 has been found to be controlled by simultaneous mass transfer across both the aqueous films and the liquid membrane (Lee, et al., 1993). Under the condition of experimentally optimised pH values of extraction (pH 6.0-6.5) and re-extraction (pH 7.0), no decomposition of penicillin-G occurs. The same SLM system has been applied for selective separation of penicillin-G from a mixture containing PhA (Lee et al., 1994). A separation factor of 1.8 was achieved by maintaining the experimental conditions under which the transport is controlled by membrane phase mass transfer in agreement with the theoretical predictions.

Tsika et al.,(1992) had studied the combined extraction of penicillin-G and enzymatic hydrolysis of 6-APA in a hollow fiber carrier (Amberlite LA-2) mediated SLM system. The selection of solvent for the carrier is crucial, since, the equilibrium constant for the complexation reaction is really influenced by the type of solvent used. The other important factors determining the permeability in the SLM system are the pH of aqueous phases, carrier concentration, penicillin-G concentration, etc. Simple mass transfer model based on simplified film theory can well describe the process of facilitated transport of penicillin across the SLM.

Effective separation of penicillin-G could be achieved in a hollow fiber contained liquid membrane (HFCLM) system utilizing Amberlite LA-2 in tri-n-butyl phosphate as
the membrane phase and a liquid-cell TEM 400/1.0 hollow-fiber membrane module (Yang et al., 1993). Due to facilitated transport of penicillin-G from an aqueous feed of citrate buffer to an alkaline stripping solution, over 90% recovery was obtained in three operating stages.

The mechanism of transport of CPC in a SLM had been studied in our laboratory (Ghosh et al., 1995). CPC could be permeated from an alkaline feed of carbonate buffer into an acidic stripping solution of acetate buffer across the membrane comprising Aliquat-336 in n-butyl acetate solution immobilized in a polypropylene (Celgard 2400) support. The transport mechanism is a case of counter-transport exhibiting overall rate dependent on solute diffusion in the membrane phase as well as the mass transfer across the aqueous boundary films. However, the stability problem of the SLM system needs attention in order that this technique could be developed further.

(c) Rotating film pertraction (RFP)

Rotating film pertraction (RFP), basically a bulk liquid membrane technique can eliminate the major shortcomings of ELM and SLM (Boyadzhiev, 1990). In RFP, the mass transfer mechanism is regarded as a typical case of an “uphill transport” or a “transport against apparent concentration gradient”. In a typical RFP device (Boyadzhiev, 1990), a rotating disc is immersed in aqueous solution of feed and receiving phase separated by the discs themselves. The space above the aqueous solution is coupled by the membrane liquid. The rotating disc, essentially a hydrophilic surface provides a moving interface as well as membrane phase agitation. The parameters affecting the mass transfer in extraction are the volume of each of the phases and rotational speed of the disc. Except the studies for extraction of phenyl alanine from dilute solution (Boyadzhiev and Atanassova, 1994), the RFP technique has not been studied for other pharmaceutical products. We perceive that the technique will be effective for extractive separation of hydrophilic beta-lactams from fermentation broth.
Non-dispersion extraction in hollow fiber membrane

Non-dispersive solvent extraction in hollow-fiber (HF) membrane has been extensively studied (Prasad and Sirkar, 1990). The technique utilizes immobilized liquid-liquid interface, at the pore mouth of microporous membrane to effect phase to phase contact and the mass transfer process. HF modules can be configured with different packing fraction, surface area membrane types providing flexibility in its selection and sizing for commercial application. They can offer height of transfer unit comparable to conventional extractor. The technique is amenable for reactive extraction and re-extraction for systems, which are sensitive to the aqueous environment (i.e. pH). A pH swing process utilizing a two HF module configuration has been suggested for separation and concentration of phenoxy acetic acid (Wald et al., 1989) based on the principle of reactive extraction with Amberlite LA-2 as the carrier. The separation efficiency is dependent on the solute distribution ratio (m) and the membrane type, which determine the overall mass transfer coefficient ($K_l$). For an $m>1$ system, an organic in pore of the hydrophobic membrane has a higher $K_l$ values of extraction than an aqueous in pore filled hydrophilic membrane. Similarly, for back extraction with $m<1$, an aqueous in the pore of a hydrophilic membrane has a higher $K_l$ values than an organic in the pore of a hydrophobic membrane. Thus, for phenoxyacetic acid, a hydrophobic membrane for extraction and a hydrophilic one for back extraction exhibits higher separation efficiency as compared to that achievable in other configurations. Study on non-dispersive extraction of cephalosporin antibiotics in this laboratory (Sahoo et al., 1998) has revealed very encouraging results.

Reactive extraction

Certain beta-lactam antibiotics are amphoteric in nature and therefore solvent extraction of these compounds is difficult. Industrial recovery of the beta-lactam by adsorption chromatography involves capital and energy intensive operation, thereby increasing the price per kg of the material. Reactive extraction can provide an attractive
method of separation and purification of beta-lactam antibiotics and is expected to be the future generation technology of promise. Since this is the topic of study in the present work, a comprehensive review of the subject is given in the section that follows.

1.3 Reactive extraction of beta-lactam antibiotics and reported studies

Most of the beta-lactams except penicillin G and V, phenyl and phenoxy acetic acids, etc. are ionic at high pH and unstable at low pH. Moreover, their distribution coefficients in most of the known solvents are relatively low. In such cases, extraction accompanied by chemical reaction with an extractant called “reactive extraction” can provide an attractive method for their separation. Two mechanisms in general can be suggested for reactive extraction of beta-lactam. In the first, called “ion-pair extraction” the extractant typically an amine, “A” dissolves in an organic phase and reacts with the anion, $P'$ of beta-lactam and a proton in the aqueous phase as shown below

$$A_{(org)} + H^+_{(aq)} \leftrightarrow HA^+_{(org)} \quad (1.2)$$
$$HA^+ + P' \leftrightarrow AHP_{(org)} \quad (1.3)$$

The first step is the activation of the carrier, i.e., protonization of the carrier. The protonized carrier now can react with beta-lactam anion ($P'$) and form a complex, $AHP$, which is soluble in organic phase. The transport of the anion from one phase to other requires the co-transport of cation ($H^+$) also. The reaction is instantaneous and the extraction rate is controlled by the mass transfer process. The other mechanism known as “liquid-liquid ion-exchange” involves water insoluble extractant such as Aliquat-336 (tricaprylylmethyl ammonium chloride) and counter transport of a second anion to provide electroneutrality. In a typical situation, the removal of beta-lactam anion, $P'$ from the aqueous phase by ion-exchange with the anion, $Cl^-$ of the extractant (QCl) dissolved in the organic phase takes place according to

26
The extraction efficiency depends on the beta-lactam type (dissociation constant), solvent and extractant (carrier) through equilibrium relationship. The extractant should be able to provide stripping of anion to another aqueous phase to affect separation. Schugerl (1994) has recently furnished a detailed analysis of the reactive extraction of penicillin G and V and precursor like phenyl and phenoxy acetic acids. For the reactive extraction of these compounds, ion-pair extraction is to be preferred because of the problem of re-extraction after ion-exchange extraction. Thirty different amines have been studied for reactive extraction of penicillins (Likidis and Schugerl, 1987) in various solvents such as butyl acetate, chloroform, di-isopropylether, kerosene, dioctylether, etc. Tertiary amines in n-butyl acetate were found to be advantageous because of their low reactivity with the solvent, but the distribution co-efficients of their complexes are significantly lower than those of secondary amines. While using quaternary ammonium salts for ion-exchange extraction, re-extraction is difficult and very large amount of anions (e.g. Cl\textsuperscript{−}) are needed to recover penicillins. The basic relationships for distribution coefficients and extraction kinetics have now been fairly well developed for secondary amine-penicillin systems (Schugerl, 1994). Experimental data for continuous extraction in standard devices such as Karr column have also been reported (Reschke and Schugerl, 1986). The extraction process carried out in a pilot plant Karr column using model media as well as fermentation broth of penicillin-G could be simulated from suitable mathematical model (Muller et al., 1988). Counter-current extraction-decanter has been proposed for commercial scale reactive extraction of penicillin-G from fermentation broth (Likidis et al., 1989). This device can provide an extraction efficiency as high as 90% under suitable physicochemical conditions. The procedure for the selection of volume ratios of the aqueous to organic phase and concentration ratio of the carrier (Amberlite LA-2) to penicillin-G at a desired degree of extraction and enrichment has recently been described (Lee and Lee, 1994). Ion-pair extraction of penicillin-V and phenoxy acetic acid with Amberlite LA-2 hydrochloride and tetrabutylammonium hydrogen sulphate can be effective in various solvents such as hexane, amyl

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QC\textsubscript{Cl}\textsubscript{(org)} + P\textsubscript{aq} \rightleftharpoons QP\textsubscript{org} + Cl\textsubscript{aq}
\]

(1.4)
acetate, octanol and chloromethane, the latter being a preferred choice of solvent (Harris et al., 1990). As reported by the above authors, cdiivanic and clavulanic acids are more effectively extracted by anion exchange with Aliquat-336 in butyl acetate and dichloromethane as the solvents. It may be noted that ion-pair extraction of zwitterionic molecules with secondary and tertiary amine is difficult. Anion exchange extraction is equally effective for CPC which could be extracted from a carbonate buffer solution using Aliquat-336 in butyl acetate and re-extracted into an acetate buffer solution without any decomposition of the beta-lactam (Hano et al., 1992). Investigations recently carried out reveal that the same carrier-solvent system is effective for reactive extraction of 6-aminopenicillanic acid (6-APA) and 7-ACA under suitable conditions (Bora et al., 1997 and 1998).

1.4 Aim of the present study

Equilibrium and kinetics of reactive extraction are important from process application point of view. These data are useful also for developing a facilitated transport liquid membrane system for separation and purification with high selectivity and rate. However, it appears that data on such important aspects are very scanty particularly for cephalosporin antibiotics. In view of this, the present thesis will cover a comprehensive study on the equilibrium and kinetics of reactive extraction of certain cephalosporin antibiotics with extractants such as secondary and primary amines and a quaternary ammonium salt.
1.4 References


