Supported Liquid Membranes with Strip Dispersion for Recovery of Cephalixin

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy
in the Graduate School of The Ohio State University

By

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2010

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Abstract

Cephalexin is an important and widely used semi-synthetic cephalosporin. Cephalosporins along with penicillins are β -lactam antibiotics and they account for the majority of the antibiotic world market. Cephalexin is traditionally produced by a 10-step chemical synthesis. An enzymatic synthesis for Cephalexin has been developed, and offers several advantages over the classical route. The enzymatic synthesis reduces energy and solvent waste, but its use for industrial production has been limited due to the difficult separation. Liquid membranes, in particular supported liquid membranes (SLMs), are a promising solution to the separation. SLMs are not used commercially, as they are still plagued with instability. SLM with the additional modification of strip dispersion has been a recent development to solve this issue. This study describes work in which Cephalexin is separated from complex reaction mixtures encountered in the enzymatic synthesis using SLMs with strip dispersion.

SLMs with strip dispersion and carrier, Aliquat 336, were used for the recovery of Cephalexin from aqueous solutions. The separation of Cephalexin from single component solutions was first investigated. Key experimental parameters were identified and Cephalexin recovery of over 99% has been achieved for most cases. In addition, the mass transfer resistances from the process were analyzed and the resistance from the extraction reaction between Cephalexin and Aliquat 336 was found to be dominant. Recovery of

Cephalexin from non-ideal solutions was also investigated, which involved co-extraction from buffer anions and other components present in the enzymatic reaction mixture during Cephalexin synthesis. *In situ* extraction was also demonstrated in which Cephalexin was removed from enzymatic reaction mixtures as it was synthesized with the enzyme penicillin acylase, and resulted with an increase in Cephalexin yield compared to synthesis without *in situ* removal. Along with these promising results, the long-term stability, the availability of large scale hollow fiber modules, and the use of inexpensive and low vapor pressure solvents make the SLM with strip dispersion process suitable for commercial applications for recovery of antibiotics and other biochemicals.

*Dedicated to my wife and my parents for the many years of love, support, and
encouragement.*

Acknowledgments

I acknowledge the faculty, staff, and my classmates at The Ohio State University's William G. Lowrie Department of Chemical and Biomolecular Engineering for their assistance during my graduate studies. I would especially like to thank my advisor Dr. W. S. Winston Ho for his guidance and support during my time at Ohio State. I would also like to thank by co-advisor, Dr. L. James Lee and my industrial advisor, Dr. Norman Li for their support.

I am grateful to all my fellow colleagues: Dr. Jin Huang, Dr. Jian Zhou, Dr. He Bai, Dr. Chi Yen, Phillip Chang, Yanan Zhao, Kartik Ramasubramanian, Lin Zhao and Prateik Singh. I would also like to acknowledge the visiting scholars and professors that I have had the pleasure to work with: Dr. Da-Ming Wang, Dr. Wei Liu, Dr. Bishnu Mandal, Zisu Hao, and Jung-Tsan Tsai. I would like to thank Kristen Weisser for her hard work and dedication in conducting experiments. I would like to give special thanks to the Turro group in the Department of Chemistry for helping with HPLC analysis. I would like to thank my wife, Lauren, for all her help and suggestions.

I am also grateful to NSF's IGERT program at Ohio State and NSF for funding my work.

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M. E. Vilt and W. S. W. Ho, Selective Separation of Cephalexin from Multiple Component Mixtures, *Ind. Eng. Chem. Res.* **2010**, doi: 10.1021/ie100073w.

M. E. Vilt and W. S. W. Ho, Supported Liquid Membranes with Strip Dispersion for the Recovery of Cephalexin, *J. Membr. Sci.* **2009**, 342, 80–87

Fields of Study

Major Field: Chemical Engineering

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List of Notations

7-ADCA = 7-amino-3-desacetoxicephalosporanic acid

A = membrane area, m^2

$Ceph$ = Cephalexin

c = concentration, mmol/L

c_f = Cephalexin concentration in the aqueous feed solution, mmol/l

c_{fo} = initial Cephalexin concentration in the aqueous feed solution, mmol/l

d_h = hydraulic diameter of the shell-side, m

d_i = inner diameter of the hollow-fiber, m

d_{lm} = log-mean diameter of the hollow-fiber, m

d_o = outer diameter of the hollow-fiber, m

D_a = diffusivity of Cephalexin monohydrate in the aqueous feed solution, m^2/s

D_m = diffusivity of Cephalexin-Aliquat 336 complex in the organic membrane solution, m^2/s

f = fraction of ionization

H_2A^+ = diprotic acid

j = flux of Cephalexin through the supported liquid membrane, m^2/s

K = overall mass transfer coefficient, m/s

K_1 = first dissociation constant, mol/L

K_2 = second dissociation constant, mol/L

k_a = mass transfer coefficient in the aqueous feed solution, m/s

k_{as} = mass transfer coefficient for the aqueous strip solution, m/s

k_e = mass transfer coefficient due to the complexation/extraction reaction, m/s

k_m = mass transfer coefficient for the membrane phase, m/s

k_o = shell-side mass transfer coefficient, m/s

k_s = mass transfer coefficient due to the decomplexation/stripping reaction, m/s

L = hollow-fiber module length, m

m_f = distribution coefficient between the organic membrane phase and the feed solution

m_s = distribution coefficient between the organic membrane phase and the strip solution

P = volumetric productivity, mM/hr

P_a = pressure on the aqueous feed solution side, Pa

P_o = pressure on the strip dispersion side, Pa

PG = phenylglycine

PGA = D-phenylglycine amide

$PGME$ = phenylglycine methyl ester

$QCeph$ = quaternary ammonium ion and Cephalexin anion complex

QCl = quaternary ammonium chloride, i.e., Aliquat 336

QS = quaternary ammonium ion and solute anion complex

S = solute

t = time, s

u_b = mean velocity of the aqueous feed solution in each hollow-fiber, m/s

V_a = volume of the aqueous feed solution, m³

V_o = volume of the organic membrane solution, m³

Y = maximum yield

Greek letters

δ = membrane thickness, m

$\Delta_a, \Delta_e, \Delta_m, \Delta_o$ = fractional resistances of the aqueous feed solution, extraction reaction, membrane, and shell-side to the overall process, respectively

ε = porosity

τ = tortuosity

α = separation factor

Chapter 1: Introduction

1.1 Antibiotics

Alexander Fleming made one of modern day medicine's most important discoveries in 1928, when he noticed a strange mold present in some old bacteria cultures that had an area surrounding the mold that was bacteria free. Fleming continued to study his discovery and was able to culture the mold and to extract a liquid that was effective at killing a variety of bacteria (Mann, 1999). He would go on to call the compound penicillin since the mold was of the genus *Penicillium*. It would take another decade of research before a team led by Howard Walter Florey would be able to show promising clinical results for using penicillin in treating infections. Finally, in 1942 the new medicine was available for clinical use, with most of the penicillin production occurring in the United States. There are now over 12,000 known antibiotics, 160 of which have been used for human clinical use (Walsh, 2003). Major classes of antibiotics, their mechanisms against bacteria, and their synthetic methods will be briefly reviewed.

Antibiotics can be categorized based on their mechanisms for killing or slowing down the growth of bacteria. Six major classes have been identified and include: β -lactam, macrolide, quinolones, tetracyclines, aminoglycosides, and glycopeptides. Common uses of antibiotics include the treatment of pneumonia, meningitis, urinary tract

infections, bronchitis, intestinal infections, skin infections, and tuberculosis (Walsh, 2003).

- *β -Lactam*: β -Lactams kill bacteria by blocking transpeptidase and transglycosylase enzymes. These enzymes play a role in providing a strong peptidoglycan layer in the cell wall of bacteria. By blocking these enzymes a build-up of peptidoglycan precursors occurs, which triggers the digestion of existing peptidoglycan by autolytic hydrolases. Without the production of new peptidoglycan cell wall destruction occurs. β -Lactam antibiotics all contain a four member β -lactam ring and include penicillins and cephalosporins, which together account for the majority of antibiotic use.
- *Macrolide*: Macrolides kill bacteria by blocking one or more of the protein biosynthetic steps on the 50S subunit of the bacterial ribosome.
- *Quinolone*: Quinolones kill bacteria by DNA replication and repair inhibition.
- *Tetracyclines*: Tetracyclines kill bacteria by blocking one or more of the protein biosynthetic steps on the 30S subunit of the bacterial ribosome.
- *Aminoglycoside*: Aminoglycosides kill bacteria by targeting regions of polyanionic 16S rRNA on the 30S ribosome, which inhibit protein synthesis.
- *Glycopeptide*: Glycopeptides kill bacteria by complexing with uncrosslinked peptides, such as acyl-D-alanyl-D-alanine, in the peptidoglycan layer in the cell wall. Production of new peptidoglycan is stopped, leading to a breakdown of the cell wall.

Antibiotics can be produced by a variety of techniques. The major methods for antibiotic synthesis include: fermentation, chemical synthesis, and enzymatic synthesis.

The bacteria, streptomycetes, are used to produce 55% of the commercially available antibiotics in fermentation processes (Walsh, 2003). A large number of antibiotic-producing strains of streptomycetes have been developed. Other key microorganisms for antibiotic fermentation include: the bacteria, *Saccharopolyspora erythraea* (Hamedi et al., 2004), the mold, *Penicillium chrysogenum* (Hossain and Dean, 2008), and the fungus, *Cephalosporium acremonium* (Sahoo et al., 1999).

The manufacture of some antibiotics, such as cephalosporins, can be accomplished by chemical synthesis. Some of these processes start with an initial reagent from a fermentation broth and the resulting products are called semi-synthetic antibiotics. Typically, the synthesis consists of several sequential steps, which take place in organic solvents and at low temperature to prevent decomposition. Two examples of synthetic processes are for the manufacture of Cephalexin and thienamycin (Elander, 2003).

Unlike fermentation, enzymatic synthesis does not use living cells. Instead, isolated enzymes are used to transform starting reagents into the desired antibiotic. The enzymatic synthetic routes are considered environmentally friendly alternatives to traditional synthesis, because the use of toxic solvents is eliminated. The enzyme penicillin G acylase is used often for the synthesis of β -lactam antibiotics (Bruggink et al., 1998). The major pharmaceutical company, DSM, currently uses enzymatic processes for the manufacture of amoxicillin, Cephalexin, and cefadroxil (DSM, 2004).

1.2 Cephalexin

The benefits of employing an enzymatic synthetic process can be seen for the manufacture of the cephalosporin, Cephalexin. Cephalexin is the most widely used

cephalosporin antibiotic with an annual use of 3,000 tons and annual sales revenue of \$850,000,000 (Barber et al., 2004). Respiratory tract infections, skin infections, bone infections, urinary tract infections, and otitis media are ailments that are commonly treated with Cephalexin. Industrial production of Cephalexin can be completed by either chemical or enzymatic synthesis. The traditional synthesis or chemical synthesis of Cephalexin involves 10 conventional chemical process steps as shown in Figure 1.1 The process starts with the synthesis of the cephalosporin precursor, 7-amino-3-desacetoxycephalosporanic acid (7ADCA), which is then coupled with an activated Dane salt. This process has many environmental issues including extensive energy use to provide cooling to low temperatures (-70°C to 10°C) at which many of the reactions take place (Bruggink and Roy, 2001). Additionally, a surplus of toxic solvents is used in the process creating 40 kg of non-recyclable waste per kg of Cephalexin (Sheldon and van Rantwijk, 2004). These disadvantages have led to development of an enzymatic synthesis of Cephalexin.

The production of substrates and their combination for the enzymatic synthesis of Cephalexin is shown in Figure 1.2. The process first involves the organic synthesis of D-phenylglycine amide (PGA) and the synthesis of 7-ADCA, which is achieved by fermentation followed by an enzymatic reaction. 7-ADCA and PGA are then combined in aqueous solution with the enzyme penicillin G acylase to produce Cephalexin. When compared to the traditional synthesis, the enzymatic method reduces solvent use by 90%, other reagents by 100%, energy consumption by 40%, and waste by 15% as shown in Figure 1.3 (Schoevaart and Kieboom, 2001). Despite these advantages, commercial use of the enzymatic process has been hindered due to a low Cephalexin yield and the

difficult separation of the reaction mixture. Most manufactures of antibiotics, particularly in China and India, still use traditional routes for antibiotic production, primarily due to the separation issue (Barber et al., 2004).

1.3 Separation of Cephalexin from Aqueous Solutions

Advances in the separation of Cephalexin from aqueous solutions would help promote the use of the enzymatic process. Purification of Cephalexin from this dilute reaction mixture is challenging due to the similar structure, acid/base properties, and solubilities of Cephalexin and the starting material 7-ADCA (Wegman et al., 2001). Major methods used in the final recovery of Cephalexin from aqueous solutions include column chromatography and crystallization/precipitation. Other methods such as nanofiltration, aqueous two-phase partitioning, and liquid membranes have also been proposed for Cephalexin recovery.

Multiple steps involving column chromatography could be used to recover Cephalexin from aqueous solutions. The following is a proposed process based on the recovery of cephalosporin C, which is similar to Cephalexin in structure and properties (Barber et al., 2004). In this process, a filtered enzyme free solution is first passed through large scale hydrophobic interaction chromatography columns to remove impurities and side products. The solution is then passed through a “scavenger” column, which removes hydrophobic colored compounds. After a pH adjustment, the solution is passed to the next “adsorber” column. In this step Cephalexin is adsorbed to the hydrophobic resin in the column. Selective elution of Cephalexin is achieved by a second pH adjustment. These steps accomplish the main separation of Cephalexin from the

solution. However, further purification is typically needed. An anion exchange chromatography column is used to remove any remaining color. A final chromatography column containing a weak cation exchanger is used to remove any possible metal contamination. A major disadvantage of chromatography methods for Cephalexin recovery is the large cost (Ghosh et al., 1997).

Selective crystallization has been successfully applied for the recovery of Cephalexin. Crystallization can be achieved by careful pH adjustment or by the addition of a complexing agent. Pure Cephalexin can be recovered from enzymatic reaction mixtures by lowering the pH of the mixture between values of 5.0 – 7.8 (Boesten et al., 1999). Crystallization from reaction mixtures must be done at an optimal pH, as there is compromise between the purity and the Cephalexin yield. This method has a low yield of pure Cephalexin that is recovered, and a second crystallization is required to further recover the remaining product. The resulting product of the second step is a mixture of Cephalexin and 7-ADCA, which may be re-used in the synthesis. The selective complexation of Cephalexin with complexing agents from an enzymatic reaction mixture has shown to recover Cephalexin in high yield and purity (Faarup, 1977). When added to a multiple component solution, complexing agents are able to specifically bind to Cephalexin forming a preprecipitated complex, which can be isolated by filtration. Cephalexin can then be released from the complex in series of acidification and extraction steps, resulting in successful recovery of Cephalexin from the original solution.

Nanofiltration uses membranes with small pore sizes around 0.5 – 2 nm in diameter and can be used to separate small organic molecules from aqueous solutions. Modified polybenzimidazole nanofiltration membranes were used to separate dilute

Cephalexin solutions. Flat sheet and hollow-fiber membranes were tested and the Cephalexin rejection was found to be dependent on the pore size and electrostatic interaction between solutes and membrane (Wang and Chung, 2006). Rejection increased with increasing pH, due to the change of ionization. Separation of more concentrated and more complex solutions needs to be investigated to determine if nanofiltration can successfully be used for the recovery of Cephalexin from enzymatic reaction mixtures.

Aqueous two-phase systems have been widely used for biochemical recovery, including Cephalexin recovery, and are formed by mixing aqueous solutions of two different polymers or by mixing aqueous polymer and concentrated salt solutions. During mixing, selective partitioning can occur and the enriched phase is recovered after phase separation. Selective partitioning of Cephalexin has been reported from solutions containing 7-ADCA and from enzymatic reaction mixtures (Hernandez-Justiz et al., 1998; Zhu et al., 2001). However, industrial scale-up of the systems is limited due to the difficult recovery of the two-phase forming components, which ideally would be recovered and reused.

Liquid membranes processes have shown potential in Cephalexin separation from aqueous solutions, but suffer from either complicated operation or long-term stability, which prohibits their use in real industrial applications. The current work uses an easy to operate, efficient, and stable liquid membrane process for the recovery of Cephalexin from aqueous solutions. For the first time, supported liquid membranes (SLMs) with strip dispersion have been used to comprehensively study the feasibility of antibiotic recovery from aqueous solutions.

1.4 Scope of Current Work

The research reported in this work is focused on demonstrating that SLM with strip dispersion can be an effective technique to recover antibiotics from aqueous solutions. The focus of this work is on the recovery of Cephalexin, which is an important antibiotic that can be synthesized in an environmentally friendly process, but is not widely used due to the difficult separation of Cephalexin from the reaction mixture.

This dissertation is divided into six chapters, in which separation of Cephalexin using SLMs with strip dispersion is primarily discussed. The topic of liquid membranes and the mass transfer mechanisms for solute transport are introduced in Chapter 2. The main configurations and applications of liquid membranes: emulsion liquid membranes (ELMs), SLMs, and SLMs with strip dispersion, are also described.

In Chapter 3, the removal and recovery of Cephalexin from aqueous solutions by SLM with strip dispersion is reported. Distribution coefficients of Cephalexin and 7-ADCA over a range of pH values are presented before results with SLMs are discussed. Several experimental parameters in the SLM system are investigated and the key parameters for efficient Cephalexin recovery are discussed. Finally, analysis of the mass transfer resistances was conducted and the major mass transfer limiting steps are identified.

The focus of Chapter 4 is on recovering Cephalexin from complex aqueous solutions. As control of the feed solution pH is important to achieve efficient Cephalexin separation, a variety of methods to maintain the pH were tested and the co-extraction of competing anions is discussed. The separation of Cephalexin from simulated enzymatic solutions with two- and four-component mixtures is also reported.

The *in situ* removal of Cephalexin from enzymatic reaction mixtures is demonstrated in Chapter 5. The effect of organic membrane solution on stability of the enzyme, penicillin G acylase, is also reported. Finally, the use of complexing agents in combination with SLM with strip dispersion is proposed for the recovery of Cephalexin, which has several advantages if implemented on an industrial scale. In Chapter 6, conclusions are presented and areas for future work are suggested.

Figure 1.1 Chemical synthesis of Cephalexin. (Sheldon and van Rantwijk, 2004)

Figure 1.2 Enzymatic process for Cephalexin synthesis. (Wegman et al. 2001)

Figure 1.3 Comparison of chemical (blue) and enzymatic (red) processes for Cephalexin synthesis. (Smolders, 2002)

Chapter 2: Liquid Membranes

2.1 Introduction

In this chapter, the following liquid membrane separation processes: ELMs, SLMs, and SLMs with strip dispersion are introduced. The transport mechanisms and examples of applications are presented. Stability has long been an issue for liquid membrane processes and will be discussed.

2.2 Facilitated Transport

Transport with liquid membranes consists of three distinct phases: feed, membrane and strip. For liquid based applications, which are the focus of this chapter, these phases can be further described as: the aqueous feed solution, organic membrane solution, and aqueous stripping solution. Facilitated transport can be classified into two types of mechanisms for solute transport, Type 1 and Type 2 facilitation. Type 1 facilitation is based on the solubility of the solute into the organic phase, while Type 2 facilitation is controlled by a chemical reaction. Further details on these facilitated transport mechanisms will be presented.

2.2.1 Type 1 Facilitation

Type 1 facilitation involves diffusion of a species from the feed phase, through the membrane phase, to the aqueous stripping solution. Once in the stripping solution, a chemical reaction occurs, prohibiting the reaction product's diffusion back through the membrane. The reaction maintains a low solute concentration in the strip solution, providing a high driving force. For example, phenol removal from waste water can be achieved with Type 1 facilitation. Phenol in the aqueous feed phase diffuses through the oil membrane phase and reacts with NaOH in the stripping phase. This results in the formation of sodium phenolate, which is not soluble in the membrane oil phase and is retained in the aqueous stripping solution (Ho and Li, 1992).

2.2.2 Type 2 Facilitation

Carrier facilitated transport is another name for Type 2 facilitation (Cahn et al., 1981; Frankenfeld et al., 1981; Fuller and Li, 1984). A mobile “carrier” compound in the liquid membrane phase transfers the target species from the feed solution to the strip solution. The transport of the target species can be broken down to the following steps:

1. Target species diffusion from the bulk phase across the aqueous boundary layer to the membrane interface.
2. Formation of carrier-target species complex.
3. Diffusion of the complexed carrier across the membrane.
4. The release of the target species at the strip interface and regeneration of the carrier.
5. The diffusion of the released target species from the membrane-strip interface to the bulk strip phase.
6. The return of the carrier across the membrane.

Commonly, carriers are referred to as extractants and solvents as dilutents. The extractant and organic solvent must be carefully selected for use in a liquid membrane process, the following general guidelines should be considered. An ideal carrier will possess the following characteristics (Klassen et al., 2005; Kislik, 2010):

- high affinity for the component(s) to be removed from the feed solution
- formation of a component-carrier complex that is soluble in the organic solvent
- low solubility in water
- low toxicity
- low viscosity
- ability to be reused or regenerated
- no side reactions
- no complexation with water
- good chemical stability.

When selecting a solvent, the following characteristics should be considered (Gu et al., 1992):

- low solubility in water
- compatibly with the extractant
- inexpensive and readily available from multiple suppliers
- low toxicity
- have a high flash point for safety reasons
- have a sufficient density difference from the aqueous phase for fast settling when used in ELM and SLM with strip dispersion processes.

Carriers can be classified into three general classes: acidic extractants, basic extractants, and neutral extractants. Table 2.1 provides some commonly used carriers for a variety of applications.

The most classic example of carrier facilitated transport is the transport of copper by an acidic extractant as shown in Figure 2.1 (Ho, 2003). The acidic extractant, HA (shown in the dimer form, $(HA)_2$, e.g., hydroxyl oxime), reacts with Cu^{2+} at the interface of the feed solution with the liquid membrane to form a copper-extractant complex, $CuA_2(HA)_2$ (Ho, 2003). The complex diffuses through the liquid membrane to the interface of the liquid membrane and the strip solution. At this interface, a decomplexation/stripping reaction occurs with a strong acid present in the strip solution. The Cu^{2+} is stripped into the strip solution, and the extractant is regenerated. The extraction and stripping reactions for the copper example are given in Equations 2.1 and 2.2, respectively.

Basic extractants such primary amines, secondary amines, tertiary amines, and quaternary ammonium salts can be used for the extraction of anionic or neutral species. Two possible mechanisms, co-transport and counter-transport, are possible, and depend on the type of amine and stripping solution that are used. Hexavalent chromium, Cr (VI), is an example of a species that can be recovered by both mechanisms. Co-transport, also referred to as ion-pair extraction, commonly occurs with secondary and tertiary amines when a basic solution is used as the stripping solution. The transport of the target species

is coupled with the transport of hydrogen ions in the same direction. Stripping with a sodium hydroxide solution leads to the regeneration of the amine and the recovery of Cr (VI). The extraction and stripping of Cr (VI) by co-transport with a tertiary amine is shown in equations 2.3 and 2.4, respectively.

Counter-transport, sometimes referred to ion-exchange, occurs when the transport of the target species is in the opposite direction as that of the counterion. The stripping solution when using tertiary amines is typically an acidic solution. The stripping solutions, when quaternary ammonium salts are used as the carrier, contain a high concentration of a counterion. The counterions are typically a strong base, strong acid, or solutions with high salt or buffer concentration. The extraction and stripping of Cr (VI) by counter-transport with a quaternary ammonium salt is shown in equations 2.5 and 2.6, respectively.

Organo-phosphoryl compounds have been applied as neutral extractants for the recovery of actinides and lanthanides (Gu et al., 1992). The neutral extractant transfers the target species to the stripping solution, where stripping regenerates the carrier and transports the target species to the aqueous strip solution. The extraction of uranium from nitric acid feed solutions with tri-*n*-butyl phosphate (TBP) has been widely studied and is an example of separation using this type of carrier. Stripping is commonly achieved with

a dilute nitric acid solution. The extraction and stripping of uranium are shown in equations 2.7 and 2.8, respectively. The driving force for this separation is the concentration gradient of the nitrate ions between the feed and stripping solutions (Cristau et al., 1999).

2.3 Emulsion Liquid Membranes

Li invented a liquid membrane process for the separation of hydrocarbons in 1968 (Li, 1968). The process used liquid surfactant membranes operating in a drop column. Droplets of a feed solution containing hydrocarbons are injected into an aqueous solution containing surfactants. A coating around the feed droplet results and a liquid membrane is formed. The coated droplet then rises through a solvent phase where selective mass transport occurs through the liquid membrane. The oil drops coalesce at the top of the column, allowing the water and surfactant to return to the aqueous phase. Li's initial process led to the development of the double emulsion method or ELM.

An ELM is formed when a stable emulsion containing two immiscible phases is dispersed in a continuous external phase. Transport of a target species in the external phase occurs through the liquid membrane phase of the emulsion into the internal phase by one of two facilitated transport mechanisms. An ELM is a three-phase system and can consist of water/oil/water (W/O/W) or oil/water/oil (O/W/O) phases. In either system, the liquid membrane is the phase separating the like phases. For a W/O/W system, the oil

phase separating the two aqueous phases is the liquid membrane phase (Ho and Li, 1992). A diagram of the W/O/W system is shown in Figure 2.2. The ELM process can be described in four steps which are shown in Figure 2.3.

The first step in forming an ELM is the formation of an emulsion between two immiscible phases. This is typically done with the use of surfactants and high-speed agitation. Emulsion droplets range from 1 – 3 μm in diameter, thus providing good stability (Ho and Li, 1992). Second, the emulsion is dispersed in a third continuous phase with constant agitation. Once dispersed, globules of the emulsion with diameters 100 – 2000 μm , form (Ho and Li, 1992). The mixing rate in the dispersion step is an important operating parameter. If the dispersing speed is too fast, emulsion breakage can occur, and if too slow, large globules can form, reducing the mass transfer area. During this second step of the ELM process, mass transfer from the external phase to the internal phase occurs by facilitated transport. After the desired separation, the emulsion and the continuous phase are separated in a settling step. The final step in the ELM process involves breaking the emulsion, whereby the internal phase is then recovered and the membrane phase can be reused. The emulsion is usually broken by the use of electric field (Hsu and Li, 1985).

2.3.1 ELM Biochemical Applications

Removal of zinc from wastewater in the viscose fiber industry, phenol removal, cyanide removal, the recovery of nickel from electroplating solutions, extraction of copper from leach solution, treatment of wastewater, biochemical processing, biomedical encapsulation and release, and use as a well control fluid are the main applications for

ELMs (Ho and Li, 1992; Vilt et al., 2010). As these applications are well established, have been extensively reviewed and are outside the focus of this work, only the applications regarding biochemical processing will be briefly reviewed.

Biochemical processing applications with ELMs include the separation of aminoacids (L-phenylalanine), biochemicals (lactic acid), and antibiotics (penicillin G) from fermentation broths. The separation of L-phenylalanine using the quaternary ammonium salt, Aliquat 336 (trioctylmethylammonium chloride), as a carrier was studied, and the solute hydrophobicity of amino acids was found to have a strong correlation with the initial flux (Thien et al., 1988). This study also addressed the issue of system optimization to provide sufficient driving force (concentration of the counterion, Cl^-) while minimizing emulsion swelling. The use of the anion extractant, D2EHPA (bis(2-ethylhexyl) phosphoric acid), for recovery of L-phenylalanine was also reported (Itoh et al., 1990). An optimized ELM system for the recovery of L-phenylalanine using D2EHPA was presented (Hong et al., 1990) and later developed into a continuous ELM process (Hong and Yang, 1994). With a fermentation solution at $\text{pH} = 2.5$ and an initial L-phenylalanine concentration of 36 g/L, a concentrated internal solution of 170 g/L can be obtained. The separation of L-phenylalanine from aspartic acid with the carrier D2EHPA (Juang and Wang, 2002) and the selective recovery of D-phenylalanine from racemic D,L-phenylalanine solutions (Pickering and Chaudhuri, 1997) was also reported.

An ELM process for the selective recovery of acrylic acid from propionic acid and acetic acid containing fermentation broths has been developed (O'Brien and Senske, 1989). The carrier Alamine 336 (tri-octyl/decyl amine) was reported for lactic acid

extraction (Chaudhuri and Pyle, 1992), and the work was later extended when the extraction of lactic acid from an actual fermentation broth was demonstrated (Scholler et al., 1993). Extraction efficiencies of lactic acid from simulated fermentation broth were almost double those of actual broth. TOPO (tri-*n*-octylphosphine oxide), TBP, TOA (tri-*n*-octylamine), and dioctylamine were compared as carriers for lactic acid recovery from fermentation broths (Hano et al., 1992). A model was developed to describe the swelling of ELMs during lactic acid extraction with the carriers TBP and TOA, and agreed well with experimental data (Yuanli et al., 2001). Extraction of lactic acid with the carrier, Amberlite LA-2 (*N*-lauryl-*N*-trialkylmethylamine) has also been investigated (Reisinger and Marr, 1992; 1993).

Citric acid extraction from fermentation broths with the carrier Alamine 336 was first studied by Boey et al. (Boey et al., 1987). A tertiary amine carrier was used in modeling and experimental studies for citric acid extraction and removal efficiency of 95 – 97% was achieved (Stoica-Guzun et al., 1999). Recent studies on citric acid extraction with the carriers Alamine 336 (Yordanov and Boyadzhiev, 2004) and Aliquat 336 (Manzak and Tutkun, 2004) have been reported.

The recovery and subsequent bioconversion of penicillin G with an ELM system was demonstrated (Scheper et al., 1987). Penicillin G was extracted through a membrane phase of Span 80 and Amberlite LA-2 in kerosene to an internal phase containing the enzyme penicillin G acylase, which converted penicillin G into the products phenylacetic acid and 6-aminopenicillanic acid. The recovery of penicillin G, with di-*n*-octylamine as a carrier and ECA 4360J (Exxon) as a surfactant with a mixed solvent of kerosene and *n*-butyl acetate and an internal phase of Na₂CO₃, was also demonstrated (Hano et al., 1990).

Penicillin G decomposition due to the high pH of the internal phase containing Na_2CO_3 was later addressed (Lee and Lee, 1992; Lee et al., 1992a; 1992b). An extraction efficiency of 95% was reported with a concentration of the internal phase nine times the original penicillin G concentration in the external phase, with the extractant Amberlite-LA 2 (Lee and Lee, 1992). Use of membrane phases containing a dilute polymer solution to improve emulsion stability resulted in highly efficient extraction of penicillin G (Lee, 2004). ELMs with the carrier Aliquat 336 for the recovery of the antibiotics, Cephalexin (Sahoo and Dutta, 1998) and Cephalosporin C (CPC) (Sahoo et al., 1999), were also reported.

2.3.2 *ELM Stability*

Due to the high rate of mechanical stirring that is required in ELMs, membrane rupture is common. As a result, the aqueous feed and strip solutions are mixed and lead to a reduction in separation efficiency (Gu et al., 1992). Stability in ELMs can be controlled by many experimental parameters such as operating time, mixing speed, and surfactant concentration. Most efforts to improve stability such as lower mixing speed or increased surfactant concentration result in reduced performance. The balance of performance and stability must be determined for each ELM system, requiring tedious experimental optimization. There have been efforts to improve ELM stability such as addition of stabilization surfactants or viscous Newtonian fluids to the membrane phase (Lee, 2008). However, the increase in stability was offset by the reduction in solute transport. It was reported that the addition of viscoelastic polymers to the membrane phase can change the membrane phase into a non-Newtonian fluid, which increased stability without a

reduction in transport efficiency (Skelland and Meng, 1999). Another technique that has been shown to increase ELM stability is to use a hollow-fiber contactor to provide the interfacial surface area between the feed solution and the organic membrane solution (Raghuraman and Wiecek; 1993; Hu and Wiecek, 1998; Fouad and Bart, 2008). Increased stability is the result of lower shear rates, compared to the typical ELM configuration that uses agitators to disperse the emulsion in the feed solution (Ho, 2003).

2.4 Supported Liquid Membranes

SLM, also referred to as immobilized liquid membrane (ILM), is a term generally used to describe a liquid membrane separation process in which a liquid membrane phase is embedded in a solid porous support, and placed between a feed and receiving phase. Traditionally, the liquid membrane phase is an organic solution and is embedded in the pores of a hydrophobic microporous polymer support. The liquid membrane solution typically contains a carrier dissolved in an organic solvent, and is immiscible with the aqueous feed and receiving solutions. When the loaded support is placed between the feed and receiving solutions, as shown in Figure 2.4, a SLM is formed. Transport of a target species in the feed solution can then occur by carrier facilitated transport. It should be noted that variations of the traditional SLM have been developed, such as: contained SLM (Sirkar, 1996), stagnant sandwich liquid membrane (SSwLM) (Molinari et al., 2005), and ionic liquid SLM (Scovazzo, 2002).

The configuration of the SLM is described by the nature of the polymeric support being used. The two main configurations are the flat sheet SLM (FSSLM) and the hollow-fiber SLM (HFSLM). Experimental studies usually start with a system that

consists of flat sheet membrane in a two-compartment cell, as shown in Figure 2.5 (Hassoune et al., 2008). FSSLM systems may also include pump induced flow of the feed and receiving solutions. HFSLMs are normally operated with thousands of fibers packed in a module under flow conditions and are used to demonstrate potential industrial applications, as this configuration provides high surface area per module volume. Some applications employ a single hollow-fiber as a support. Figure 2.6 shows a typical configuration for a single fiber SLM, used for sample enrichment in analytical analysis applications. Industrial use of SLMs has been hampered by instability.

2.4.1 SLM Biochemical Applications

Major applications of SLMs include the removal and recovery of metals, the management of nuclear waste, and the recovery of biological and biochemical species. The applications of metal recovery and nuclear waste management are well established and have several reviews available (de Gyves and Rodriguez de San Miguel, 1999; Mohapatra and Manchanda, 2003). The recovery of many biological and biochemical species have been accomplished with the use of SLMs. These include antibiotics (penicillin G), organic acids (citric and lactic acid), amino acids (L-phenylalanine), and sugars. The recovery of biological and biochemical species will be reviewed in detail.

The majority of SLM systems for antibiotic recovery have focused on the recovery of penicillin G. Marchese et al. first transported penicillin G (benzylpenicillin) through a SLM using a flat sheet polytetrafluoroethylene (PTFE) support (Marchese et al., 1989). The extraction of penicillin G and Penicillin V with subsequent enzymatic hydrolysis to 6-aminopenicillanic acid was studied subsequently (Tsikas et al., 1992).

When polypropylene (PP) hollow-fibers were used as a support, Amberlite LA-2 demonstrated superior stability in long-term studies compared to Adogen 464 (1-methyltrioctylammonium chloride) (Tsikas et al., 1992). Amberlite LA-2, a secondary amine, has been the most common carrier used in penicillin recovery with SLMs. Investigation of an SLM with Amberlite LA-2 in 1-decanol as the membrane phase with a PP flat sheet support determined that the mass transfer of penicillin G was controlled by both the aqueous film layer and the liquid membrane phase (Lee et al., 1993). This work was extended to show the strong transport competition between penicillin G and phenylacetic acid when both are in the feed solution (Lee et al., 1994c). Amberlite LA-2 was also used as a carrier for determination of the transport mechanism and rates of transport of penicillin G through a SLM (Juang et al., 1998). Another study utilizing SLMs included the use of hexafluorophosphates of 1-*n*-butyl-, 1-*n*-hexyl- and 1-*n*-octyl-3-methyl imidazolium, and trioctylmethylammonium chloride (TOMAC) as ionic liquids in a polyvinylidene fluoride (PVDF) SLM (Matsumoto et al., 2007). The ionic liquids in this study; however, did not improve extraction or stripping compared to conventional solvent extraction with butyl acetate. CPC extraction through a PP SLM containing the carrier Aliquat 336 was reported (Ghosh et al., 1995). When increasing the carrier concentration, the transport of CPC increased until a maximum was reached. After this point, increasing carrier concentration decreased transport due to a large decrease in diffusion coefficients in the membrane phase, resulting from an increase in viscosity.

The removal of other drugs not classified as antibiotics has also been investigated. For example, the removal of diclofenac, a nonsteroidal anti-inflammatory drug, from wastewaters is of interest since it is not removed during standard water treatment

procedures (Molinari et al., 2006). A SLM system composed of TBP dissolved in *n*-decane yielded a higher diclofenac flux value compared to an SSwLM, 2.4 vs. 1.7 mmol/hm². However, the long-term stability of the SSwLM was ~2.5 times higher.

The recovery of citric acid from a fermentation broth with a PP support was demonstrated by Friesen et al. with the carrier trilaurylamine (Friesen et al., 1991). Citric acid extraction, with the carrier Alamine 336, was studied with the solvents heptane and xylene, in which heptane led to instability (Sirman et al., 1990). Systems composed of xylene gave acceptable performance and the limiting transport step was determined to be diffusion across the membrane. In another study, TOA and TOA salts were compared for citric acid extraction, and it was found that all the TOA salts studied increased citric acid transport, with the citrate salt having the highest enhancement (Juang and Chen, 1997). The extraction reaction of citric acid with TOA can be classified into two reaction regions, fast and slow. It was reported that the extraction reaction is part of the slow reaction region in a SLM with a PTFE support (Park et al., 2006). SLM systems for citric acid recovery that were subjected to a thermal gradient were proposed by Rockman et al. (Rockman et al., 1997). In a SLM system with the carrier Alamine 336, increasing the temperature of the receiving solution over the range of 30 – 80°C resulted in increased citric acid flux. The extraction of citric and maleic acids from kiwi fruit juice was carried out by Schaefer and Hossain (Schaefer and Hossain, 1996). Alamine 336 and Aliquat 336 were compared for acid removal, and Alamine 336 was recommended due to higher permeability at a pH of 3.3, which is the pH of untreated kiwi juice.

Liquid polyorganosiloxanes functionalized with amine, ether, ester, and alkyl organo-functional groups were used for the recovery of lactic acid and ethyl lactate

(Yahaya and Brisdon, 2000). In this study, lactic acid transport was poor, but stable operation of 100 hours was observed for ethyl lactate with ether and amine functionalized polyorganosiloxanes. Using TOA as a carrier with a PVDF support, Juang et al. determined that the rate controlling steps for lactic acid transport were the interfacial chemical reaction and membrane diffusion (Juang et al., 1997). The ionic liquid tetradecyl(trihexyl)phosphonium bis(2,4,4-trimethylpentyl)phosphinate (IL-104) was dissolved in *n*-dodecane and used in a SLM that was in a spiral channel flat sheet module (Martak et al., 2008). In this study, the transport of lactic acid due to the back transport of water from the receiving phase was reported for the first time. SLM systems for the enantiomer separation of L,D-lactic acid has also been reported (Hadik et al., 2005; Yang and Chung, 2007). HFSLM studies using the chiral selector, N-3,5-dinitrobenzoyl-L-alanine octylester, reported that when toluene was removed from the SLM by a nitrogen purge, the chiral selector remained in the membrane pores and resulted in increased enantioselectivity but a decrease in flux (Hadik et al., 2005). The use of a modified carbonate form of Aliquat 336 was another approach to the separation of D,L- lactic acid mixtures in which L-lactic acid was recovered with a separation factor of 2.3 (Yang and Chung, 2007). High purity aconitic acid was recovered from cane molasses solutions by a SLM process. The carrier TBP was able to selectively extract aconitic acid from a feed solution containing oxalic, maleic, and citric acids (McMurray and Griffin, 2002).

L-phenylalanine is an important precursor to the artificial sweetener Aspartame (Thien et al., 1988) and its separation by SLMs has been demonstrated. The extraction of phenylalanine hydrochloride was achieved with various crown ethers (Bryjak et al., 1991). The extractant D2EHPA, was studied for phenylalanine separation in a HFSLM

(Choi et al., 2003) and a spiral channel SLM (Kertesz et al., 2004). Aliquat 336 has also been used as an extractant in various studies (Molinari et al., 1992; Campbell et al., 1994; Adarkar et al., 1997).

A variety of SLM systems have been applied to the enatio-separation of D,L-phenylalanine. A SLM process for the optical resolution of amino acids was proposed, which contained an encapsulated surfactant-protease complex in the membrane phase (Miyako et al., 2005). L-phenylalanine was enantioselectively esterified by the protease complex. After the L-phenylalanine ethyl ester diffused through the membrane phase, an α -chymotrypsin complex in the receiving phase catalyzed ester hydrolysis, re-forming the initial L-phenylalanine. Resolution of a racemic mixture of phenylalanine was achieved with a separation factor of 1.8, with the chiral carrier, N-decyl-(l)-hydroxyproline, which was complexed with copper ions (Clark et al., 2005). A two-stage continuous SLM process was tested for the enatio-separation of leucine and phenylalanine (Maximini et al., 2006). After five separation steps, leucine products consisting of 99% D-enantiomer and 99% L-enantiomer could be produced.

SLMs have been used to recover sugars from aqueous solutions. Of particular interest is the selective recovery of fructose over glucose during the production of high fructose syrup. An extensive review from 2004 summarizes the early work done in this field, and showed that boronic acid carriers have the potential to achieve this separation (Duggan, 2004). More recent work showed that the addition of Aliquat 336 with the carrier 2-(aminomethyl)-phenylboronic acid increased fructose flux and increased selectivity over glucose in a FSSLM system (Duggan et al., 2008). The boronic acid derivative, 4-[8-(2-nitrophenoxy)octyloxycarbonyl] benzeneboronic acid, was used in

both flat sheet and hollow-fiber SLM configurations for the selective extraction of fructose from fermentation broths (Di Luccio et al., 2000). The HFSLM achieved longer stability and a selectivity up to 20, compared to only 14 in the FSSLM system.

Carriers other than boronic acids have also been used in SLM systems for the facilitated transport of sugars. A resorcinarene carrier, a derivative of calixarene, was used in a FSSLM system, and it yielded higher selectivity for pentoses compared to fructoses, which is not typically seen with boronic acid carriers (Rhialou et al., 2005). A SLM system composed of methyl cholate dissolved in cyclohexane and supported with a PVDF flat sheet was studied for the extraction of various sugars and was found to be stable for a period up to 23 days (Hassoune et al., 2008).

2.4.2 SLM Stability

SLMs have seen limited commercial success due to the lack of long-term stability. The main mechanisms for SLM instability and recent approaches taken to solve this problem will be addressed. Six mechanisms for SLM instability were identified and include (Kemperman et al., 1996):

- pressure difference over the membrane
- solubility of the liquid membrane phase in aqueous feed and strip solutions
- wetting of pores in polymer support by aqueous phases
- blocking of membrane pores by water or carrier precipitation
- osmotic pressure difference over the membrane
- emulsification of the liquid membrane phase from lateral shear forces.

Emulsification of the liquid membrane phase and the osmotic pressure difference over the membrane are considered to be the two major mechanisms of SLM instability (Dreher and Stevens, 1998). The other mechanisms can be avoided by properly designing and operating a SLM system.

There have been many attempts to improve SLM stability and they include:

- use of room temperature ionic liquids (RTILs) in SLMs (Fortunato et al., 2005)
- re-impregnation of the liquid membrane phase in the support (Nakao et al., 1987; Tanigaki et al., 1988; Teramoto et al., 2000)
- use of composite supports that have a barrier or stabilization layer.

For the last attempt, the barrier layer has been formed by applying hydrophilic coatings on hydrophobic supports (Wijerset et al., 1998; He et al., 2004), interfacial polymerization (Kemperman et al., 1998; Wang et al., 1998), phase-inversion (Yang et al., 2007), and plasma polymerization (Yang et al., 2000).

2.5 Supported Liquid Membranes with Strip Dispersion

To overcome instability with traditional SLMs, Ho et al. developed SLMs with strip dispersion (Ho and Poddar, 2001; Ho, 2003). The technique has also been referred to as “emulsion pertraction”, “pseudo-emulsion based hollow-fiber strip dispersion technique”, and “surfactant-free supported emulsion liquid membrane extraction”. This technique maintains a constant supply of organic membrane solution to the SLM, to ensure long-term stability. The SLM with strip dispersion system is shown in Figure 2.7, and an enlarged view is shown in Figure 2.8. As shown in these figures, an aqueous strip solution is dispersed in an organic membrane solution by a mixer, and passed onto one

side of a membrane support. When a microporous hydrophobic support is used, the organic phase of the dispersion becomes imbedded in the pores of the support, typically hollow-fiber modules, forming a stable SLM. Stability is maintained by having a constant supply of organic membrane solution to the pores. An aqueous feed solution is passed on the other side of the membrane support, and the target species is extracted into the organic solution by a selective carrier. The target species is then stripped by the aqueous strip solution. For final recovery of the target species, the mixer is turned off or a settler is used for the loaded strip dispersion (not shown), and the dispersion quickly separates. SLMs with strip dispersion processes offer several advantages compared to other liquid membrane configurations, including (Klaassen et al., 2005):

- no emulsion formation in the water phase
- flexible process parameters
- phase separation is not necessary
- compact modular equipment
- low energy consumption
- large specific surface areas due to hollow-fiber contactors.

2.5.1 Applications for Heavy Metal Removal and Recovery

Applications for SLMs with strip dispersion are similar to those found for traditional SLMs. Heavy metal removal and recovery has been the focus of current literature.

It is possible to remove Cr (VI) and recover it in the form of chromate using commercial size hollow-fiber modules (Ho and Poddar, 2001). In a two-step process, Cr

(VI) is removed, reducing its concentration from 100 ppm to 0.05 ppm in the acidic feed solution, and concentrating it to 200,000 ppm in the aqueous strip solution. The organic membrane solution contains Amberlite LA-2, 1-dodecanol, and PLURONIC L31 in Isopar L. Stripping is achieved with NaOH. Sulfuric acid competes with chromic acid for complexation with the amine carrier at Cr (VI) concentrations less than 100 ppm.

A mathematical model for the removal and recovery of Cr (VI) was optimized (Ortiz et al., 2003) and the proposed configuration involves a two-step process similar to that described by Ho (Ho and Poddar, 2001), consisting of an organic membrane solution that is formed by Alamine 336, 1-dodecanol, and PLURONIC L31 in Isopar L. Optimal operating conditions were reported, and simulations showed good comparison with experimental results.

In the recovery of Cr (VI) from simulated industrial waste in hydrochloric acid solutions with the carrier Cyanex 923, the stripping solution consisted of 10 g/L hydrazine sulphate, which reduces Cr (VI) to the less toxic Cr (III) (Sonawane et al., 2010). This process has shown selective recovery of Cr (VI) over other metals, as transport of Cu(II), Fe (II), Ni (II), and Zn (II) were all less than 3.4% and transport of Cr (VI) over the same time span was >95%.

The transport of Cr (III) from alkaline aqueous solutions with trioctyl methylammonium chloride (TOMACl) in a HFSLM strip dispersion process was recently demonstrated (Alguacil et al., 2009). The influence on the organic solvent was investigated and *n*-decane and *n*-heptane (aliphatic diluents) had higher permeability values compared with aromatic diluents such as toluene and cumene.

Pilot plant studies for the removal and recovery of copper showed that with a 4''-diameter hollow-fiber module the copper in the feed could be reduced from 150 ppm to < 0.1 ppm using the carrier LIX 973N (Ho et al. 2001, 2002). The aqueous strip solution was concentrated to 3,700 ppm, which is suitable for copper metal recovery via electro-winning, with no significant contamination from other metals present in the feed.

The recovery of copper catalyst from the wet peroxide oxidation process was reported (Urtiaga et al., 2006). LIX 622N in kerosene was used as the membrane solution and was stripped with sulfuric acid. The final aqueous strip solution, containing copper sulfate, was found to be non-ecotoxic and suitable for recycle to the oxidation reactor (Urtiaga et al., 2006). One hollow-fiber module using SLMs with strip dispersion was compared to non-dispersive solvent extraction using two modules, one for extraction and one for stripping.

The separation of copper from zinc containing feed solutions with the carrier 1-phenyl-3-methyl-4-benzoyl-pyrazolone-5 in xylene was reported using a flat sheet polypropylene support (Gu et al., 2009). Increased membrane stability, improved flux, and higher recovery of copper in the aqueous stripping solution were observed for the SLM with strip dispersion process compared to a similar SLM process. Rheological experiments have shown the organic membrane solution becomes non-Newtonian during a 6 hour transport time. The organic solution exhibited shear thickening properties and is also a thixotropic fluid.

Ho et al. presented laboratory and scale-up results for the removal and recovery of zinc with the carrier Cyanex 301(Ho and Wang, 2001; Ho et al., 2002). The feed solution was from the Berkeley Pit (Butte, Montana) and was previously treated for copper

removal. After a four-hour run time in recycle mode, 121 L of feed solution was reduced from 526 ppm to 0.65 ppm, while achieving a strip solution concentration of 20,000 ppm, which is suitable for reuse.

The selective removal and recovery of zinc from copper containing feed solutions was studied using a flat sheet polypropylene support (He et al., 2006). An optimum pH = 2 – 2.5 was reported for the separation of zinc from copper, using the carrier D2EHPA in kerosene.

The removal and recovery of zinc from spent pickling acidic baths in a hot-dip galvanizing process was demonstrated with the carrier TBP (Carrera et al., 2009). A mathematical model that considers the mass transfer resistance shared between the organic liquid membrane and the organic phase boundary layer surrounding the stripping droplets was found to have satisfactory agreement with experimental results.

The selective extraction of zinc and iron from passivating bath liquids from galvanizing processes has been demonstrated (Klaassen et al., 2005). A Cyanex extractant is used to remove zinc and iron while not extracting chromium or cobalt. The process has been tested on the pilot plant scale and two full-scale installations are planned in the Netherlands. The system has been fully automated and is estimated to save galvanic firms around \$40,000 a year.

In a system for the removal and recovery of cobalt, an organic solution containing Cyanex 301, 1-dodecanol, and Isopar L was employed to effectively recover cobalt at high concentrations (Ho, 2003). A low feed pH = 2 was used to avoid proton transfer over the length of the hollow-fiber module.

The removal and recovery of gold from alkaline cyanide solutions using SLM with strip dispersion has been studied using the organic extractant LIX-79 (N,N-bis(2-ethyl hexyl)guanidine) in *n*-heptane (Sonawane et al., 2008). Selectivity of metal cyanide salts was reported as: $\text{Au}(\text{CN})_4^{2-} > \text{Zn}(\text{CN})_4^{2-} > \text{Ag}(\text{CN})_2^- > \text{Ni}(\text{CN})_4^{2-} > \text{Fe}(\text{CN})_6^{2-} > \text{Cu}(\text{CN})_4^{3-}$.

The advantages of SLMs with strip dispersion over traditional SLMs are exhibited in the removal and recovery of cadmium (Gu et al. 2006; He et al., 2007). In the selective removal of cadmium from zinc, the loss of membrane solution was reported to be 0.6 – 1.4% for SLM with strip dispersion and 12 – 14% for SLM (Gu et al. 2006). The low loss of membrane solution suggests long stability. Increased recovery, higher selectivity, and increased flux were also reported. Similar advantages were reported for the simultaneous removal of cadmium and cyanide from simulated electroplating rinse water (He et al., 2007). An organic solution containing the carriers trialkyl-phosphine oxide (TRPO) and D2EHPA in kerosene reduced CN^- and Cd (II) from initial concentrations of 30 ppm and 20 ppm to 0.05 ppm and 0.02 ppm, respectively. In another study, cadmium was recovered from HCl solutions in a SLM with strip dispersion system with the carrier Cyanex 923 (Sastre et al., 2008). It was reported that Cd (II) was selectively transported from binary solutions containing Cr (VI) and Cu (II), but not from solutions containing Fe (III) and Zn (II).

The ionic liquid $(\text{PJMTH}^+)_2(\text{SO}_4^{2-})$ was synthesized by reacting the primary amine, Primene JMT, with sulfuric acid and used for the removal and recovery of iron (III) from sulfuric acid containing feed solutions (Alguacil et al., 2010). Analysis of the overall mass transfer coefficients indicate that the mass transfer resistance for the

membrane phase is negligible and the interfacial mass transfer resistances due to the extraction and stripping reactions were found to be dominant. Several configurations of SLM with strip dispersion were attempted and the highest iron (III) transport occurred with the feed solution in the tube side of the hollow-fiber module and operation in counter-current mode.

The extractant 2-ethyl hexyl phosphonic acid-mono-2-ethyl hexyl ester (PC-88A) was employed in a FSSLM strip dispersion system with a polyvinylidene fluoride support for the recovery of thulium ions from HCl solutions (Pei et al., 2009). Compared to sulfuric acid and nitric acid, hydrochloric acid had the most efficient recovery of thulium at 92.2% when used as the stripping solution.

New extractants were synthesized for the removal of radioactive strontium, Sr-90 (Ho and Wang, 2002). The extractants were branched alkyl phenylphosphonic acids and contained the following four functional groups: 2-butyloctyl, 2-hexyldecyl, 2-octyldecyl/2-hexyldodecyl, and 2-octyldodecyl. Efficient stripping was achieved with hydrochloric acid compared to sulfuric acid. The new extractants were more effective in strontium removal than the conventional extractant D2EHPA.

Uranium recovery was achieved in a SLM with strip dispersion system, consisting of the carrier TBP, dissolved in n-paraffin hydrocarbon and a stripping solution of 0.01 M HNO₃ (Roy et al., 2008). When using an untreated oxalate supernatant as the feed solution, U (VI) was recovered with an efficiency of 84%. Plutonium, which was present in the feed, was extracted by TBP but was not recovered in the strip solution.

2.5.2 Applications for Removal and Recovery of Organics

Ho recovered penicillin G with an efficiency of 93% using the secondary amine, Amberlite LA-2, in Isopar L (Ho, 2003). With a feed solution concentration of 8,840 ppm, the aqueous strip solution containing sodium carbonate was concentrated to 41,000 ppm.

The extraction of pharmacologically active alkaloids from medicinal plants was demonstrated with the extractant D2EHPA (Ouyang et al., 2010). The tertiary isoquinoline alkaloids (protopine and allocryptopine) and quaternary isoquinoline alkaloids (sanguinarine and chelerythrine) were extracted from the *Macleaya cordata* plant. Optimal conditions for the SLM system were reported as: pH = 3 for the feed solution, 0.5 M HCl concentration in the strip solution, 0.3 M for the carrier (D2EHPA) concentration, and 0.67 for the volume ratio of the aqueous strip solution to the organic membrane solution.

The recovery of phenol from phenolic resin waste water containing formaldehyde was reported by Urtiaga et al. (Urtiaga et al., 2009). As described earlier in 2.2.1, when using a stripping solution composed of NaOH the recovered phenol is in the form of sodium phenolate. Phenol removal was conducted by both Type 1 (pure kerosene) and Type 2 (Cyanex 923) facilitation. The process was selective for the separation of phenol from formaldehyde when kerosene was used as the organic solution as no formaldehyde was detected in the aqueous strip solution. When the carrier, Cyanex 923, was added to the organic solution at a concentration of 10% v/v an increase in extraction kinetics was observed. However, the process was non-selective as formaldehyde was transferred to the strip solution. Acidification of the stripping phase was conducted for the final recovery of

phenol, and a 71 wt% phenol solution was recovered, which can be reused as raw materials in the production of phenolic resins.

The removal and recovery of fumaric acid from industrial waste water was achieved with the carrier trialkylamine (N₇₃₀₁). The effect of adding “inert” or “active” co-solvents or modifiers to the organic solution containing N₇₃₀₁ and kerosene was investigated and the Hansen solubility parameter theory was applied to analyze the results. The highest degree of extraction was obtained with the modifier *n*-octanol due to its higher solubility of amine–acid complexes which resulted in the better transport of fumaric acid. With optimal conditions the removal of fumaric acid was 89.5% and stability was demonstrated for a period of 25 hours, in which 5 consecutive cycles were completed by renewing the feed and stripping solutions every 5 hours (Li et al., 2009).

2.6 Conclusions

Liquid membranes can provide selective transport of species by facilitated transport. ELMs and SLMs have demonstrated the wide variety of applications that exist with liquid membranes. However, commercialization of these processes has been limited, due to lack of long-term stability. SLM with strip dispersion is a liquid membrane process, which is efficient, easy to operate, and provides operational stability. Applications for SLM with strip dispersion have shown significant growth in the last ten years and continue to grow, but have mainly focused on removal and recovery of heavy metals.

Table 2.1 Examples of Extractants.

Carrier type	Carrier	Common applications
Acidic	organophosphoric acids (D2EHPA)	metallic cations
-	crown ethers	metallic cations
-	hydroxyoximes (LIX [®] 84)	metallic cations (copper, nickel and palladium)
Basic	primary amine (Primene JMT)	anionic species
-	secondary amine (Amberlite LA-2)	penicillin G
-	tertiary amine (tri- <i>n</i> -octylamine)	anionic species at low pH (cadmium, cobalt, and organic acids)
-	quaternary ammonium salt (Aliquat 336)	anionic species (metallic anions and organic acids at high pH)
Neutral	tri- <i>n</i> -butyl phosphate (TBP)	uranium, plutonium, and thorium
-	trioctylphosphine oxide (Cyanex 921)	uranium and acetic acid

Figure 2.1 Carrier facilitated transport mechanism for copper.

Figure 2.2 Enlarged view of emulsion liquid membrane.

Figure 2.3 Emulsion liquid membrane process. (Correia and de Carvalho, 2000)

Figure 2.4 Schematic diagram of supported liquid membrane.

Figure 2.5 Typical two-compartment cell for flat sheet supported liquid membrane experiments. Where F, M, and R are the feed, membrane, and receiving solutions, respectively. (Adapted from Hassoune et al., 2008)

Figure 2.6 Single hollow-fiber supported liquid membrane used for sample enrichment in analytical applications. (Pedersen-Bjergaard and Rasmussen, 2008)

Figure 2.7 Schematic diagram of the supported liquid membrane with strip dispersion process. (Ho and Wang, 2002)

Figure 2.8 Enlarged view of the supported liquid membrane with strip dispersion. (Ho and Wang, 2002)

Chapter 3: Supported Liquid Membranes for Cephalexin Recovery

3.1 Introduction

Liquid membrane extraction has demonstrated effective recovery of Cephalexin from dilute aqueous solutions using the carrier Aliquat 336. A bulk liquid membrane (BLM) system was first reported for the uphill transport of Cephalexin (Sahoo et al., 1997). The BLM used the strong pH dependence of the distribution coefficient of Cephalexin between Aliquat 336 and aqueous solution to transport Cephalexin from a feed phase of high pH to a receiving phase of lower pH. This work was extended to an ELM system (Sahoo and Dutta, 1998). The ELM was able to selectively recover Cephalexin from an aqueous solution containing 7-ADCA and Cephalexin in a 1:1 mixture. Liquid membrane processes are a promising solution to the separation of Cephalexin, but traditional liquid membrane processes such as ELMs and SLMs are not suited for large-scale industrialization due to problems with long-term stability. The issue of instability must be overcome for liquid membrane processes to be considered for the recovery of antibiotics in commercial applications.

SLM with strip dispersion first reported by Ho et al. (Ho et al., 1999a; 1999b) is a promising solution to the problem of membrane stability. Previous literature on SLM with strip dispersion has focused on the removal and recovery of heavy metals such as

chromium (Ho and Poddar, 2001), copper (Ho, 2004), zinc (Ho, 2004), cobalt (Ho, 2004), strontium (Ho and Wang, 2002), uranium (Roy et al., 2008), gold (Sonawane et al., 2008), and cadmium (Gu et al., 2006). Studies have ranged from lab to pilot plant scales and have demonstrated that SLM with strip dispersion can efficiently remove metals from aqueous solutions, while also recovering them in high concentrations in the aqueous strip solution. Gu et al. compared SLM with strip dispersion and traditional SLMs for the selective removal of cadmium from feed solutions containing zinc (Gu et al., 2006). SLM with strip dispersion had lower organic membrane solution loss, higher flux, higher selectivity, and increased recovery compared to traditional SLMs (Gu et al., 2006). In this chapter, SLM with strip dispersion is used for the recovery and concentration of Cephalexin from dilute aqueous solutions with the carrier Aliquat 336. The influences of various experimental parameters on Cephalexin separation were investigated. In addition, the mass transfer resistances from the process were analyzed.

Cephalexin is a zwitterionic molecule with pK_a values of 2.56 and 6.88 (Yamana and Tsuji, 1976), with its ionic state determined by the pH of the solution, as shown in Figure 3.1. At a pH 6.88 and above, Cephalexin is anionic, at pH values below 2.56 it is cationic, and exists as a zwitterion between the two pK_a values. Extraction can occur by two mechanisms, ion-pair extraction and ion-exchange. Ion-pair extraction occurs when neutrally charged Cephalexin is extracted by a secondary or tertiary amine (Dutta and Sahoo, 2002). Extraction of Cephalexin in an anionic form can be done by the ion-exchange mechanism with a quaternary amine. Extraction with quaternary amines, such as Aliquat 336, is preferred due to higher extraction rates and the formation of the antibiotic-amine complex in a 1:1 ratio (Bora et al., 1998). The extraction and stripping

reactions of Cephalexin with the carrier Aliquat 336 (QCl) are shown in Equations 3.1 and 3.2, respectively.

3.2 Experimental

3.2.1 Materials

Cephalexin monohydrate of BP USP grade and 7-ADCA were obtained from MP Biomedicals, Solon, OH. Citric acid, sodium citrate, sodium carbonate, sodium bicarbonate, and 2-propanol were all of ACS reagent grade and were purchased from Fisher Scientific, Pittsburgh, PA. Potassium chloride of ACS reagent grade was obtained from GFS Chemicals, Columbus, OH. Isopar L (isoparaffin hydrocarbon solvent) was purchased from ExxonMobil Chemical, Houston, TX. 1-Decanol and dodecanol were obtained from Sigma-Aldrich, Milwaukee, WI. Aliquat 336 was kindly donated by Cognis Corporation, Tucson, AZ. All chemicals were used as received.

3.2.2 Distribution Coefficient Experiments

Studies to determine the distribution coefficient (extraction equilibrium) were conducted in a 125-ml round-bottom glass flask that contained 30 ml of 15 mM of Cephalexin or 7-ADCA and 30 ml of organic solution containing 2.5 – 5 wt% (48 – 96 mM) Aliquat 336 and 1.25 – 5 wt% of 1-decanol or dodecanol in Isopar L. The pH values of the aqueous solutions were varied from 4.0 – 10.0 using common buffer

solutions. Various buffers depending on the desired pH range were used, which included citrate for pH = 4.0 – 6.0, phosphate for pH = 6.5 – 8.0, and carbonate-bicarbonate for pH = 9.0 – 10.0. Citrate buffers were prepared by mixing 33.0, 20.5, and 9.5 ml of 0.1 M citric acid with 17.0, 29.5, and 40.5 ml of 0.1 M sodium citrate to obtain pH values of 4.0, 5.0, and 6.0, respectively. Phosphate buffers were prepared by mixing 68.5, 39, and 5.3 ml of 0.1 M monobasic sodium phosphate with 31.5, 61.0, and 94.7 ml of dibasic sodium phosphate to obtain pH values of 6.5, 7.0, and 8.0, respectively. Carbonate-bicarbonate buffers of pH values of 9.0, 9.5, and 10.0 were prepared by mixing 4.0, 13.0, 27.5 ml of 0.1 M sodium carbonate with 46.0, 37.0, and 22.5 ml of 0.1 M sodium bicarbonate, respectively. Initial and equilibrium pH values were measured with a digital pH meter (Model Accumet AB15, Fisher Scientific, Pittsburgh, PA). Mixing for six hours with a magnetic stirrer ensured equilibrium was obtained. After equilibrium was reached, the phases were separated. The concentration of Cephalexin or 7-ADCA was determined using a UV-spectrophotometer (Model UV-1700, Shimadzu, Columbia, MD), which was calibrated at 262 nm and 265 nm for Cephalexin and 7-ADCA, respectively. The distribution coefficient between the organic membrane solution and the feed solution was calculated by:

$$m_f = \frac{c_{QCeph}}{c_f} \quad (3.3)$$

in which c_{QCeph} was calculated from Equation 3.4, which is a mass balance of Cephalexin between the organic and aqueous phases.

$$c_{QCeph} = \frac{V_a}{V_o} (C_{fo} - C_f) \quad (3.4)$$

3.2.3 Supported Liquid Membrane Experiments

SLM with strip dispersion experiments were conducted with the feed solution and strip dispersion in recycle mode. The experimental set-up is shown schematically in Figure 3.2. Digitally controlled peristaltic pumps (Masterflex 7524-40 from Cole-Parmer, Vernon Hills, IL) were used to pump the aqueous feed solution on the tube side and the strip dispersion on the shell-side of a hollow-fiber module. The feed solution and strip dispersion were pumped at rates of 150 – 450 ml/min. The feed solution was pumped first with an inlet pressure on the tube side of the module of 34.5 kPa (5 psi), while the inlet pressure of the strip dispersion on the shell-side was 6.9 kPa (1 psi).

The hollow-fiber module (Model G453) was purchased from Membrana, Charlotte, NC. The module was 2.5'' (6.35 cm) in diameter and 8'' (20.3 cm) in length, with an effective length of 15 cm. The module contained ~10,800 polypropylene fibers, which provided 1.4 m² of surface area. Each hollow-fiber had an outer diameter of 300 µm and an inner diameter of 220 µm. Hollow-fiber pore size was 0.03 µm, and porosity was approximately 40%. Some SLM experiments used a smaller 1.7'' x 5.5'' hollow-fiber module, which contained the same hollow-fibers as the 2.5'' x 8'' module. Experiments in which the smaller module was used are specifically described.

Feed solutions containing 5 – 25 mM of Cephalexin were prepared by mixing Cephalexin monohydrate with 500 ml of 0.05 M carbonate-bicarbonate buffer at pH = 9.5. This resulted in Cephalexin solutions with a pH of 8.0, which were constantly mixed with a magnetic stirrer. The strip dispersion was prepared by dispersing 100 – 300 ml of aqueous strip solution containing KCl (0 – 3 M) in buffer solution in 600 ml of organic

membrane solution using an overhead mixer (Model BDC2002, Caframo, Warton, Ontario) at 200 – 400 RPM and a 3"-diameter three-blade impeller. Organic membrane solutions contained 1.25 – 12.5 wt% of Aliquat 336 and 0.6 – 6.25 wt% of 1-decanol in Isopar L. 1-Decanol was added to the organic membrane solution due to the poor solubility of Aliquat 336 in Isopar L and was always at one half the concentration of carrier. During the course of an experiment, samples from the feed solution and strip dispersion were taken. Samples from the strip dispersion were allowed to settle for about 1 minute, before the aqueous strip solution was sampled. The pH values of the feed solution and aqueous strip solution were measured along with the Cephalexin concentration, which was measured by a UV-spectrophotometer. The Cephalexin concentration in the organic phase was determined by mass balance. After each experimental run, the hollow-fiber module was cleaned with a 50/50 mixture of 2-propanol and deionized water, followed by flushing with deionized water. The module was then dried overnight with dry filtered air.

3.3 Results and Discussion

3.3.1 pH Dependence of Distribution Coefficient

Figure 3.3 shows the distribution coefficients of Cephalexin and 7-ADCA with an organic solution consisting of 2.5 wt% Aliquat 336 as a function of pH. Distribution coefficients for Cephalexin were higher than those for 7-ADCA, suggesting that selective separation may be achieved with the carrier Aliquat 336. The results were as expected and show similar trends to those reported previously (Sahoo and Dutta, 1998). The distribution coefficients rise with increasing pH up to a pH ~9 due to the increased

dissociation of the solutes. Cephalosporins have shown enhanced degradation at pH values of 10 and above, which can explain the decrease in distribution coefficients which was observed when the pH was increased from 9 to 10 (Yamana and Tsuji, 1976). To limit Cephalexin decomposition, while still maintaining a high distribution coefficient, dilute carbonate-bicarbonate buffer was used to obtain an initial pH = 8.0 in SLM experiments, which is also the optimal pH for Cephalexin synthesis (Schroën et al., 2002a). It has been reported that carbonate buffer solutions have lower co-extraction with Aliquat 336 compared to other buffer systems in the pH range of interest (Hano et al., 1991).

3.3.2 Optimization of Solvent and Modifier Concentration

Previous reports that have investigated the extraction of Cephalexin with Aliquat 336 using various types of solvents. Examples of solvents that have been studied include: benzene, butyl acetate, chloroform, cyclohexane, dichloromethane, heptane, kerosene, and octanol (Bora et al., 2000). These solvents are not preferred for a large scale commercial process due to either toxicity, high vapor pressure, or a combination of the two. We have chosen to use the isoparaffin, Isopar L, as the solvent for our SLM system due to its low cost, low toxicity, and low vapor pressure. However, Aliquat 336 has a low solubility in Isopar L, which results in a three-phase system during extraction experiments. To overcome this issue, a modifier was added to the solvent to ensure that the Aliquat 336 is fully dissolved and third phase formation was eliminated. Two logical candidates for use as a modifier are 1-decanol and dodecanol due to their low water solubility. The minimum concentration of modifier that eliminated third phase formation

was found to be 50% of 1-decanol or dodecanol compared to the Aliquat 336 concentration. The effect of the concentration of modifier in the solvent was investigated in extraction equilibrium experiments with a feed pH = 8.0 and results are presented in Table 3.1. An increase of modifier concentration from 50% of the Aliquat 336 concentration to equal amounts of modifier and carrier resulted in a reduction in Cephalexin extraction for both 1-decanol and dodecanol. The modifiers themselves were also tested as solvents and had less extraction compared to the Isopar L plus modifier cases. Overall, the use of 1-decanol at 50% Aliquat 336 concentration had the highest extraction and was used as the standard organic solution for extraction and SLM experiments.

3.3.3 Strip Dispersion Mixing Rate

To investigate the effect of mixing speed, 600 ml of organic solution and 300 ml of aqueous stripping solution were mixed at 200, 300, and 400 RPM with a 3"-diameter three-blade impeller. As shown in Figure 3.4, an increase in the mixing speeds from 200 to 300 RPM improved recovery from 90% to 95%. An additional increase from 300 to 400 RPM resulted in no further improvement in Cephalexin recovery. As a result, all further experiments utilized a 400 RPM mixing rate for the strip dispersion, to ensure a high mass transfer surface area, which allows for efficient stripping.

3.3.4 Effect of KCl Concentration

The influence of Cl⁻ counterion concentration in the aqueous strip solution was investigated. Figure 3.5 shows the concentration profiles of the feed and aqueous strip

phases with varying concentrations of KCl in aqueous strip solutions containing 0.1 M citrate buffer. Uphill transport was observed for all conditions, even in the absence of Cl^- in the stripping solution. Without Cl^- in the stripping solution, Cephalexin transport is possible as buffer anions in the stripping solution can strip the Cephalexin-Aliquat 336 complex. Increasing the counterion concentration greater than 1.0 M KCl resulted in negligible improvement in transport, as observed with a KCl concentration of 3.0 M. The large excess of counterion needed for efficient recovery was typical when using Aliquat 336 in liquid membrane processes due to the ion-exchange mechanism taking place. Previous works, which used Aliquat 336 for the reactive extraction of β -lactam antibiotics, suggested that stripping efficiency was independent of the counterion concentration and found their observations difficult to explain (Bora et al, 2008; Hano et al., 1992; Sahoo et al, 1997; Sahoo and Dutta, 1998). Previously reported studies also used a citrate buffer as the receiving phase, however, the Cl^- concentration in the stripping solutions were limited to the range of 0 to 30 mM (Sahoo et al, 1997; Sahoo and Dutta, 1998). In studies which did investigate a wide range of Cl^- concentration (0 to 2 M), the low pH of the receiving phase ($\text{pH} = 4$) was a possible reason that poor stripping was observed (Hano et al., 1992). In regard to recovery of other fermentation products with an quaternary ammonium salt as the carrier, high Cl^- concentration in the stripping solution has been reported for the recovery of penicillin G (Reschke and Schügerl, 1984) and for the recovery of L-phenylalanine in both ELM (Thien et al., 1988) and SLM processes (Molinari et al., 1992).

3.3.5 pH in Aqueous Strip Solution

Maintaining a pH gradient between the aqueous feed solution and the aqueous strip solution has been suggested as the possible driving force when using the carrier Aliquat 336 to recover Cephalexin in liquid membrane processes (Bora et al, 2008; Hano et al., 1992; Sahoo et al, 1997; Sahoo and Dutta, 1998). The following is a discussion of the role of the stripping solution pH on the extraction and recovery of Cephalexin. When using a weak buffer solution, the pH of the aqueous strip solution is susceptible to change, due to the co-transport of buffer ions present in the feed and strip solutions. To determine the effect of the strip solution pH on the recovery of Cephalexin, aqueous strip solutions were varied in buffer concentration at volumes of 300, 200, and 150 ml with a KCl concentration of 1.0 M.

Figures 3.6 – 3.8 show the Cephalexin concentration in the feed and stripping solutions over time, along with the pH in the stripping solution. In these figures, the feed solution pH is not shown but always had an initial pH = 8.0. Small changes to the feed solution pH were observed over time, but never reached a value above 9.0. It is observed in Figure 3.6 and Figure 3.7 that as the volume of aqueous strip solution is decreased, a higher concentration of citrate buffer is needed to maintain efficient transport of Cephalexin. The most profound effects of citrate buffer concentration can be seen for the case with employing a strip volume of 150 ml (Figure 3.6). A buffer concentration of 0.5 M in the stripping solution is able to resist major pH change, and high Cephalexin recovery is obtained throughout the experiment. Buffer concentrations of 0.2 and 0.1 M were not resistant to changes in pH, and recovery of Cephalexin soon declined due to an increased pH of the stripping solution. This can be explained by the distribution coefficient of Cephalexin with Aliquat 336. As the stripping solution pH is increased, the

distribution coefficient also increases, resulting in Cephalexin accumulation in the membrane phase.

The results in Figure 3.8 indicate that the pH was relatively stable with an aqueous strip volume of 300 ml, and a recovery of 97% was obtained for all buffer concentrations. From the data presented in Figures 3.6 – 3.8, it appears that optimal recovery of Cephalexin occurs between a pH of 5 – 6. Maintaining the stripping solution in this optimal range allows the anionic form of Cephalexin to partake in the stripping reaction while keeping the distribution coefficient between the organic membrane solution and the strip solution low.

Further evidence for the existence of an optimal pH range is provided by Figure 3.9, which compares aqueous strip volumes of 150 ml with varying values of initial pH. Maximum Cephalexin recovery was achieved using strip solutions with initial pH = 5.2 by citrate and phosphate buffers, which had recoveries of 96% and 94%, respectively. When the pH of the strip solution was raised to 8.0, the pH gradient between the feed and the strip solutions was eliminated, and the total recovery of Cephalexin dropped to 73%. Extraction and recovery rates were drastically reduced when the stripping solution consisted of a citrate buffer with an initial pH = 3.3. Even though this value provides a large pH gradient between the feed and stripping solutions, little Cephalexin transport is observed, as Cephalexin is not able to participate in the stripping reaction shown in Equation 3.2 due to the zwitterionic form of Cephalexin being predominant. From the data presented, it appears a pH gradient is not the driving force for uphill transport, but an appropriate pH can lead to enhanced recovery of Cephalexin with Aliquat 336.

Data are also shown in Figure 3.9 for an experiment that used an aqueous stripping solution that contained no buffer, and only contained an excess of KCl (1.0 M). Compared to a phosphate buffer of pH 8.0, a higher Cephalexin recovery was observed when no buffer was used, 73% and 87.6%, respectively. This is surprising because the pH of the stripping solution is slightly higher when no buffer is being used, which contradicts the previously made conclusion that a higher pH leads to lower recovery. This can be explained by electron charge density of the anions present in the stripping solution, as chloride ions have a higher affinity for ion-exchange with quaternary ammonium salts compared to phosphate ions (Mulder, 1996).

3.3.6. Volume of Aqueous Strip Solution

A major advantage of using SLM with strip dispersion for the recovery of Cephalexin is that the final strip solution can result in a higher concentration than the initial feed solution due to the uphill transport that occurs. To determine how high of a concentration enhancement can be achieved, the volume of the aqueous strip solution was studied between 50 – 300 ml. As seen in Figure 3.10, a reduction in the volume of the aqueous strip solution results in a higher Cephalexin concentration in the strip solution. As the volume of the strip solution was reduced there was also a reduction in the efficiency of the Cephalexin recovery as shown in Table 3.2. For the case of 50 ml volume of strip solution Cephalexin crystallization in the shell-side of the hollow-fiber module occurred. The crystallization can be explained by the low solubility of Cephalexin in aqueous solutions.

3.3.7. Carrier Concentration

The effect of the Aliquat 336 concentration in the organic membrane solution was studied over the range of 1.25 to 12.50 wt% (24 to 243 mM). Viscosities of organic solutions were measured with a Brookfield digital viscometer (Model DV-E) and are presented in Table 3.3. The extraction of Cephalexin increased with increasing Aliquat 336 concentration up to a value of 10.00 wt% (194 mM) as shown in Figure 3.11. A further increase in carrier concentration resulted in a decrease in extraction due to the increase in viscosity which increases the membrane resistance.

3.3.8 Mass Transfer Analysis

If it is assumed that Cephalexin is extracted from the feed solution while passing through the hollow-fiber module, then the Cephalexin transport can be described by a mass balance on the feed solution (Ho and Wang, 2002):

$$\frac{dc_f}{dt} = -\frac{A}{V_a} j \quad (3.5)$$

By selecting an appropriate extractant, one that has a high distribution coefficient between the organic membrane and feed solution and a low distribution coefficient between the organic membrane and aqueous strip solution, the flux through a SLM can be expressed as (Ho and Wang, 2002):

$$j = Kc_f \quad (3.6)$$

The combination of Equations 3.5 and 3.6 followed by integration results in:

$$\ln \frac{c_f}{c_{fo}} = -\frac{KA}{V_a} t \quad (3.7)$$

which can be applied to experimental data to obtain an experimental overall mass transfer coefficient. The overall mass transfer resistance through the SLM is equal to the sum of the following individual mass transfer resistances: the feed-side resistance, the interfacial resistance due to the extraction reaction, the membrane-phase resistance, the shell-side resistance, the resistance due to the stripping reaction, and the strip-side resistance.

$$\frac{1}{K} = \frac{1}{k_a} + \frac{1}{k_e} + \frac{1}{m_f k_m} + \frac{1}{m_f k_o} + \frac{1}{m_f k_s} + \frac{1}{(m_f / m_s) k_{as}} \quad (3.8)$$

Because flow in the tube side of the hollow-fiber module is laminar, the mass transfer coefficient in the aqueous phase is calculated by the Leveque equation (Leveque, 1928; Wickramasinghe et al., 1992):

$$Sh = \frac{k_a d_i}{D_a} = 1.62 \left(\frac{d_i^2 u_b}{D_a L} \right)^{\frac{1}{3}} \quad (3.9)$$

The diffusion coefficient of Cephalexin in aqueous solution was previously reported as $4.56 \times 10^{-10} \text{ m}^2/\text{s}$ (Seki et al, 2003). This value agrees well with the value of $4.66 \times 10^{-10} \text{ m}^2/\text{s}$ obtained by using the Wilke-Chang equation (Wilke and Change, 1955), with an estimate of molar volume at the normal boiling point for Cephalexin obtained from the Tyn and Calus method (Reid et al., 1987).

The mass transfer coefficient of the membrane phase can be calculated by (Ho and Poddar, 2001):

$$k_m = \frac{D_m}{\delta} \frac{\varepsilon}{\tau} \frac{d_{lm}}{d_o} \quad (3.10)$$

The value of D_m , $3.9 \times 10^{-10} \text{ m}^2/\text{s}$, was estimated by using the Haydauk and Minhaus equation for non-aqueous solutions (Reid et al., 1987). Parachors for the Cephalexin-Aliquat 336 complex and Isopar L are estimated by additive group contributions to be 1745.6 and $511 \text{ cm}^3 \text{ g}^{1/4}/\text{s}^{1/2}$, respectively. The molar volume at the normal boiling point for Isopar L is calculated to be $274 \text{ cm}^3/\text{mol}$ by the Tyn and Calus method (Reid et al., 1987). Viscosities of organic solutions from section 3.3.7 were used in the calculations.

For liquid-liquid extraction in a Liqui-Cel[®] hollow-fiber module, the mass transfer coefficient on the shell-side can be calculated by (Baudot et al., 2001):

$$Sh = \frac{k_o d_h}{D_m} = 0.56 Re^{0.62} Sc^{0.33} \quad (3.11)$$

Where d_h is the hydraulic diameter of the shell-side, defined in Equation 3.12, where the diameter of the fiber bundle, d_a , is 0.047 m (Baudot et al., 2001).

$$d_h = \frac{d_a^2 - d_i^2 - nd_o^2}{nd_o} \quad (3.12)$$

As described in Equation 3.8, the total mass transfer resistance can be attributed to six individual resistances. If the stripping reaction is assumed to be instantaneous, the overall mass transfer coefficient can be expressed as:

$$\frac{1}{K} = \frac{1}{k_a} + \frac{1}{k_e} + \frac{1}{m_f k_m} + \frac{1}{m_f k_o} \quad (3.13)$$

The assumption of instantaneous stripping reaction was justified in cases that mass balances showed a very high stripping efficiency of 99 – 100% when an excess of the counterion, Cl^- , was used in the stripping solution and the appropriate stripping pH of 5 –

6 was maintained. The cases where this assumption for the data presented held were for the following:

- excess of Cl^- ion (1 M or greater) with a strip volume of 300 ml
- excess of Cl^- ion and a high citrate buffer concentration with strip volumes less than 300 ml.

The assumption is not always valid, such as the data shown in Figure 3.6. When the low citrate buffers of 0.1 and 0.2 M were used in the strip solution, poor stripping resulted, and the instantaneous stripping assumption could not be applied. During the mass transfer analysis, Equation 3.13 was only applied to experiments where stripping efficiency was high (99 – 100%).

The values of k_a , k_m , and k_o can be calculated from Equations 3.9, 3.10, and 3.11, respectively. An estimate of the mass transfer resistance from the extraction reaction can be obtained by difference of the calculated mass transfer resistances and the experimental overall mass transfer resistance. The fractional resistance (Δ) of each contributing step, the aqueous feed-side resistance (Δ_a), extraction reaction resistance (Δ_e), membrane phase resistance (Δ_m), and organic phase shell-side resistance (Δ_o), are displayed in Table 3.4 for different Aliquat 336 concentrations. For all Aliquat 336 concentrations, the mass transfer resistance from the extraction reaction between Cephalexin and Aliquat 336 is controlling the mass transfer resistance, accounting for over 90% of the resistance. As a majority of the experiments conducted in this work used an Aliquat 336 concentration of 2.5 wt%, the mass transfer analysis for this condition will be further discussed. Figure 3.12 displays the dimensionless concentration of the feed solution over time. The nearly linear slope indicates that the data agrees well with the mass transfer coefficient

expression shown in Equation 3.7 and results in an initial overall mass transfer coefficient of 1.48×10^{-7} m/s. The low amounts of fractional resistances calculated for the aqueous feed-side and the organic shell-side are verified by Figures 3.13 and 3.14, in which the effect of adjusting the feed solution and strip dispersion flow rates on the extraction rate was studied, respectively. Adjusting the feed solution and strip dispersion flow rates had little effect on the extraction of Cephalexin, validating the low resistances due to the aqueous feed-side and shell-side what were calculated.

3.3.6 Cephalexin Concentration in Feed Solution

The influence of Cephalexin concentration in the feed solution was investigated in the range of 5 to 25 mM or 1.83 to 9.14 g/L. Concentrations in the feed solutions and stripping solutions over time are shown in Figure 3.15. A slower extraction rate is observed with an increase of Cephalexin concentration. However, as shown in Table 3.5, the final efficiency of extraction for all feed concentration values remained high. The amount of Cephalexin recovery in the aqueous strip solution was highly efficient in all cases, but slightly declined as the feed concentration was increased.

3.4 Conclusions

The carrier Aliquat 336 was used in a SLM with strip dispersion system for the recovery of the antibiotic, Cephalexin, from aqueous solutions. Results have shown that the counterion concentration in the stripping solution has a significant effect on the extraction and recovery of Cephalexin. An excess of Cl^- along with an appropriate pH in the stripping solution results in the most efficient recovery of Cephalexin. Calculation of

the mass transfer resistances indicate that the rate-controlling step in this process is the extraction reaction between Cephalexin and the carrier.

Table 3.1 Extraction with 5 wt% Aliquat 336 in Organic Membrane Solution.

Solvent	Modifier	Extraction (%)
Isopar L	2.5 wt% 1-decanol	87.0
Isopar L	5 wt% 1-decanol	82.2
Isopar L	2.5 wt% dodecanol	86.8
Isopar L	5 wt% dodecanol	81.1
1-decanol	-	70.0*
dodecanol	-	76.5

*Emulsion was formed

Table 3.2 Cephalexin Recovery with Varying Volume of Aqueous Strip Solution.

Volume of aqueous strip solution	Concentration enhancement ratio	Total Cephalexin recovery (%)
300 ml	1.65	98.90
200 ml	2.46	98.40
150 ml	3.21	96.20
100 ml	4.60	91.91
50 ml	—*	—*

* Not measured due to crystallization of Cephalexin in aqueous strip solution

Table 3.3 Viscosity of Organic Solutions at Different Concentrations of Aliquat 336.

Organic membrane solution	Viscosity (cp)
Isopar L	1.56
1.25 wt% Aliquat 336	1.58
2.50 wt% Aliquat 336	1.70
5.00 wt% Aliquat 336	1.99
7.50 wt% Aliquat 336	2.10
10.00 wt% Aliquat 336	2.71
12.50 wt% Aliquat 336	3.17

Table 3.4 Mass Transfer Fractional Resistances.

Aliquat 336 (wt%)	Fractional resistances			
	Δ_a	Δ_e	Δ_m	Δ_o
1.25	0.010	0.923	0.045	0.022
2.50	0.022	0.926	0.035	0.017
5.00	0.038	0.909	0.035	0.018

Table 3.5 Effect of Initial Cephalexin Concentration on SLM with Strip Dispersion Performance. (Same experimental conditions as described in Figure 3.15)

Cephalexin concentration (mM)	$K \times 10^7$ (m/s)	Initial flux (g/m ² /hr)	Extraction (%)	Recovery (%)
5	4.29	2.82	99.7	99.7
8	2.72	2.86	99.7	99.1
15	1.52	2.98	99.1	97.1
25	0.96	3.17	98.8	96.7

Figure 3.1 Cephalexin ionization states. ($\text{pK}_{a1} = 2.56$, $\text{pK}_{a2} = 6.88$, isoelectric point = 4.5)

Figure 3.2 Schematic view of the supported liquid membrane with strip dispersion system in recycle mode with a hollow-fiber module support.

Figure 3.3 Cephalexin and 7-aminodeacetylcephalosporanic acid (7-ADCA) distribution coefficients at varying pH.

Figure 3.4 Effect of aqueous strip solution mixing rate on Cephalexin recovery. Feed solution: 15 mM Cephalexin. Organic membrane solution: 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L. Aqueous strip solution: 1 M KCl in 0.1 M citrate buffer (pH = 6.0).

Figure 3.5 Cephalexin concentration in the feed (solid lines) and aqueous strip solution (dotted lines) with varying KCl concentration. Initial Cephalexin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 300 ml, 0.1 M citrate buffer (pH = 6.0).

Figure 3.6 Cephalexin concentration in the feed (solid lines) and aqueous strip solutions (dashed lines) with varying buffer concentration. Initial Cephalexin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 150 ml, 1 M KCl. Open symbols represent strip solution pH.

Figure 3.7 Cephalixin concentration in the feed (solid lines) and aqueous strip solutions (dashed lines) with varying buffer concentration. Initial Cephalixin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 200 ml, 1 M KCl. Open symbols represent strip solution pH.

Figure 3.8 Cephalixin concentration in the feed (solid lines) and aqueous strip solutions (dashed lines) with varying buffer concentration. Initial Cephalixin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 300 ml, 1 M KCl. Open symbols represent strip solution pH.

Figure 3.9 Cephalixin concentration in the feed (solid lines) and aqueous strip solutions (dashed lines) with varying pH of stripping solution (symbols). Initial Cephalixin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 150 ml, 1 M KCl.

Figure 3.10 Effect of aqueous strip solution volume on Cephalexin recovery. Feed solution: 15 mM Cephalexin. Organic membrane solution: 2.50 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 1 M KCl in 0.5 M citrate buffer (pH = 6.0).

Figure 3.11 Effect of Aliquat 336 concentration on the extraction rate of Cephalexin in 1.7'' x 5.5'' hollow-fiber module. Initial Cephalexin concentration of 15 mM. Organic membrane solution: 600 ml. Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Figure 3.12 Plot of the initial overall mass transfer coefficient. Initial Cephalexin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Figure 3.13 Percent extraction of Cephalexin with varying feed solution flowrate. Initial Cephalexin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Figure 3.14 Percent extraction of Cephalexin with varying strip dispersion flowrate. Initial Cephalexin concentration of 15 mM. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Figure 3.15 Concentration profiles with varying Cephalixin concentration in the feed solution. Organic membrane solution: 600 ml, 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L. Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Chapter 4: Cephalexin Separation from Complex Mixtures

4.1 Introduction

The industrial production of antibiotics has been an intensely studied subject in the field of biocatalysis for the past 20 years. β -Lactam antibiotics, which consist of penicillins and cephalosporins, have been of particular interest as they represent 65% of the world's antibiotic market (Elander, 2003). Only recently have synthesis schemes using biocatalysts been robust, efficient, and cost effective enough to meet the criteria for industrial use. The enzymatic synthesis of Cephalexin, which is the most widely produced cephalosporin, can be either kinetically or thermodynamically controlled, with the kinetic process being the most widely used (Sheldon and van Rantwijk, 2004). The kinetic process involves the combination of a reactive side-chain donor such as PGA or phenylglycine methyl ester (PGME) with the 7-ADCA nucleus in the presence of the enzyme, penicillin G acylase, to produce Cephalexin (Schroën et al., 2002a). When compared to traditional organic synthesis, the enzymatic method reduces solvent use by 90%, other reagents by 100%, energy consumption by 40%, and waste by 15% (Schoevaart and Kieboom, 2001). Despite these advantages, commercial use of the enzymatic process has been hindered due to a low Cephalexin yield and the difficult separation of the reaction mixture (Bruggink and Roy, 2001). Along with separation from

the starting materials, Cephalexin separation from reaction side-products such as phenylglycine (PG) needs to be considered. Approaches to the separation of Cephalexin have included the formation of an insoluble complex (Zwanenburg et al., 2001), nanofiltration (Wang and Chung, 2006), solvent extraction (Schügerl, 2005), adsorption (Dutta et al., 2004), aqueous two-phase partitioning (Zhu et al., 2001), and liquid membranes (Dutta and Sahoo, 2002).

Aliquat 336 has been used to successfully recover Cephalexin in liquid membrane systems. Previous studies have focused on bulk liquid membrane and ELM configurations, which are not suitable for large-scale industrialization (Sahoo et al., 1997; Sahoo and Dutta, 1998). It was shown in Chapter 3 that Cephalexin can be efficiently removed and recovered from aqueous solutions using SLM with strip dispersion (Vilt and Ho, 2009). Extraction and recovery of 99% were achieved when an appropriate pH gradient was maintained between the feed and strip solutions in combination with an excess of Cl^- in the stripping solution (Vilt and Ho, 2009). The separation of Cephalexin with liquid membranes from multi-component feed solutions has been limited. An ELM with Aliquat 336 was able to selectively recover Cephalexin from an aqueous solution containing 7-ADCA and Cephalexin in a 1:1 mixture (Sahoo and Dutta, 1998). The separation of Cephalexin with the carrier Aliquat 336 from complex feed solutions containing multiple solutes has yet to be reported.

In this chapter, the separation of Cephalexin from feed solutions containing a mixture of solutes was investigated. To achieve the separation, we used a SLM with strip dispersion process that used hollow-fiber modules and the extractant Aliquat 336. Feed solutions were prepared to simulate enzymatic reaction mixtures for the production of

Cephalexin and contained a single-component (Cephalexin), two-components (Cephalexin and 7-ADCA) or four-components (Cephalexin, 7-ADCA, PGA and PG).

As described in Chapter 3, extraction of Cephalexin in its anionic form can be done by the ion-exchange mechanism with a quaternary amine, such as Aliquat 336. As solutions involved with the enzymatic synthesis of Cephalexin will contain other amino acids, their co-extraction must also be considered. The structures of Cephalexin and other components that may be present in the enzymatic synthesis of Cephalexin are displayed in Table 1 (Schroën et al., 2002a; Vieira et al., 2003; Yamana and Tsuji, 1976). The extraction and stripping reactions of an anionic solute with the carrier Aliquat 336 (QCl) are shown in equations 4.1 and 4.2, respectively.

The specificity of Aliquat 336 has been shown to be related to the hydrophobicity of the anion (Thien et al., 1988). Because Cephalexin is one of the most hydrophobic cephalosporin antibiotics, high selectivity over 7-ADCA can be expected (Bora et al., 2000). Phenylglycine extraction with Aliquat 336 in feed solutions with a high pH has been demonstrated with a pH = 12.0 (Gonzalez-Munoz et al., 2006). Minimal phenylglycine is expected to be extracted when a target pH = 8.0 is set for the Cephalexin producing solutions. Other inorganic anions present in the feed solution from buffers are also a concern for competition with Cephalexin. Relatively low equilibrium constants for SO_4^{2-} , CO_3^{2-} , and PO_4^{3-} have been reported with Aliquat 336 when compared to

hydrophobic amino acids, suggesting that high selectivity of the target solute is possible (Hano et al., 1991).

4.2 Experimental

4.2.1 Materials

Cephalexin monohydrate of BP USP grade and D-alpha-phenylglycine were obtained from MP Biomedicals, Solon, OH. PGA was obtained from AK Scientific Inc., Mountain View, CA. L-Phenylalanine and D-2-phenylglycine methyl ester hydrochloride were obtained from Sigma-Aldrich, Milwaukee, WI. 7-Aminodesacetoxycephalosporanic acid was obtained from TCI America, Portland, OR. Citric acid, sodium citrate, sodium carbonate, sodium bicarbonate, sodium hydroxide and 2-propanol were all of ACS reagent grade and were purchased from Fisher Scientific, Pittsburgh, PA. Potassium chloride of ACS reagent grade was obtained from GFS Chemicals, Columbus, OH. Isopar L (isoparaffin hydrocarbon solvent) was purchased from ExxonMobil Chemical, Houston, TX. 1-Decanol was obtained from Sigma-Aldrich. Aliquat 336 was kindly donated by Cognis Corporation, Tucson, AZ. All chemicals were used as received.

4.2.2 Extraction Equilibrium Experiments

Studies to determine the extractive equilibrium between solutes and Aliquat 336 were conducted in a 125-ml round-bottom glass flask that contained 20 ml of feed solution and 20 ml of organic solution. Initial and equilibrium pH values were measured with a digital pH meter (Model Accumet AB15, Fisher Scientific, Pittsburgh, PA).

Mixing for 4 hours with a magnetic stirrer ensured equilibrium was obtained. After equilibrium was reached, the phases were separated with a separatory funnel. Concentrations were then measured by HPLC for multiple component solutions and UV-Vis for solutions containing a single component.

The distribution coefficient between the organic membrane solution and the feed solution was calculated by:

$$m_f = \frac{c_{QCeph}}{c_f} \quad (4.3)$$

in which c_{QCeph} was calculated from Equation 4.4, which is a mass balance of Cephalexin between the organic and aqueous phases.

$$c_{QCeph} = \frac{V_a}{V_o} (c_{fo} - c_f) \quad (4.4)$$

To quantify the selective extraction of Cephalexin over other solutes present in feed solutions, a separation factor, α , can be defined (Juang et al., 2004). For extraction experiments, we defined:

$$\alpha = \frac{[QCeph]/[QS]}{[Ceph_{(initial)}]/[S_{(initial)}]} \quad (4.5)$$

4.2.3 Stripping Equilibrium Experiments

Stripping equilibrium experiments were conducted between complexed organic solutions and aqueous strip solutions for Cephalexin, 7-ADCA, PG, and PGA. Extraction equilibrium experiments were conducted with organic solution containing 2.5 wt% Aliquat 336. As extraction of each solute varies with Aliquat 336, the feed

concentrations were varied in an attempt to achieve the same solute-Aliquat 336 concentration. Feed concentrations of 12 mM Cephalexin, 30 mM 7-ADCA, 15 mM PG and 60 mM PGA were used to obtain solute-Aliquat 336 concentrations between 7.2 – 8.7 mM. The complexed organic solution was then separated from the feed solution by a separatory funnel. The organic solution was then mixed with an aqueous stripping solution that contained 1 M KCl and 0.1 M of various buffers. Buffers were prepared as previously reported in Chapter 3 (Vilt and Ho, 2009).

4.2.4 Aliquat 336 Modification

60 mL of organic solution was mixed with 60 mL of 2 M Na₂CO₃ for 90 minutes to allow for the conversion of Aliquat 336 in the chloride form to a carbonate form. The phases were then separated by a separatory funnel.

4.2.5 Supported Liquid Membrane Experiments

SLM with strip dispersion experiments were conducted with the feed solution and strip dispersion in recycle mode. The experimental set-up is similar to that described in Chapter 3. Digitally controlled peristaltic pumps (Masterflex 7524-40 from Cole-Parmer, Vernon Hills, IL) were used to pump the aqueous feed solution on the tube side and the strip dispersion on the shell-side of a hollow-fiber module. The feed solution and strip dispersion were pumped at rates of 200 – 300 ml/min. The feed solution was pumped first with an inlet pressure on the tube side of the module of 34.5 kPa (5 psi), while the inlet pressure of the strip dispersion on the shell-side was 6.9 kPa (1 psi). This prevents the organic solution from passing through the pores and into the feed solution. The

hollow-fiber modules (Models G453 and G542) were purchased from Membrana, Charlotte, NC. Specifications for the modules are shown in Table 4.2.

500 ml feed solutions contained one-component (Cephalexin), two-components (Cephalexin and 7-ADCA) or four-components (Cephalexin, 7-ADCA, PG, and PGA). The pH of the feed solution was controlled by either a buffer or by continuous NaOH titration. This resulted in feed solutions with a pH of 8.0 – 9.5, which were constantly mixed with a magnetic stirrer. The strip dispersion was prepared by dispersing 300 ml of aqueous strip solution containing 1 M KCl in buffer solution (citrate buffer pH = 6.0) in 600 ml of organic membrane solution using an overhead mixer (Model BDC2002, Caframo, Wiarton, Ontario) at 400 RPM and a 3"-diameter three-blade impeller. It should be noted that the pH of the buffer drops to 5.2 after 1 M KCl addition, and is the typical pH of the initial stripping solution. Organic membrane solutions contained 1.25 – 5 wt% of Aliquat 336 and 0.6 – 2.5 wt% of 1-decanol in Isopar L. 1-Decanol was added to the organic membrane solution due to the poor solubility of Aliquat 336 in Isopar L and was always at one half the concentration of the carrier.

During the course of an experiment, samples from the feed solution and strip dispersion were taken at predetermined time intervals. Samples from the strip dispersion were allowed to settle for about 1 minute, before the aqueous strip solution was sampled. The pH values of the feed solution and aqueous strip solution were also measured at schedule time intervals during the experiments, except when the feed solution was constantly titrated with NaOH in which the feed solution pH was constantly measured. Concentrations were measured by UV-Vis or HPLC. The concentrations in the organic

phase were determined by mass balance. For SLM experiments, we defined the separation factor as:

$$\alpha = \frac{J_{Ceph(initial)} / J_{solute(initial)}}{[Ceph_{(initial)}] / [S_{(initial)}]} \quad (4.6)$$

After each experimental run, the hollow-fiber module was cleaned with a 50/50 mixture of 2-propanol and deionized water, followed by flushing with deionized water. The module was then dried overnight with dry filtered air.

4.2.6 HPLC Analysis

The HPLC system used was a Hewlett Packard 1100 Series consisting of a binary pump (Model G1312A) and a diode-array detector (DAD) (Model G1315A). A reverse phase Kromasil C18 (4.6 mm x 250 mm) column (Eka Chemicals) was purchased from Fisher Scientific. The particle size was 5 μm , and the pore size was 100 Å. Samples were diluted based on their estimated concentrations from previous experiments, and 20 μL were used for injection. Samples were eluted under a 50/50 gradient of methanol and 44.6 mM sodium-phosphate buffer of pH = 6.0 at a flow rate of 1 mL/min. The effluent was analyzed at wavelengths of 265 nm and 223 nm. Elution times were 2.7, 2.9, 3.2, 3.4, 3.9, and 5.0 min for 7-ADCA (265 nm), PG (223 nm), PGA (223 nm), phenylalanine (223 nm), Cephalexin (265 nm), and PGME (223 nm), respectively. Phenylalanine was an internal standard (223 nm). Samples were always run in duplicate. Concentrations were then calculated using calibration curves which were obtained from stock solutions. A sample chromatograph of a four-component mixture containing Cephalexin, 7-ADCA, PG, and PGA plus the internal standard is shown in Figure 4.1

4.2.7 UV-Vis Analysis

The concentration of a solute in one-component aqueous solutions was determined using a UV-Vis spectrophotometer (Model UV-1700, Shimadzu, Columbia, MD). It was calibrated at 262 nm, 258 nm, 214 nm, and 214 nm for Cephalexin, 7-ADCA, PG, and PGA, respectively.

4.3 Results and Discussion

4.3.1 Extra-Flow Module and Mini-Module Comparison

In Chapter 3, the recovery of Cephalexin using SLM with strip dispersion used 2.5" x 8" Liqui-Cel[®] Extra-Flow hollow-fiber modules from Membrana (Vilt and Ho, 2009). These modules are widely used as liquid-liquid contactors due to the cross-flow configuration, which minimizes liquid bypassing on the shell-side of the module. Smaller lab scale modules, which operate in a parallel flow configuration, are also commercially available. Liqui-Cel[®] Extra-Flow hollow-fiber modules and Mini-Modules[®], whose properties are listed in Table 4.2, have been used for Cephalexin extraction over a range of experimental conditions. A hollow-fiber mean velocity of 0.0122 m/s was used, which correlated to the feed solution flow rates of 300 ml/min and 200 ml/min for the Extra-flow module and Mini-module, respectively. The shell-side flow for each module was the same as the feed solution flow rate.

The resulting overall mass transfer coefficients for the two types of modules with each set of experimental conditions are shown in Table 4.3. To statistically analyze the data collected, an Analysis of Variance for a Randomized Complete Block Design can be

done (Montgomery, 2001). Using the statistical software JMP 8.0 (JMP 8.0, 2008) the response (mass transfer coefficient) was analyzed by the type of module and with the blocking factor of experimental condition. It can be concluded that at a significance level of 0.05, there is a statistical difference between the two hollow-fiber modules. It should be noted that if we choose a significance level of 0.03, the conclusion changes and no statistical difference between the modules is obtained. Possible factors that can be contributed to the calculated statistical difference in the two modules include: (1) the low number of samples, as each experiment was only run one time, and (2) the environmental conditions such as room temperature and humidity which were used for the experiments and could be expected to have daily fluctuations.

The Cephalexin concentrations in the feed and strip solutions for the first set of experimental conditions in Table 4.3 are shown in Figure 4.2. As expected, Cephalexin extraction and recovery were slower when using the smaller Mini-Module, compared to the Extra-Flow module due to the reduction of surface area, which could be accounted for by Equation 3.7. The degree of extraction and recovery for the two modules was very comparable. Extraction of 99.0% and 98.9% and recovery of 97.8% and 97.6% were obtained for the Mini-Module and the Extra-Flow module, respectively. Since results between the two modules were similar, Mini-Modules were used for the reminder of the SLM experimental data presented in this chapter.

4.3.2 Feed Buffer

The optimal conditions for the enzymatic synthesis of Cephalexin have been reported to occur in the pH range of 7.5 – 8.5 (Schroën et al., 2002a). Different buffer

solutions and constant titration with 2 M NaOH were investigated as methods to maintain the pH within the optimal range during the recovery of Cephalexin using the SLM with strip dispersion. The extraction and recovery of Cephalexin from feed solutions containing carbonate buffer, phosphate buffer, or NaOH is shown in Figure 4.3. The feed pH in all cases was maintained at 7.5 – 8.5 as intended.

Carbonate buffers with concentrations of 0.05 M and 0.10 M had the best extraction and recovery of Cephalexin, in which recovery for both cases was over 99%. A further increase in the carbonate buffer concentration to 0.20 M resulted in a reduction of extraction and recovery, with efficiencies of 97% and 93%, respectively. With a feed solution of 0.20 M carbonate buffer, an increase in the extraction of CO_3^{2-} occurs, which is indicated by the increased pH in the stripping solution as shown in Figure 4.4. The increase of pH in the stripping solution increases the distribution coefficient of Cephalexin between the organic membrane phase and the aqueous strip solution, which favors the Cephalexin-Aliquat complexation and results in a reduction in stripping efficiency. The stripping solution pH values for the 0.05 M and 0.10 M cases stay within the optimal stripping pH range of 5 – 6, where stripping is nearly complete (Vilt and Ho, 2009).

The competition of buffer anions with Cephalexin is another explanation for the reduction in extraction and recovery efficiencies that was observed for the 0.20 M carbonate buffer case. The reduction of extraction efficiency and solute flux with high concentrations of competing inorganic ions has been reported when using Aliquat 336 as a carrier (Thien et al., 1988). As the buffer concentration is increased, the competition between the buffer and Cephalexin also increases which can lead to a decrease in

Cephalexin flux. In the case of 0.20 M carbonate buffer, the flux was reduced to 2.14 g/m²h, which was a reduction of 27% compared to the 0.05 M carbonate buffer case.

The use of phosphate buffers to control the pH of the feed solution resulted in poor extraction and recovery. This can be attributed to the higher extraction of phosphate ions, compared to carbonate ions, with Aliquat 336 (Hano et al., 1991). The reduction in Cephalexin flux cannot be explained by a large increase in the stripping solution pH, as was observed with the 0.2 M carbonate buffer case as shown in Figure 4.4. The small shift in pH is difficult to explain without phosphate ion concentrations in the strip solutions, which were not analyzed in this study. Possible explanations include poor stripping of the phosphate ions from the Aliquat 336-phosphate complex or the transfer of less basic species such as HPO_4^{2-} compared to PO_4^{3-} or CO_3^{2-} .

The final approach to control the feed solution pH was continual titration with 2 M NaOH. Initial extraction and recovery of Cephalexin was high, but declined after a period of 45 minutes, and at 4 hours the recovery was at 94.3%. It is hypothesized that the slow continual addition of NaOH led to an accumulation of Aliquat 336-OH complex in the organic phase. This is supported by Figure 4.4, in which the stripping solution pH was relatively constant throughout the experiment, suggesting that OH^- ions were not transported to the stripping solution and stayed complexed with the carrier.

4.3.3 Cephalexin / 7-ADCA Separation

Cephalexin and 7-ADCA distribution coefficient data with Aliquat 336 suggest that selective Cephalexin separation can be achieved, but extraction data from mixed feed solutions have yet to be reported. Keeping an initial total solute concentration of 30 mM,

the effect of Cephalexin : 7-ADCA molar ratio in the feed solution on the 2-component mixture separation was investigated. Figure 4.5 shows the results which were obtained with the molar ratios ranging from 1:10 to 10:1. The highest separation factor resulted when the feed solution had a molar ratio of 1.25:1, which contained 16.7 mM Cephalexin and 13.3 mM 7-ADCA.

It was recently reported by Bora et al. that 7-ADCA had more efficient stripping compared to Cephalexin with Aliquat 336 complexes when using chloride free stripping solutions (Bora et al., 2008). As stripping studies with high chloride concentrations have yet to be reported, stripping experiments for 7-ADCA and Cephalexin in 1 M KCl solutions were conducted. The percentages of stripping for Cephalexin and 7-ADCA over a range of pH values are presented in Table 4.4. As shown in this table, 7-ADCA stripping with 1 M KCl solutions was more efficient compared to Cephalexin over the range of pH values tested. These findings are similar to the previous studies that used only buffered solutions for stripping of Cephalexin and 7-ADCA, which suggest that efficient stripping without large chloride concentrations is possible (Bora et al., 2008). However, based on our previous SLM experiments with Cephalexin, it was demonstrated that a high chloride concentration is indeed needed for efficient recovery (Vilt and Ho, 2009).

The separation of Cephalexin from 7-ADCA-containing feed solutions was carried out on lab scale SLM experiments. Table 4.5 summarizes the results from experiments that used various feed conditions to control the feed solution pH. The feed solution was maintained at a pH = 8.0 using carbonate buffer, phosphate buffer, or NaOH titration, and the resulting separation factors for Cephalexin vs. 7-ADCA were 5.5, 4.8,

and 4.7, respectively. An increase in the separation factor was observed when a feed solution pH = 9.5 containing carbonate buffers was used. The enhanced separation can be attributed to the increase of the distribution coefficient of Cephalexin between the organic membrane phase and the aqueous feed solution, which has a maximum at this pH (Vilt and Ho, 2009).

The optimal separation between Cephalexin and 7-ADCA occurred with a feed carbonate buffer concentration of 0.2 M, in which a separation factor of 12.1 was obtained. Concentration profiles for Cephalexin and 7-ADCA in the feed and stripping solutions are shown in Figure 4.6 for the feed solution containing 0.2 M carbonate buffer. Buffer concentrations lower than 0.2 M were not able to maintain the feed pH at the initial value due to co-extraction of the carbonate ions from the feed solution. In the case of the 0.1 M carbonate buffer at pH = 9.5, the feed solution pH was reduced to 8.0 after a period of 1 hour, whereas the feed pH was only reduced to pH = 9.0 with a carbonate buffer concentration of 0.2 M after the same time. A large excess of carbonate ions, as in the case of 0.4 M carbonate buffer, was able to maintain the feed solution pH at a constant value, but it also enhanced the co-extraction of buffer ions, resulting in the reduction of Cephalexin extraction. As previously discussed in Section 4.3.2, the competition of buffer ions can explain why the Cephalexin flux decreases with increasing carbonate buffer concentration. The flux dropped 14% and 29% when the carbonate buffer concentration was increased from 0.10 M to 0.20 M and 0.40 M, respectively. The difference in optimal concentration of carbonate buffer and pH between feed solutions containing Cephalexin (Section 4.3.2) and those containing both Cephalexin and 7-ADCA shows the complexity that can occur when using non-ideal feed solutions.

Kyuchoukov et al. obtained more efficient extraction of lactic acid by using a modified extractant, by exchanging the chloride anion in Aliquat 336 with a carbonate ion (Kyuchoukov et al., 2004). A modified form of Aliquat 336 was studied for the separation of Cephalexin from 7-ADCA-containing solutions. Aliquat 336 was modified by simple anion exchange of the chloride ion with a carbonate ion as described earlier. With the modification procedure, over 90% conversion of the extractant to the modified form has been reported (Kyuchoukov et al., 2004). Equilibrium extraction results for unmodified and modified forms of Aliquat 336 are compared in Table 4.6. An increase in the separation factor resulted from the use of the modified form of Aliquat 336 at concentration of 2.5 wt%. This can be attributed to the increased pH of the feed solution, which increases the distribution coefficient of Cephalexin. Even though the separation of Cephalexin over 7-ADCA was enhanced, SLM experiments using the modified Aliquat 336 were not conducted due to the insufficient amount of this modified carrier at this time.

4.3.4 Simulated 4-Component Enzymatic Reaction Mixtures

Equilibrium experiments using Aliquat 336 were conducted with feed solutions containing four-components which are present in the enzymatic synthesis of Cephalexin. Feed solutions contained equal concentrations of Cephalexin, 7-ADCA, PG, and PGA or PGME, each at 15 mM. Table 4.7 shows the resulting separation factors for each component for the two feed solutions containing either PGA or PGME. The selective extraction of Cephalexin from PGME was relatively low, compared to that of PGA, and is difficult to explain. At the pH of the feed solution (pH = 9.5), PGME is predominantly

in the form of a neutral ester which should not take place in the extraction reaction. However, Aliquat 336 has been reported to extract neutral species which may be occurring with the extraction of PGME (Kyuchoukov et al., 2005; Yang et al., 1991). The extraction reaction that is removing the PGME can be described as:

Feed solutions containing PGA were used to simulate the enzymatic synthesis of Cephalexin for the remainder of this chapter.

Similar to Cephalexin and 7-ADCA, the stripping efficiencies of PG and PGA from Aliquat 336 complexes were investigated. Stripping efficiencies of 97% and 95% for PG and PGA were obtained, respectively, when a stripping solution containing 1 M KCl and 0.1 M citrate buffer (pH = 6.0) was used.

The enzymatic synthesis of Cephalexin can occur by the use of immobilized or free enzyme. Cephalexin synthesis carried out with free enzymes have advantages of higher 7-ADCA yield and lower hydrolysis of PGA; however, immobilized enzymes are commonly used due to the ease of product removal from the enzyme (Schroën et al. 2002a). Schroën et al. compared the immobilized enzymes Assemblase 3750 and Assemblase 7500 for Cephalexin production using 100 mM concentrations of 7-ADCA and PGA (Schroën et al. 2002a). Final concentrations in the reaction vessel were approximately 40 mM, 60 mM, 60 mM, and 10 mM for Cephalexin, 7-ADCA, PG, and PGA, respectively (Schroën et al. 2002a). We used this composition as a basis for the simulated feed solutions, which were scaled down to concentrations of 15 mM, 15 mM, 15 mM, and 3 mM for Cephalexin, 7-ADCA, PG, and PGA, respectively.

The simulated solutions were scaled down to match Cephalexin concentrations from our previous work involving the recovery of Cephalexin from single component solutions with a 1.7'' x 5.5'' hollow-fiber module (Section 4.3.1). This was used to estimate the sample dilution required for HPLC analysis and to ensure an experimental time frame that allowed for the SLM experiment and subsequent HPLC analysis to be completed in a timely manner in order to minimize Cephalexin decomposition.

Based on results from SLM experiments containing 7-ADCA and Cephalexin, initial four-component SLM experiments used a feed pH = 9.5, and the extraction and stripping results are shown in Figures 4.7 and 4.8, respectively. Cephalexin was selectively extracted from the feed solution with an initial flux of 2.3 g/m²h; however, co-extraction of PG was also very high with an initial flux of 0.60 g/m²h. The high co-extraction can be explained by analyzing the fraction of ionization of PG. With diprotic acids, the amount of ionization between the different ionization states as a function of pH can be predicted by the following equations (Harris, 2003):

$$f_{H_2A^+} = \frac{[H^+]^2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (4.8)$$

$$f_{HA} = \frac{K_1[H^+]}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (4.9)$$

$$f_{A^-} = \frac{K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \quad (4.10)$$

in which the sum of $f_{H_2A^+}$, f_{HA} , and f_{A^-} is equal to 1. Since PG is amphoteric, its ionic state is pH dependent, as calculated by Equations 4.5 – 4.7 and shown in Figure 4.9. At a pH = 9.5, 76% of PG is in an anionic state, which can easily react with Aliquat 336.

A feed solution of $\text{pH} = 8.0$, which is in the optimal range for Cephalexin enzymatic synthesis, was also used in four-component SLM experiments, and the extraction and stripping results are shown in Figures 4.10 and 4.11, respectively. Again, Cephalexin was selectively extracted from the feed solution, and an initial flux of $3.1 \text{ g/m}^2\text{h}$ was obtained. The co-extraction of PG was significantly reduced, and the separation factor was increased to 17.4, which is an improvement of 10.2 times compared to the feed solution $\text{pH} = 9.5$. The large reduction in PG co-extraction can be attributed to the reduction of the anionic form of PG, which at a $\text{pH} = 8.0$ is only 9%. Extraction of 7-ADCA and PGA were also increased compared to a feed $\text{pH} = 9.5$. However, due to the larger increase in Cephalexin extraction, the separation factors of Cephalexin vs. both solutes also increased. At feed $\text{pH} = 8.0$, separation factors of 6.3, 17.4, and 4.4 were obtained for the separation of Cephalexin from 7-ADCA, PG, and PGA, respectively.

The stripping data in Figure 4.11 shows that the aqueous strip solution had a higher concentration of PG compared to PGA. At first, this may seem unexpected as PGA had a higher extraction rate from the feed solution. However, the initial concentration of PG was 15 mM compared to only 3 mM for PGA, which can explain the low PGA concentration in the strip solution. When the results from the SLM experiment with a feed $\text{pH} = 8.0$ are compared to the batch equilibrium extraction results in Table 4.7, the SLM results have higher separation values for 7-ADCA, PG, and PGA. This can be explained by the non-equilibrium based separation process used for the SLM.

The obtained separation factors are competitive with other separation processes for Cephalexin recovery from reaction mixtures. Separation factors have ranged from 1.5 – 3.0 for crystallization by pH adjustment (Boesten et al., 1999), 1.3 – 6.0 for adsorption

(Dutta et al., 2004; Schroën et al. 2002b) and 3.2 – 8.4 for aqueous two-phase partitioning systems (Zhu et al., 2001). The most specific removal method for Cephalexin from reaction mixtures is complexation with a complexing agent. However, this method has its drawbacks as common complexing agents are environmentally toxic and also deactivate the enzyme (Kemperman et al., 2001; Schroën et al., 2002b). Non-toxic complexing agents have been identified but they still exhibit enzyme deactivation (Kemperman et al., 2001). Future work may explore the SLM in conjunction with a non-toxic complexing agent in which the complexing agent recovers Cephalexin from the SLM strip solution, avoiding deactivation of the enzyme.

4.4 Conclusions

In this chapter, it was demonstrated that SLMs with strip dispersion can be used for the extraction and recovery of Cephalexin from feed solutions that are non-ideal. Competition from feed solution buffers and other components in the enzymatic reaction mixture were investigated. Among the feed pH control methods studied, carbonate buffers with concentrations of 0.05 M and 0.10 M gave the highest extraction and recovery, in which recovery for both cases was over 99%. Optimal extraction of Cephalexin from feed solutions containing equimolar concentrations of 7-ADCA occurred at a feed solution pH = 9.5, resulting in a separation factor of 12.1. At a feed solution of pH = 8.0, which is in the optimal range for Cephalexin enzymatic synthesis with the product solution containing also 7-ADCA, PG, and PGA, separation factors of 6.3, 17.4, and 4.4 were obtained for the separation of Cephalexin from 7-ADCA, PG, and

PGA, respectively. Improved Cephalexin extraction was achieved at this feed pH when compared to a feed pH = 9.5 due to high co-extraction of PG at the latter.

Table 4.1 Components of Enzymatic Synthesis of Cephalexin.

Name	Structure	MW	pK _a Values
Cephalexin		347.4	2.5 6.9
7-ADCA		214.2	3.0 4.9
phenylglycine amide		150.2	<1.0 7.2
phenylglycine		151.2	2.0 9.0
phenylglycine methyl ester		165.3	6.9

Table 4.2 Hollow-Fiber Module Specifications.

Specification	Module	
	1.7'' x 5.5'' Mini-Module	2.5'' x 8'' Liqui Cell Module
Configuration	Parallel flow	Extra-flow
Membrane material	Polypropylene	Polypropylene
Membrane porosity	0.40	0.40
Membrane pore size (μm)	0.03	0.03
Membrane tortuosity	2.6	2.6
Number of fibers	7400	10800
Effective length (cm)	12.2	15
Inner diameter/outer Diameter (μm)	220/300	220/300
Effective surface area (m^2)	0.58	1.4

Table 4.3 Overall Mass Transfer Coefficients for Mini-Module and Extra-Flow Module at Various Experimental Conditions.

Experimental conditions	$K \times 10^5$ (cm/s)	
	Mini-Module	Extra-Flow Module
15 mM feed concentration 2.5 wt% Aliquat 336 1 M KCl, 0.1 M citrate buffer	1.45	1.44
5 mM feed concentration 2.5 wt% Aliquat 336 1 M KCl, 0.1 M citrate buffer	2.86	2.82
8 mM feed concentration 2.5 wt% Aliquat 336 1 M KCl, 0.1 M citrate buffer	2.82	2.72
15 mM feed concentration 5 wt% Aliquat 336 1 M KCl, 0.1 M citrate buffer	2.75	2.69
15 mM feed concentration 2.5 wt% Aliquat 336 0.1 M KCl, 0.1 M citrate buffer	1.15	1.03

Table 4.4 Equilibrium Stripping Values of Cephalexin and 7-ADCA over a Range of pH Values.

Initial strip solution pH	% Stripping	
	Cephalexin ^a	7-ADCA ^b
4	66	90
5	68	90
7	62	87
9	65	90

^a [*QCeph*] = 8.7 mM

^b [*Q7-ADCA*] = 8.2 Mm

Table 4.5 Results of SLM Experiments for the Separation of Cephalixin from Solutions Containing 7-ADCA with Different Methods to Control the Feed Solution pH. ^a

Feed solution	α	Cephalixin flux (g/m ² h)
pH = 8.0, 0.1 M PO ₄ buffer	4.8	0.8
pH = 8.0, NaOH	4.7	4.4
pH = 8.0, 0.1 M CO ₃ buffer	5.5	3.6
pH = 9.5, 0.1 M CO ₃ buffer	6.2	4.2
pH = 9.5, 0.2 M CO ₃ buffer	12.1	3.6
pH = 9.5, 0.4 M CO ₃ buffer	9.5	3.0
pH = 9.5, NaOH	5.1	4.5

^a Initial Cephalixin and 7-ADCA concentrations: 15 mM each; Organic membrane solution: 600 ml, 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Table 4.6 Equilibrium Extraction Results on the Comparison of Aliquat 336 and Aliquat 336-CO₃ for the Separation of Cephalexin from 7-ADCA-Containing Solutions. ^a

Extractant	α	Initial pH	Final pH
2.5% Aliquat 336	3.6	7.7	8.2
2.5% Aliquat 336-CO ₃	3.9	7.7	8.5
10% Aliquat 336	2.1	7.7	7.6
10% Aliquat 336-CO ₃	2.0	7.7	8.3

^a Initial Cephalexin and 7-ADCA concentrations in 20 ml aqueous feed solution: 15 mM each; Organic membrane solution: 20 ml.

Table 4.7 Equilibrium Extraction Results on the Separation Factors of Cephalexin from Four-Component Feed Solutions. ^a

	7-ADCA	PG	PGA	PGME
α	3.1	5.8	4.9	-
	3.6	6.2	-	3.3

^a Initial Cephalexin, 7-ADCA, PG, and PGA or PGME concentrations in 20 ml aqueous feed solution: 15 mM each; Organic membrane solution: 20 ml, 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L.

Figure 4.1 HPLC chromatograph of four-component sample.

Figure 4.2 Cephalalexin concentration in the feed (solid lines) and aqueous strip solutions (dashed lines) with different hollow-fiber module configuration and size. Initial Cephalalexin concentration: 15 mM; Organic membrane solution: 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Figure 4.3 Cephalexin concentration in the feed (solid lines) and aqueous strip solutions (dashed lines) for various methods of feed pH control. Initial Cephalexin concentration: 8 mM; Organic membrane solution: 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Figure 4.4 Stripping solution pH vs. time for various methods of feed pH control. Initial Cephalaxin concentration: 8 mM; Organic membrane solution: 2.5 wt% Aliquat 336 and 1.25 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.1 M citrate buffer (pH = 6.0).

Figure 4.5 Separation factor of Cephalixin vs. 7-ADCA as a function of varying Cephalixin : 7-ADCA molar ratio. Initial total solute concentration in the feed solution was constant at 30 mM; Organic solution: 2.5 wt% Aliquat 336, 1.25 wt % 1-decanol, in Isopar L.

Figure 4.6 Cephalixin and 7-ADCA concentrations in the feed (solid lines) and aqueous strip solutions (dashed lines) with a feed solution of 0.2 M carbonate buffer. Initial Cephalixin and 7-ADCA concentrations: 15 mM each; Organic membrane solution: 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Figure 4.7 Extraction results from four-component feed solution with an initial pH = 9.5. Initial concentrations: 15 mM Cephalexin, 15 mM 7-ADCA, 15 mM PG, and 3 mM PGA in 0.2 M carbonate buffer; Organic membrane solution: 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Figure 4.8 Stripping results from four-component feed solution with an initial pH = 9.5. Initial concentrations: 15 mM Cephalexin, 15 mM 7-ADCA , 15 mM PG, and 3 mM PGA in 0.2 M carbonate buffer; Organic membrane solution: 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Figure 4.9 Fraction of ionization of phenylglycine.

Figure 4.10 Extraction results from four-component feed solution with an initial pH = 8.0. Initial concentrations: 15 mM Cephalexin, 15 mM 7-ADCA, 15 mM PG, and 3 mM PGA in 0.05 M carbonate buffer; Organic membrane solution: 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Figure 4.11 Stripping results from four-component feed solution with an initial pH = 8.0. Initial concentrations: 15 mM Cephalalexin, 15 mM 7-ADCA, 15 mM PG, and 3 mM PGA in 0.05 M carbonate buffer; Organic membrane solution: 5 wt% Aliquat 336 and 2.5 wt% 1-decanol in Isopar L; Aqueous strip solution: 300 ml, 1 M KCl and 0.5 M citrate buffer (pH = 6.0).

Chapter 5: *In Situ* Removal of Cephalexin by SLM

5.1 Introduction

The enzyme, penicillin G acylase, is used for the enzymatic synthesis of Cephalexin. The reaction scheme for Cephalexin production using 7-ADCA and PGA as initial reagents is shown in Figure 5.1. PGA first binds to the enzyme, forming an enzyme-acyl complex. This complex is then reacted with 7-ADCA to form Cephalexin or is hydrolyzed to form the side product PG. Cephalexin can also bind to the enzyme and reform the enzyme-acyl complex ($E - PG$). The enzyme is most commonly produced from *Escherichia coli* and can be used in either immobilized or liquid (free) forms for enzymatic synthesis of Cephalexin. The enzyme can be immobilized on solid supports such as polymeric resins, biopolymers or inorganic particles (Kallenberg et al., 2005). The immobilized enzyme is typically used as it can be easily removed from the reaction mixture by simple filtration. Although the liquid enzyme has achieved higher yield and a reduction in side-product formation (Schroën et al., 2002a), its use in Cephalexin synthesis has been limited due to its difficult separation from the reaction mixture.

It has been suggested if instantaneous and irreversible *in situ* product removal is achieved, Cephalexin yield would increase and side-product formation will be reduced (Hernandez-Justiz et al., 1998). The yield of Cephalexin increases as a result of shifting

the reaction equilibrium. *In situ* removal has been widely utilized in applications such as ethanol fermentation (Kim et al., 1999; Schügerl, 2000), but studies for the *in situ* removal of Cephalexin have been limited.

Using PGME and 7-ADCA as initial substrates and penicillin G acylase immobilized on glyoxyl agarose, *in situ* removal of Cephalexin using two-phase partitioning was demonstrated (Hernandez-Justiz et al., 1998). The two-phase system, had a polyethylene glycol (MW = 600) top phase and a 3 M ammonium sulfate bottom phase. Enzymatic synthesis with the two-phase system had an overall yield of 72% compared to 31% for a one-phase system, when 100 mM concentrations of both substrates were used. The use of solid polymeric adsorbents has also been investigated for the *in situ* removal of Cephalexin (Schroën et al., 2002b). Ten materials were screened for their adsorption of Cephalexin compared to 7-ADCA, and three were selected for *in situ* synthesis experiments with immobilized penicillin G acylase and 100 mM concentrations of 7-ADCA and PGA. Cephalexin synthesis in the presence of Amberlite XAD-16 and NH₂-functionalized polystyrene-co-divinylbenzene was very poor due to 7-ADCA and enzyme binding to the adsorbents. Synthesis with Amberlite IRA-400 was successful, however, the Cephalexin yield was about 14% less than the control case with no adsorbent, due to binding of 7-ADCA.

The application of complexing agents for *in situ* removal of Cephalexin from enzymatic reaction mixtures has also been reported. The complexing agent, β -naphthol, was used for the *in situ* removal of Cephalexin from a reaction mixture of 7-ADCA and PGME at both low and high substrate concentrations (Li et al., 2008). At low substrate concentrations the Cephalexin yield was increased from 61% to 88% when the

complexing agent was present in the reaction mixture. At high substrate concentrations the Cephalexin yield was increased from 81% to 98% with the addition of β -naphthol. Schroën et al. studied β -naphthol, 1,5-dihydroxy-naphthalene, 2,3-dihydroxy-naphthalene, and 2,7-dihydroxy-naphthalene, which are all efficient complexing agents, for the *in situ* removal of Cephalexin from PGA and 7-ADCA solutions with both free and immobilized forms of penicillin G acylase (Schroën et al., 2002b). Results from simple Cephalexin complexation experiments did not predict which complexing agent was best suited for *in situ* removal, as β -naphthol had the highest yield during *in situ* synthesis but did not have the highest complexation. The presence of β -naphthol decreased the formation of side product, PG, and increased the Cephalexin yield to 66%, compared to 42% for the control case with no complexing agent.

The results with complexing agents for *in situ* removal are promising; however, there is a drawback of high enzyme deactivation from the complexing agents. For example, β -naphthol immediately reduces the enzyme activity to 28%, and after a period of one day the enzyme activity is reduced to 2% (Schroën et al., 2002b). This high degree of enzyme deactivation requires large increases in enzyme concentration to achieve the same enzyme activity that is observed without the complexing agent. One complexing agent, 1,5-dihydroxy-naphthalene, has shown no enzyme deactivation when an immobilized form is used; however, the liquid enzyme suffered approximately 50% deactivation.

An ideal separation process for *in situ* removal of Cephalexin from enzymatic reaction mixtures would be able to selectively remove Cephalexin, not exhibit enzyme deactivation, and provide separation of the product from the enzyme. *In situ* extraction by

SLMs is a process that can be used for efficient removal of Cephalexin from enzymatic reaction mixtures and is demonstrated for the first time in this chapter.

5.2 Experimental

5.2.1 Materials

Cephalexin monohydrate of BP USP grade and D-alpha-phenylglycine were obtained from MP Biomedicals, Solon, OH. PGA was obtained from AK Scientific Inc., Mountain View, CA. L-Phenylalanine was obtained from Sigma-Adlrich, Milwaukee, WI. 7-ADCA was obtained from TCI America, Portland, OR. Citric acid, sodium citrate, sodium carbonate, sodium bicarbonate, and hydrochloric acid were all of ACS reagent grade and were purchased from Fisher Scientific, Pittsburgh, PA. Potassium chloride of ACS reagent grade and anhydrous dibasic sodium phosphate were obtained from GFS Chemicals, Columbus, OH. Monobasic sodium phosphate monohydrate was obtained from Mallinckrodt Baker, Philipsburg, NJ. Isopar L (isoparaffin hydrocarbon solvent) was purchased from ExxonMobil Chemical, Houston, TX. 1-Decanol was obtained from Sigma-Adlrich. Aliquat 336 (trioctylmethylammonium chloride) was kindly donated by Cognis Corporation, Tucson, AZ. Ethanol of USP grade was obtained from Decon Labs, King of Prussia, PA. Methanol and 2-propanol of HPLC grade were obtained from Fisher Scientific.

The enzyme, Assemblase liquid (penicillin G acylase), was kindly donated by DSM Anti-Infectives B. V., Delft, The Netherlands. The activity of the enzyme was unknown. β -Naphthol was obtained from MP Biomedicals. 2-Phenylphenol and methyl

2-aminobenzoate were from Sigma-Aldrich. All chemicals were used as received, excluding β -naphthol which was ground with a mortar and pestle before use.

5.2.2 Cephalixin Synthesis

Cephalixin synthesis was carried out in a temperature-controlled vessel at 20°C. The initial pH of the solution was 8.0 and was maintained by manual addition of 4 M HCl. 50 ml of solution containing PGA and 7-ADCA, both at 50 mM was first brought to the desired temperature and pH. The enzyme was then added at 0.25 – 1.0% v/v. Samples were then taken every 10 minutes and analyzed by HPLC. Due to the delay in HPLC analysis and the time of sampling, an enzyme deactivation procedure was used to stop the enzymatic reaction. Because the enzyme is deactivated at low pH, addition of 20 μ l of sample to 0.25 ml of 1 M HCl was used for deactivation. After a period of 1 minute, the solution was neutralized by adding 2.4 ml of 0.1 M NaOH. Final dilution was achieved by adding 0.33 ml of deionized water, resulting in a dilution factor of 150.

The synthesis of Cephalixin can be evaluated in terms of the maximum molar conversion yield, Y (%), which is commonly based on 7-ADCA as it is the more expensive of the two initial substrates:

$$Y = \frac{[Ceph]_{max}}{[7-ADCA]_{initial}} \times 100 \quad (5.1)$$

Volumetric productivity, P (mM/hr), can be defined as the amount of Cephalixin produced per unit of time and unit of volume at the time of maximum yield:

$$P = \frac{[Ceph]_{max}}{t_{max}} \quad (5.2)$$

5.2.3 In Situ Removal by SLM

In situ extraction experiments by SLM with strip dispersion experiments were conducted with the feed solution and strip dispersion in recycle mode. The SLM experimental set-up was similar to that described in Chapter 3. 500 ml feed solutions initially contained 50 mM 7-ADCA and 50 mM PGA. The enzyme was added at a concentration of 0.25 – 0.50% v/v. The pH of the feed solution was controlled by continuous NaOH titration. The strip dispersion was prepared by dispersing 300 ml of aqueous strip solution containing 1 M KCl in buffer solution (citrate buffer pH = 6.0) in 600 ml of organic membrane solution using an overhead mixer (Model BDC2002, Caframo, Warton, Ontario) at 400 RPM and a 3"-diameter three-blade impeller. Organic membrane solutions contained 5 – 10 wt% of Aliquat 336 and 2.5– 5.0 wt% of 1-decanol in Isopar L.

During the course of an experiment, samples from the feed solution and strip dispersion were taken at predetermined time intervals. Samples from the strip dispersion were allowed to settle for about 1 minute before the aqueous strip solution was sampled. Samples were prepared using the enzyme deactivation procedure described in 5.2.2. The pH values of the feed solution and aqueous strip solution were also measured at schedule time intervals during the experiments. Concentrations were measured by HPLC. The concentrations in the organic phase were determined by mass balance.

After each experimental run, the hollow-fiber module was cleaned with a 50/50 mixture of 2-propanol and deionized water, followed by flushing with deionized water. The module was then dried overnight with dry filtered air.

5.2.4 Enzyme Stability

The amount of hydrolysis of PGA over time was used to measure the activity of the enzyme. Enzyme activity testing was carried out in a temperature-controlled vessel at 20°C. An enzyme concentration of 0.10% v/v was added to 50 mL of 50 mM PGA solution composed of 0.1 M sodium phosphate buffer at a pH = 8.0. The feed pH was maintained at 8.0 by manual addition of 1 M HCl. The hydrolysis of PGA to PG was monitored by sampling the solution every 10 minutes for a period of 1 hour. In testing the enzyme stability, 0.10% of enzyme was added to 50 mL of phosphate buffer and was stored at 20°C, 5°C, or mixed with 5 mL of 5 wt% Aliquat 336 organic solution at 20°C for a period of 4 hours. PGA (0.0025 mols) was then added to the enzyme solution. In the case of mixing with Aliquat 336 organic solution, the buffer solution was separated from the organic solution with a separatory funnel before the PGA was added. Samples were taken every 10 minutes to measure the degree of hydrolysis, which was compared to the control. Samples used the enzyme deactivation procedure described in 5.2.2.

5.2.5 Complexation Experiments

50 mL solutions of 30 mM Cephalexin were used for complexation experiments. The solutions were composed of phosphate or citrate buffers to simulate aqueous strip solutions in SLM experiments. Complexing agents (3 – 9 mmol) were added to the Cephalexin solution and mixed for 90 minutes with a magnetic stirrer at either 5°C or room temperature. Some experiments used 2 mL of methanol as a co-solvent for the complexing agent. The solutions were then filtered and analyzed by HPLC.

In order to demonstrate that pure Cephalexin can be recovered, it can be purified from the complexing agent-Cephalexin complex. The complex was dissolved in a

mixture of water and ethyl acetate. The pH was adjusted to 1.5, at which the complex readily dissolves. The aqueous phase was then separated with a separatory funnel and washed twice with ethyl acetate. The pH of the aqueous phase was then adjusted to the isoelectric point of 4.7 with 4 M NaOH, while simultaneously seeded the solution with Cephalexin monohydrate and cooling to 5°C. The precipitated material was recovered by filtration and washed with a 50/50 mixture of 2-propanol and water. The recovered solid was then dried over night in a vacuum oven at room temperature. The solid was characterized by Fourier transform infrared (FTIR) spectrometry, using a Nexus 470 FTIR spectrometer (Thermo Nicolet, Madison, WI), using Smart MIRacle Single Reflection Horizontal ATR (attenuated total reflectance).

5.2.6 HPLC Analysis

The HPLC system used was a Hewlett Packard 1100 Series consisting of a binary pump (Model G1312A) and a diode-array detector (DAD) (Model G1315A). A reverse phase Kromasil C18 (4.6 mm x 250 mm) column (Eka Chemicals) was purchased from Fisher Scientific. The particle size was 5 μm , and the pore size was 100 Å. Samples were diluted and 20 μL were used for injection. Samples were eluted under a 50/50 gradient of methanol and 44.6 mM sodium-phosphate buffer of pH = 6.0 at a flow rate of 1 mL/min. The effluent was analyzed at wavelengths of 265 nm and 223 nm. Elution times were 2.7, 2.9, 3.2, 3.4, and 3.9 for 7-ADCA (265 nm), PG (223 nm), PGA (223 nm), phenylalanine (223 nm), and Cephalexin (265 nm), respectively. Phenylalanine was an internal standard (223 nm). Samples were always run in duplicate. Concentrations were then calculated using calibration curves which were obtained from stock solutions.

5.3 Results and Discussion

5.3.1 *Cephalexin Synthesis*

Experiments were performed with free penicillin G acylase, 7-ADCA, and PGA as initial reagents, to demonstrate the enzymatic synthesis of Cephalexin. Initially 50 mM solutions of 7-ADCA and PGA were used in experiments that had enzyme concentrations of 0.25, 0.50, and 1.00% v/v. Figures 5.2 – 5.4 show the reagent concentrations over time for the three different enzyme concentrations. As expected, an increase in enzyme concentration increased the volumetric productivity, as the maximum Cephalexin concentration was observed at earlier times. The maximum overall yield slightly declined with increasing enzyme concentration, and yields of 32.6, 29.4, and 29.1% for enzyme concentrations of 0.25, 0.50, and 1.00% v/v were observed, respectively. It has been reported that increasing initial PGA concentrations results in increased Cephalexin yields (Schroën et al., 2001). The initial PGA concentration was raised to 100 mM, while keeping the 7-ADCA concentration at 50 mM and experiments were tested at the same enzyme concentrations as the previous synthesis experiments. As expected, an increase in yield was observed compared to the experiments with the lower PGA concentration as indicated in Figures 5.5 – 5.7. The maximum overall yields were 57.3, 53.5, and 54.3% for enzyme concentrations of 0.25, 0.50, and 1.00% v/v, respectively. The yields observed are slightly larger compared to literature, in which the immobilized form of enzyme was used (Schroën et al., 2001).

5.3.2 *In Situ Extraction*

The use of a SLM with strip dispersion process for the *in situ* removal and recovery of Cephalexin from reaction mixtures has been demonstrated. Results from a 5 wt% Aliquat 336 organic membrane solution for a 0.50% v/v enzyme concentration are shown in Figure 5.8. The Cephalexin synthesis and the extraction were started simultaneously. The extraction had a maximum Cephalexin yield of 30.2%, which occurred at 50 minutes. Compared to the synthesis without *in situ* removal of Cephalexin, which had a yield of 29.4%, the benefits of *in situ* extraction were minimal. The low increase in yield can be attributed to the slow removal rate of Cephalexin and the co-extraction of substrates. During early times of the experimental run, there was very little Cephalexin that was synthesized, and as a result a large degree of co-extraction of 7-ADCA was observed.

In an attempt to increase the removal rate of Cephalexin and reduce the amount of co-extraction, modifications were made to the experimental procedure. *In situ* extraction with an increased carrier concentration of 10 wt% Aliquat 336 was employed. Also, a time lag of 20 minutes between the start of the synthesis and the start of the extraction was used. This allowed for Cephalexin to be present when the extraction began and to reduce the co-extraction of 7-ADCA that occurred during early times of the previous *in situ* extraction experiment. The experimental results with the higher carrier concentration and time lag are shown in Figure 5.9. The maximum yield of 31.5% was observed at 60 minutes, which like the previous experiment was a minimal increase from the control. However, the implantation of the time lag, did reduce the co-extraction of 7-ADCA.

A lower enzyme concentration of 0.25% v/v and the use of two hollow-fiber modules in series was conducted and the results are shown in Figure 5.10. The yield was

increased to 42.3%, which is an increase of 10% from normal enzymatic synthesis. Additional experiments with reduced enzyme concentration and increased hollow-fiber surface area are expected to further match the rate of Cephalexin synthesis and removal, possibly leading to a further increase in Cephalexin yield.

5.3.3 Enzyme Stability

Table 5.1 presents the results of residual activity of penicillin G acylase after storage in phosphate buffer solutions at 20°C, 5°C, and mixed with 5 wt% Aliquat 336 organic solution at 20°C. When the enzyme was stored in phosphate buffer at a pH = 8.0 a reduction in enzyme activity was observed. This was observed because the maximal stability of the enzyme occurs at pH = 6.0, which is near its isoelectric point (Grinberg et al., 2008). When the enzyme was stored at 20°C, a further increase in enzyme inactivation was observed compared to 5°C due to an increase in the inactivation rate constant (Guranda et al., 2004). The addition of organic solution slightly lowered the enzyme activity compared to similar storage conditions in the absence of organic solution. Further investigation into the mechanism of enzyme deactivation by the organic solution is required. Possible causes for the lower enzymatic activity by organic solutions have been identified (Klibanov, 1997).

5.3.4 Complexation of Cephalexin from Strip Solutions

Complexation of Cephalexin has been reported as a highly specific method to recover Cephalexin from enzymatic reaction mixtures. After recovery of the complex, pure Cephalexin can be obtained by a few simple steps that involve pH adjustment and

solvent extraction. However, many of the complexing agents are naphthalene derivatives and are considered toxic, which are not desired in the environmentally friendly enzymatic synthesis. Furthermore, most complexing agents significantly reduce the enzyme activity, eliminating the chance for enzyme reuse. Nontoxic alternatives, such as 2-phenylphenol and methyl 2-aminobenzoate have been suggested (Kemperman et al., 2001), but detailed studies for Cephalexin complexation have yet to be reported. 2-Phenylphenol is commonly used as an agricultural fungicide and disinfectant while methyl 2-aminobenzoate is used in food flavoring and perfumes. In this work, complexing agents for recovery of Cephalexin from aqueous strip solutions are reported. The combination of complexation and SLM with strip dispersion can provide the following advantages:

- prevent enzyme deactivation as complexation occurs in the strip solution where the enzyme is not present
- the use of a continuous process
- the handling of solids in a separate downstream processing step.

Tables 5.2, 5.3, and 5.4 present the degrees of Cephalexin complexation for β -naphthol, 2-phenylphenol, and methyl 2-aminobenzoate, respectively. Experimental parameters such as pH, temperature, chloride concentration, concentration of complexing agent, and addition of a co-solvent were investigated. Complexation was done in strip solutions containing either phosphate (pH = 7.0) or citrate buffer (pH = 5.6) to study the effect of pH. Complexation was higher for all three complexing agents at a lower pH, and was significantly higher for methyl 2-aminobenzoate, which increased from 6% to 63%. The increase in the degree of complexation resulted because Cephalexin is closer to its isoelectric point at a lower pH, which enhances crystallization.

Stripping solutions in the SLM process require a large excess of chloride ions for efficient stripping, so the effect of chloride concentration on complexation was studied. The addition of 1 M chloride resulted in reduced Cephalexin complexation for all the complexing agents, but the effect was minimal for β -naphthol and methyl 2-aminobenzoate. The complexation of Cephalexin with 2-phenylphenol was severely reduced, from 84% to 48%. The large drop in complexation with 2-phenylphenol can be attributed to the formation of 2-phenylphenol potassium salt.

The concentration of complexing agent was varied to determine the effect on Cephalexin complexation. Although increasing the concentration of complexing agent had no effect on the degree of complexation for β -naphthol and methyl 2-aminobenzoate, the degree of complexation increased with increasing 2-phenylphenol concentration. The addition of a hydrophilic solvent, such as methanol, ethanol or acetone has been recommended to increase the aqueous solubility of the complexing agent (Kemperman et al., 1999). Therefore, the effect of adding methanol to the complexing agents was investigated, which resulted in increased complexation with 2-phenylphenol attributable to the low solubility of 2-phenylphenol in water.

The last parameter that was investigated for the complexation of Cephalexin was temperature. Complexation at 5°C reduced the complexation with β -naphthol and 2-phenylphenol, but increased that with methyl 2-aminobenzoate. The reduction in temperature results in competing factors that influence the degree of complexation. At lower temperatures the Cephalexin and complexing agents have reduced solubilities, which enhance and inhibit complexation, respectively. It is hypothesized that the reduced degree of complexation with β -naphthol and 2-phenylphenol, as compared to methyl 2-

aminobenozate, was due to their reduced aqueous solubility at low temperature. As methyl 2-aminobenozate is a liquid, the reduction in solubility might have been small, and the increased complexation resulted due to the reduced solubility of Cephalexin in aqueous solution, which enhanced complexation.

Complexation with β -naphthol from a four-component stripping solution containing 30 mM Cephalexin, 10 mM 7-ADCA, 2 mM PGA, and 5 mM PG was conducted. This composition was based on strip solution data from four-component SLM experiments (Section 4.3.4). After complexation from the four-component mixture, the recovered complex was purified as described in Section 5.2.5 and a yellowish white substance was isolated. The purified substance matched the FT-IR spectra of pure Cephalexin monohydrate as shown in Figure 5.11.

The following are key observations from the complexation results presented:

- 2-phenylphenol has high complexation with addition of co-solvent or high concentration of complexing agent
- methyl 2-aminobenozate complexation was increased at low temperature
- β -naphthol and 2-phenylphenol complexation was decreased at low temperature
- complexation is specific to Cephalexin in multiple component mixtures.

5.4 Conclusions

In situ removal of Cephalexin by SLMs with strip dispersion has been achieved. The maximum yield of Cephalexin was increased from 32% to 42% when *in situ* removal was conducted using two hollow-fiber modules and an organic membrane solution

containing 10 wt% Aliquat 336. Enzyme stability experiments were performed and showed that the organic membrane solution did not significantly deactivate the liquid enzyme. The use of complexing agents for final recovery of Cephalexin from the aqueous stripping solution was investigated, and the combination of using the SLM process with complexation from the strip solution allowed for the recovery of Cephalexin without deactivation of the enzyme. The recovered substance matched the FT-IR spectra of pure Cephalexin monohydrate.

Table 5.1 Enzyme Stability Results.

Condition	Relative enzyme activity
Control T = 20°C	100%
Stored 4 hours T = 5°C	95.5%
Stored 4 hours T = 20°C	91.9 %
Mixed 4 hours with organic solution T = 20°C	87.2 %

Table 5.2 Complexation of Cephalexin with β -Naphthol. ^a

Condition	Complex ratio	[Ceph] _f	% Complexation
PO ₄ buffer, 0 M KCl, pH = 7.0	2:1	6.1	79
Citrate buffer, 0 M KCl, pH = 5.6	2:1	3.4	89
Citrate buffer, 1 M KCl, pH = 5.0	2:1	4.5	85
Citrate buffer, 1 M KCl, pH = 5.0	4:1	3.3	88
Citrate buffer, 1 M KCl, pH = 5.0	6:1	4.2	86
Citrate buffer, 1 M KCl, pH= 5.0, MeOH addition	2:1	3.8	87
Citrate buffer, 1 M KCl, pH = 5.0, T = 5°C	2:1	13.9	53

^a aqueous solubility = 0.74 g/L

Table 5.3 Complexation of Cephalexin with 2-Phenylphenol. ^a

Condition	Complex ratio	[Ceph] _f	% Complexation
PO ₄ buffer, 0 M KCl, pH = 7.0	2:1	7.6	74
Citrate buffer, 0 M KCl, pH = 5.6	2:1	4.9	84
Citrate buffer, 1 M KCl, pH = 5.0	2:1	15.6	48
Citrate buffer, 1 M KCl, pH = 5.0	4:1	9.7	68
Citrate buffer, 1 M KCl, pH = 5.0	6:1	4.3	86
Citrate buffer, 1 M KCl, ph = 5.0, MeOH addition	2:1	4.1	86
Citrate buffer, 1 M KCl, pH = 5.0, T = 5°C	2:1	21.1	29

^a aqueous solubility = 0.17 g/L

Table 5.4 Complexation of Cephalexin with Methyl 2-Aminobenzoate. ^a

Condition	Complex ratio	[Ceph] _f	% Complexation
PO ₄ buffer, 0 M KCl, pH = 7.0	2:1	27.7	6
Citrate buffer, 0 M KCl, pH = 5.6	2:1	11.2	63
Citrate buffer, 1 M KCl, pH = 5.0	2:1	12.7	57
Citrate buffer, 1 M KCl, pH = 5.0	4:1	12.8	57
Citrate buffer, 1 M KCl, pH = 5.0	6:1	13.1	56
Citrate buffer, 1 M KCl, pH = 5.0, MeOH addition	2:1	13.3	55
Citrate buffer, 1 M KCl, pH = 5.0, T = 5°C	2:1	9.5	69

^a aqueous solubility = 1.8 g/L

Figure 5.1 Reaction scheme for the enzymatic synthesis of Cephalexin. E = Enzyme, PGA = D-phenylglycine amide, E – PG = enzyme-acyl complex, 7-ADCA = 7-Amino-3-desacetoxicephalosporanic acid, PG = phenylglycine, CEPH = Cephalexin.

Figure 5.2 Enzymatic synthesis of Cephalexin with 0.25% v/v liquid enzyme, 50 mM 7-ADCA, and 50 mM PGA.

Figure 5.3 Enzymatic synthesis of Cephalexin with 0.50% v/v liquid enzyme, 50 mM 7-ADCA, and 50 mM PGA.

Figure 5.4 Enzymatic synthesis of Cephalexin with 1.00% v/v liquid enzyme, 50 mM 7-ADCA, and 50 mM PGA.

Figure 5.5 Enzymatic synthesis of Cephalexin with 0.25% v/v liquid enzyme, 50 mM 7-ADCA, and 100 mM PGA.

Figure 5.6 Enzymatic synthesis of Cephalexin with 0.50% v/v liquid enzyme, 50 mM 7-ADCA, and 100 mM PGA.

Figure 5.7 Enzymatic synthesis of Cephalexin with 1.00% v/v liquid enzyme, 50 mM 7-ADCA, and 100 mM PGA.

a) Feed solution: 50 mM 7-ADCA and 50 mM PGA with 0.50% v/v enzyme.

b) Strip solution: 1 M KCl and 0.2 M citrate buffer.

Figure 5. 8 *In situ* extraction with 5 wt% Aliquat 336.

a) Feed solution: 50 mM 7-ADCA and 50 mM PGA with 0.50% v/v enzyme.

b) Strip solution: 1 M KCl and 0.2 M citrate buffer.

Figure 5.9 *In situ* extraction with 10 wt% Aliquat 336.

a) Feed solution: 50 mM 7-ADCA and 50 mM PGA with 0.25% v/v enzyme.

b) Strip solution: 1 M KCl and 0.2 M citrate buffer.

Figure 5.10 *In situ* extraction with 10 wt% Aliquat 336 using two modules in series.

Figure 5.11 FT-IR spectra of the recovered Cephalixin and the pure Cephalixin monohydrate.

Chapter 6: Conclusions and Recommendations for Future Work

6.1 Conclusions

SLM with strip dispersion has been used as a separation process for recovery of the antibiotic Cephalexin. The process is able to recover and concentrate the Cephalexin in a simple manner, while providing long-term stability, which is a requirement for industrial use. Selective transport of the solute is achieved by carrier facilitated transport, in which the carrier Aliquat 336 was used in this work.

Several experimental parameters were investigated for the recovery of Cephalexin from one-component (Cephalexin) aqueous solutions. Efficient recovery was obtained when a high chloride concentration was used in the stripping solution in combination with the use of a citrate buffer, which had a pH of 5 – 6. Analysis of the mass transfer resistances was conducted and the kinetics from the extraction reaction was found to be dominant.

The separation of Cephalexin from multi-component feed solutions containing 7-ADCA, PGA, and PG was also studied. Improved Cephalexin extraction was achieved at a feed pH = 8.0 when compared to a feed pH = 9.5 due to high co-extraction of PG at the latter. At a feed pH = 8.0 and a feed solution composition of 15 mM, 15 mM, 15 mM, and 3 mM for Cephalexin, 7-ADCA, PG, and PGA, respectively, an initial Cephalexin

flux of 3.1 g/m²h was achieved. The co-extraction of anions from different feed buffers was investigated and carbonate buffers with concentrations of 0.05 M and 0.10 M gave the highest extraction and recovery, in which recovery for both cases was over 99%.

In situ extraction has been demonstrated during the enzymatic synthesis of Cephalexin by using SLMs with strip dispersion. The *in situ* removal process resulted in an increase of 10% in the overall Cephalexin yield compared to synthesis without Cephalexin removal. Enzyme stability results show that the organic membrane solution does not significantly deactivate the enzyme. The use of complexing agents for final recovery of Cephalexin from the aqueous stripping solution was investigated, and the combination of using the SLM process with complexation from the strip solution allowed for the recovery of Cephalexin without deactivation of the enzyme. The recovered substance matched the FT-IR spectra of pure Cephalexin monohydrate.

6.2 Future Work

6.2.1 Cephalexin Kinetics with Aliquat 336

The strong contribution of the interfacial extraction reaction to the overall mass transfer resistance which was described in Chapter 3 is rarely seen in liquid membrane transport (Juang et al., 1998). Further study on the interfacial reaction kinetics on the formation of the Cephalexin-Aliquat 336 complex at the interface between the feed solution and the liquid membrane phase, including the forward reaction rate constant and the reaction equilibrium constant could be conducted.

6.2.2 Long-Term Stability Testing

SLMs with strip dispersion have been successful in many applications, as described in Chapter 2. A few studies have shown that the organic membrane loss, which at significant levels can lead to instability, is reduced with SLMs with strip dispersion compared to other liquid membrane configurations (Gu et al., 2006). However, there have been no long-term stability tests using the process, which show constant performance during continuous operation and reuse of the organic membrane solution. An experiment lasting 30 – 60 days would demonstrate that the process is truly stable and can be run continuously on an industrial scale.

6.2.3 Additional Biological Applications

As SLMs with strip dispersion has demonstrated the successful recovery of Cephalexin, other similar structured antibiotics can also be studied. Figure 6.1 presents other commercially important β -lactam antibiotics that should have high recovery rates using SLMs with strip dispersion.

The recovery of CPC from fermentation broths may also be an area of future work. CPC is an important antibiotic and the separation from deacetyl cephalosporin is difficult, but may be possible with SLMs with strip dispersion.

Butyric acid, lactic acid, citric acid, and propionic acid are several organic acids that are produced by fermentation and all have the potential to be recovered by SLMs with strip dispersion.

Cefaclor

Cephradine

Loracarbef

Figure 6.1 Antibiotics for future studies.

References Cited

- Adarkar, J. A., S. B. Sawant, J. B. Joshi, and V. G. Pangarkar. 1997. Extraction of amino acids using an immobilized liquid membrane. *Biotechnol. Prog.* 13: 493–496.
- Alguacil F. J., M. Alonso, F. A. Lopez, and A. Lopez-Delgado. 2009. Application of pseudo-emulsion based hollow fiber strip dispersion (PEHFSD) for recovery of Cr(III) from alkaline solutions. *Sep. Purif. Technol.* 66: 586–590.
- Alguacil, F. J., M. Alonso, F. A. Lopez, A. Lopez-Delgado, I. Padilla, and H. Tayibi. 2010. Pseudo-emulsion based hollow fiber with strip dispersion pertraction of iron(III) using $(\text{PJMTH}^+)_2(\text{SO}_4^{2-})$ ionic liquid as carrier. *Chem. Eng. J.* 157: 366–372
- Barber, M., U. Giesecke, A. Reichert, and W. Minas. 2004. Industrial enzymatic production of cephalosporin-based β -lactams. *Adv. Biochem. Eng. Biotechnol.* 88: 179–215.
- Baudot, A., J. Flourey, and H. E. Smorenburg. 2001. Liquid-liquid extraction of aroma compounds with hollow fiber contactor. *AIChE J.* 47: 1780–1793.
- Boesten, W. H. J., E. C. Roos, and W. J. J. van den Tweel. 1999. Process for the recovery of Cephalexin. U.S. Patent 5,874,571, Feb 23.
- Boey, S. C., M. C. Garcia del Cerro, and D. L. Pyle. 1987. Extraction of citric acid by liquid membrane extraction. *Chem. Eng. Res. Des.* 65: 218–223.
- Bora, M. M., N. N. Dutta, and K. G. Bhattacharya. 1998. Extraction of 7-aminocephalosporanic acid with secondary, tertiary, and quaternary amines. *J. Chem. Eng. Data* 43: 318–324.
- Bora, M. M., N. N. Dutta, and K. G. Bhattacharya. 2000. Extraction equilibria of cephalosporin antibiotics with Aliquat 336. *J. Chem. Eng. Data* 45: 399–403.
- Bora, M. M., S. Borthakur, P. G. Rao, and N. N. Dutta. 2008. Study on the reactive extraction and stripping kinetics of certain β -lactam antibiotics. *Chem. Eng. Process.* 47: 1–8.

- Bruggink, A., E. C. Roos, and E. de Vroom. 1998. Penicillin acylase in the industrial production of β -lactam antibiotics. *Org. Process Res. Dev.* 2: 128–133.
- Bruggink, A. and P. D. Roy. 2001. Industrial synthesis of semisynthetic antibiotics. In *Synthesis of β -lactam Antibiotics*. A. Bruggink, Ed. Dordrecht, The Netherlands: Academic Publishers. pp. 13–54.
- Bryjak, M., P. Wieczorek, P. Kafarski, and B. Lejczak. 1991. Transport of amino acids and their phosphonic acid analogs through supported liquid membranes containing macrocyclic carriers. Experimental parameters. *J. Membr. Sci.* 56: 167–180.
- Campbell, M. J., R. P. Walter, R. Singleton, and C. J. Knowles. 1994. Investigation of the stability and selectivity of phenylalanine transport across a supported liquid membrane. *J. Chem. Technol. Biotechnol.* 60: 263–273.
- Carrera, J. A., E. Bringas, M. F. San Roman, and I. Ortiz. 2009. Selective membrane alternative to the recovery of zinc from hot-dip galvanizing effluent. *J. Membr. Sci.* 326: 672–680.
- Chaudhuri, J. B. and D. L. Pyle. 1992. Emulsion liquid membrane extraction of organic acids. II. Experimental. *Chem. Eng. Sci.* 47: 49–56.
- Choi, J. W., K. S. Cho, B. K. Oh, Y. K. Kim, I. J. Youn, and W. H. Lee. 2003. Separation and concentration of L-phenylalanine using hollow fiber supported liquid membrane. *J. Ind. Eng. Chem.* 9: 294–300.
- Clark, J. D., B. Han, A. S. Bhowan, and S. R. Wickramasinghe. 2005. Amino acid resolution using supported liquid membranes. *Sep. Purif. Technol.* 42: 201–211.
- Correia, P. F. M. M. and J. M. R. de Carvalho. 2000. Recovery of 2-chlorophenol from aqueous solutions by emulsion liquid membranes: Batch experimental studies and modeling. *J. Membr. Sci.* 179 : 175–183.
- Cristau, H. J., D. Virieux, J. F. Dozol, and H. Rouquette. 1999. Selective extraction of actinides by polyphosphine polyoxides through supported liquid membrane. *J. Radioanal. Nucl. Chem.* 241 : 543–547.
- de Gyves, J. and E. Rodriguez de San Miguel. 1999. Metal ion separations by supported liquid membranes. *Ind. Eng. Chem. Res.* 38: 2182–2202.
- Di Luccio, M., B. D. Smith, T. Kida, C. P. Borges, and T. L. M. Alves. 2000. Separation of fructose from a mixture of sugars using supported liquid membranes. *J. Membr. Sci.* 174: 217–224.
- Dreher, T. M. and G. W. Stevens. 1998. Instability mechanisms of supported liquid

membranes. *Sep. Sci. Technol.* 33: 835–853.

DSM. 2004. DSMPureactives A new standard in Antibiotics. Press Release, December, 7.

Duggan, P. J. 2004. Fructose-permeable liquid membranes containing boronic acid carriers. *Aust. J. Chem.* 57: 291–299.

Duggan, P. J., T. A. Houston, M. J. Kiefel, S. M. Levonis, B. D. Smith, and M. L. Szydzik. 2008. Enhanced fructose, glucose and lactose transport promoted by a lipophilic 2-(aminomethyl)-phenylboronic acid. *Tetrahedron* 64: 7122–7126.

Dutta, N. N. and G. C. Sahoo. 2002. Perspectives in liquid membrane extraction of cephalosporin antibiotics. *Adv. Biochem. Eng. Biotechnol.* 75: 209–242.

Dutta, M., M. M. Borah, and N. N. Dutta. 2004. Adsorptive separation of β -lactam antibiotics: Technological perspectives. *Adv. Biochem. Eng. Biotechnol.* 86: 255–278.

Elander, R. P. 2003. Industrial production of β -lactam antibiotics. *Appl. Microbiol. Biotechnol.* 61: 385–392.

Faarup, P. 1977. Method of preparing a sparingly soluble complex of Cephalexin. US Patent 4,003,896, Jan. 18.

Fortunato, R., C. A. M. Afonso, J. Benavente, E. Rodriguez-Castellon, and J. G. Crespo. 2005. Stability of supported ionic liquid membranes as studied by X-ray photoelectron spectroscopy. *J. Membr. Sci.* 256: 216–223.

Fouad, E. A. and H.-J. Bart. 2008. Emulsion liquid membrane extraction of zinc by a hollow-fiber contactor. *J. Membr. Sci.* 307: 156–168

Friesen, D. T., W. C. Babcock, D. J. Brose, and A. R. Chambers. 1991. Recovery of citric acid from fermentation beer using supported-liquid membranes. *J. Membr. Sci.* 56: 127–141.

Ghosh, A. C., S. Borthakur, M. K. Roy, and N. N. Dutta. 1995. Extraction of cephalosporin C using supported liquid membrane. *Sep. Technol.* 5: 121–126.

Ghosh, A. C., R. K. Mathur, and N. N. Dutta. 1997. Extraction and purification of cephalosporin antibiotics. *Adv. Biochem. Eng. Biotechnol.* 56: 111–145.

Gonzalez-Munoz, M. J., S. Luque, J. R. Alvarez, and J. Coca. 2006. Reactive extraction of D,L- α -phenylglycine with a quaternary ammonium salt: Effect of hydroxide anions and water coextraction. *Sep. Purif. Technol.* 51: 18–23.

Grinberg, V. Y., T. V. Burova, N. V. Grinberg, T. A. Shcherbakova, D. T. Guranda, G. G. Chilov, and V. K. Švedas. Thermodynamic and kinetic stability of penicillin acylase from *Escherichia coli*. *Biochimica et Biophysica Acta* 1784: 736–746.

Gu, Z., W. S. W. Ho, and N. N. Li. 1992. Emulsion liquid membranes: Design considerations. In *Membrane Handbook*. W. S. W. Ho and K. K. Sirkar, Eds. New York, NY: Chapman and Hall. pp. 656–700.

Gu, S., Y. Yu, D. He, and M. Ma. 2006. Comparison of transport and separation of Cd(II) between strip dispersion hybrid liquid membrane (SDHLM) and supported liquid membrane (SLM) using tri-n-octylamine as carrier. *Sep. Purif. Technol.* 51: 277–284.

Gu, S., D. He, and M. Ma. 2009. Analysis of extraction of Cu (II) by strip dispersion hybrid liquid membrane (SDHLM) using PMBP as Carrier. *Solvent Extr. Ion Exch.* 27: 513–535.

Guranda, D. T., T. S. Volovik, and V. K. Švedas. pH stability of penicillin acylase from *Escherichia coli*. *Biochemistry (Moscow)* 69: 1700–1705.

Hadik, P., L.-P. Szabo, E. Nagy, and Z. Farkas. 2005. Enantioseparation of D,L-lactic acid by membrane techniques. *J. Membr. Sci.* 251: 223–232.

Hamed, J., F. Malekzadeh, and A. E. Saghafi-nia. 2004. Enhancing of erythromycin production by *Saccharopolyspora erythraea* with common and uncommon oils. *J. Ind. Microbiol. Biotechnol.* 31: 447–456.

Hano, T., T. Ohtake, M. Matsumoto, S. Ogawa, and F. Hori. 1990. Extraction of penicillin with liquid surfactant membrane. *J. Chem. Eng. Jpn.* 23: 772–775.

Hano, T., T. Ohtake, M. Matsumoto, D. Kitayama, F. Hori., and F. Nakashio. 1991. Extraction equilibria of amino acids with quaternary ammonium salt. *J. Chem. Eng. Jpn.* 24: 20–24.

Hano, T., M. Matsumoto, T. Ohtake, K. Sasaki, F. Hori, and Y. Kawano. 1992a. Application of liquid membrane technique to the recovery of fermented organic acids. *Process Metallurgy* 7B: 1887–1892.

Hano, T., M. Matsumoto, T. Ohtake, and F. Hori. 1992b. Reactive extraction of cephalosporin C. *J. Chem. Eng. Jpn.* 25: 293–297.

Harris, D. C. 2003. *Quantitative Chemical Analysis, Sixth Ed.* New York, NY: W. H. Freeman and Company. pp. 216–217.

- Hassoune, H., T. Rhlalou, and J.-F. Verchere. 2008. Studies on sugars extraction across a supported liquid membrane: Complexation site of glucose and galactose with methyl cholate. *J. Membr. Sci.* 315: 180–186.
- He, D., X. Luo, C. Yang, M. Ma, and Y. Wan. 2006. Study of transport and separation of Zn(II) by a combined supported liquid membrane/strip dispersion process containing D2EHPA in kerosene as the carrier. *Desalination* 194: 40–51.
- He, D., S. Gu, and M. Ma. 2007. Simultaneous removal and recovery of cadmium (II) and CN[−] from simulated electroplating rinse wastewater by a strip dispersion hybrid liquid membrane (SDHLM) containing double carrier. *J. Membr. Sci.* 305: 36–47.
- He, T., L. A. M. Versteeg, M. H. V. Mulder, and M. Wessling. 2004. Composite hollow fiber membranes for organic solvent-based liquid–liquid extraction. *J. Membr. Sci.* 234: 1–10.
- Hernandez-Justiz, O., R. Fernandez-Lafuente, M. Terrei, and J. M. Guisan. 1998. Use of aqueous two-phase systems for in situ extraction of water soluble antibiotics during their synthesis by enzymes immobilized on porous supports. *Biotechnol. Bioeng.* 59: 73–79.
- Ho, W. S. W. and N. N. Li. 1992. Emulsion liquid membranes: Definitions. In *Membrane Handbook*. W. S. W. Ho and K. K. Sirkar, Eds. New York, NY: Chapman and Hall. pp. 597–610.
- Ho, W. S. W., T. K. Poddar, R. Pusic, and J. Roller. 1999a. Unique membrane technology for removal/recovery of metals from wastewaters and process streams. *American Electroplaters and Surface Finishers Society/EPA Conference for Environmental Excellence*. Lake Buena Vista, FL.
- Ho, W. S. W., T. K. Poddar, R. Pusic, and J. Roller. 1999b. Unique membrane technology for removal/recovery of metals from wastewaters and process streams. In *Proceedings of the 1999 International Congress on Membranes and Membrane Processes*, Toronto, Canada.
- Ho, W. S. W., B. Wang, T. E. Neumuller, and J. Roller. 2001. Supported liquid membranes for removal and recovery of metals from waste waters and process streams. *Environ. Prog.* 20: 117–121.
- Ho, W. S. W. and T. K. Poddar. 2001. New membrane technology for removal and recovery of chromium from waste waters. *Environ. Prog.* 20: 44–52.
- Ho, W. S. W., T. K. Poddar, and T. E. Neumuller. 2002. Removal and recovery of copper and zinc by supported liquid membranes with strip dispersion. *J. Chin. Inst. Chem. Engrs.* 33: 67–76.

- Ho, W. S. W. and B. B. Wang. 2002. Strontium removal by new alkyl phenylphosphonic acids in supported liquid membranes with strip dispersion. *Ind. Eng. Chem. Res.* 41: 381–388.
- Ho, W. S. W. 2003. Removal and recovery of metals and other materials by supported liquid Membranes with strip dispersion. *Ann. N. Y. Acad. Sci.* 984: 97–122.
- Hong, S. A., H. J. Choi, and S. W. Nam. 1992. Concentration of amino acids by a liquid emulsion membrane with a cationic extractant. *J. Membr. Sci.* 70: 225–235.
- Hong, S. A. and J. W. Yang. 1994. Process development of amino acid concentration by a liquid emulsion membrane technique. *J. Membr. Sci.* 86: 181–192.
- Hossain, M. M and J. Dean. 2008. Extraction of penicillin G from aqueous solutions: Analysis of reaction equilibrium and mass transfer. *Sep. Purif. Technol.* 62: 437–443.
- Hu, S.-Y.B. and J. M. Wiencek. 1998. Emulsion-liquid-membrane extraction of copper using a hollow-fiber contactor. *AIChE J.* 44: 570–581.
- Itoh, H., M. P. Thien, T. A. Hatton, and D. I. C. Wang. 1990. A liquid emulsion membrane process for the separation of amino acids. *Biotechnol. Bioeng.* 35: 853–860.
- JMP 8.0.* 2008. SAS Institute Inc.: Cary, NC.
- Juang, R.-S. and L.-J. Chen. 1997. Transport of citric acid across a supported liquid membrane containing various salts of a tertiary amine. *J. Membr. Sci.* 123: 81–87.
- Juang, R.-S., S.-H. Lee, and R.-C. Shiau. 1997. Mass-transfer modeling of permeation of lactic acid across amine-mediated supported liquid membranes. *J. Membr. Sci.* 137: 231–239.
- Juang, R.-S., S.-H. Lee, and R.-C. Shiau. 1998. Carrier-facilitated liquid membrane extraction of penicillin G from aqueous streams. *J. Membr. Sci.* 146: 95–104.
- Juang, R.-S. and Y.-Y. Wang. 2002. Amino acid separation with D2EHPA by solvent extraction and liquid surfactant membranes. *J. Membr. Sci.* 207: 241–252.
- Juang, R.-S., H.-C. Kao, and W.-H. Wu. 2004. Analysis of liquid membrane extraction of binary Zn(II) and Cd(II) from chloride media with Aliquat 336 based on thermodynamic equilibrium models. *J. Membr. Sci.* 228: 169–177.
- Kallenberg, A. I., F. van Rantwijk, and R. A. Sheldon. 2005. Immobilization of penicillin G acylase: The key to optimum performance. *Adv. Synth. Catal.* 347: 905–926.

- Kemperman, A. J. B., D. Bargeman, T. Van Den Boomgaard, and H. Strathmann. 1996. Stability of supported liquid membranes: State of the art. *Sep. Sci. Technol.* 31: 2733–2762.
- Kemperman, A. J. B., H. H. M. Rolevink, T. Van Den Boomgaard, and H. Strathmann. 1998. Stabilization of supported liquid membranes by interfacial polymerization top layers. *J. Membr. Sci.* 138: 43–55.
- Kemperman, G. J., R. de Gelder, F. J. Dommerholt, P. C. Raemakers-Franken, A. J. H. Klunder, and B. Zwanenburg. 1999. Clathrate-type complexation of cephalosporins with β -naphthol. *Chemistry – A Eur. J.* 5: 2163–2168.
- Kemperman, G. J., R. de Gelder, F. J. Dommerholt, C. G. P. H. Schroën, R. Bosma, and B. Zwanenburg. 2001. Compexants for the clathration mediated synthesis of the antibiotic cephradine. *Green Chem.* 3: 189 – 192.
- Kertesz, R., S. Schlosser, and M. Simo. 2004. Mass-transfer characteristics of a spiral-channel SLM module in pertraction of phenylalanine. *Desalination* 163: 103–117.
- Klaassen, R., P. H. M. Feron, and A. E. Jansen. 2005. Membrane contactors in industrial applications. *Chem. Eng. Res. Des.* 83: 234–246.
- Klibanov, A. M. 1997. Why are enzymes less active in organic solvents than in water? *Trends Biotechnol.* 15: 97–101.
- Kim, J. K., E. L. Iannotti, and R. Bajpai. 1999. Extractive recovery of products from fermentation broths. *Biotechnol. Bioprocess Eng.* 4: 1–11.
- Kislik, V. S. 2010. Carrier-facilitated coupled transport through liquid membranes: general theoretical considerations and influencing parameters. In *Liquid Membranes*. V. S. Kislik, Ed. Amsterdam, The Netherlands: Elsevier. pp. 17–71.
- Kyuchoukov, G., M. Marinova, J. Albet, and J. Molinier. 2004. New method for the extraction of lactic acid by means of a modified extractant (Aliquat 336). *Ind. Eng. Chem. Res.* 43: 1179–1184.
- Kyuchoukov, G., D. Yankov, J. Albet, and J. Molinier. 2005. Mechanism of lactic acid extraction with quaternary ammonium chloride (Aliquat 336). *Ind. Eng. Chem. Res.* 44: 5733–5739.
- Lee, C. J., H. J. Yeh, W. J. Yang, and C. R. Kan. 1993. Extractive separation of penicillin G by facilitated transport via carrier supported liquid membranes. *Biotechnol. Bioeng.* 42: 527–534.

- Lee, C. J., H. J. Yeh, W. J. Yang, and C. R. Kan. 1994c. Separation of penicillin G from phenylacetic acid in a supported liquid membrane system. *Biotechnol. Bioeng.* 43: 309–313.
- Lee K. H., S. C. Lee, and W. K. Lee. 1994a. Penicillin G extraction from model media using an emulsion liquid membrane: A theoretical model of product decomposition. *J. Chem. Technol. Biot.* 59: 365–370.
- Lee K. H., S. C. Lee, and W. K. Lee. 1994b. Penicillin G extraction from model media using an emulsion liquid membrane: Determination of optimum extraction conditions. *J. Chem. Technol. Biot.* 59: 371–376.
- Lee S. C. and W. K. Lee. 1992. Extraction of penicillin G from simulated media by an emulsion liquid membrane process. *J. Chem. Technol. Biot.* 55: 251–261.
- Lee, S. C. 2004. Comparison of extraction efficiencies of penicillin G at different w/o ratios in the emulsion liquid membrane systems with dilute polymer solutions. *J. Membr. Sci.* 237: 225–232.
- Leveque, M. A. 1928. Les Lois de la transmission de Chaleur par convection. *Ann. Mines* 13: 201–299.
- Li, D., Y. Zhang, S. Cheng, Q. Gao, and D. Wei. 2008. Enhanced enzymatic production of Cephalexin at high substrate concentration *in situ* product removal by complexation. *Food Technol. Biotechnol.* 46: 461–466.
- Li, N. N. 1968. Separating hydrocarbons with liquid membranes. U.S. Patent 3,410,794, Nov. 12.
- Li, S.-J., H.-L. Chen, and L. Zhang. 2009. Recovery of fumaric acid by hollow-fiber supported liquid membrane with strip dispersion using trialkylamine carrier. *Sep. Purif. Technol.* 66: 25–34.
- Mann, J. 1999. *The Elusive Magic Bullet: The Search for the Perfect Drug*. Oxford, UK: Oxford University Press.
- Manzak, A. and O. Tutkun. 2004. Extraction of citric acid through an emulsion liquid membrane containing Aliquat 336 as carrier. *Sep.Sci. Technol.* 39: 2497–2512.
- Marchese, J., J. L. Lopez, and J. A. Quinn. 1989. Facilitated transport of benzylpenicillin through immobilized liquid membrane. *J. Chem. Tech. Biotechnol.* 46: 149–159.
- Martak, J., S. Schlosser, and S. Vlckova. 2008. Pertraction of lactic acid through supported liquid membranes containing phosphonium ionic liquid. *J. Membr. Sci.* 318: 298–310.

- Matsumoto, M., T. Ohtani, and K. Kondo. 2007. Comparison of solvent extraction and supported liquid membrane permeation using an ionic liquid for concentrating penicillin G. *J. Membr. Sci.* 289: 92–96.
- Maximini, A., H. Chmiel, H. Holdik, and N. W. Maier. 2006. Development of a supported liquid membrane process for separating enantiomers of N-protected amino acid derivatives. *J. Membr. Sci.* 276: 221–231.
- McMurray, S. H. and G. J. Griffin. 2002. Extraction of aconitic acid from mixtures of organic acids and cane molasses solutions using supported liquid membranes. *J. Chem. Technol. Biotechnol.* 77: 1262–1268.
- Miyako, E., T. Maruyama, F. Kubota, N. Kamiya, and M. Goto. 2005. Optical resolution of various amino acids using a supported liquid membrane encapsulating a surfactant-protease complex. *Langmuir* 21: 4674–4679.
- Mohapatra, P. K. and V. K. Manchanda. 2003. Liquid membrane based separations of actinides and fission products. *Indian J. Chem.* 42A: 2925–2938.
- Mok, Y. S., S. C. Lee, and W. K. Lee. 1995. Synergistic effect of surfactant on transport rate of organic acid in liquid emulsion membranes. *Sep.Sci. Technol.* 30: 399–417.
- Molinari, R., L. De Bartolo, and E. Drioli. 1992. Coupled transport of amino acids through a supported liquid membrane. I. Experimental optimization. *J. Membr. Sci.* 73: 203–215.
- Molinari, R., P. Argurio, and F. Pirillo. 2005. Comparison between stagnant sandwich and supported liquid membranes in copper (II) removal from aqueous solutions: flux, stability and model elaboration. *J. Membr. Sci.* 256: 158–168.
- Molinari, R., A. Caruso, P. Argurio, and T. Poerio. 2006. Diclofenac transport through stagnant sandwich and supported liquid membrane systems. *Ind. Eng. Chem. Res.* 45: 9115–9121.
- Montgomery, D. C. 2001. *Design and Analysis of Experiments, 5th Ed.* Hoboken, NJ: John Wiley and Sons. pp. 126–144.
- Mulder, M. 1996. *Basic Principles of Membrane Technology.* Dordrecht, The Netherlands: Kluwer Academic Publishers. pp. 340–347.
- Nakao, M., K. Takahashi, and H. Takeuchi. 1987. A method for continuous operation of supported liquid membranes. *J. Chem. Eng. Jpn.* 20: 326–328.

O'Brien, D. J. and G. E. Senske. 1989. Separation and recovery of low molecular weight organic acids by emulsion liquid membranes. *Sep. Sci. Technol.* 24: 617–628.

Ortiz, I., M. Fresnedo San Roman, S. M. Corvalan, and A. M. Eliceche. 2003. Modeling and optimization of an emulsion pertraction process for removal and concentration of Cr(VI). *Ind. Eng. Chem. Res.* 42: 5891–5899.

Ouyang, L., X. Su, D. He, Y. Chen, M. Ma, Q. Xie, and S. Yao. 2010. A study on separation and extraction of four main alkaloids in *Macleaya cordata* (Wild) R. Br. with strip dispersion hybrid liquid membrane. *J. Sep. Sci.* 33: 2026–2034.

Park, S.-W., B.-S. Choi, S.-S. Kim, and J.-W. Lee. 2006. Facilitated transport of organic acid through a supported liquid membrane with a carrier. *Desalination* 193: 304–312.

Pei, L., B. Yao, and C. Zhang. 2009. Transport of Tm(III) through dispersion supported liquid membrane containing PC-88A in kerosene as the carrier. *Sep. Purif. Technol.* 65: 220–227.

Pickering, P. J. and J. B Chaudhuri. 1997. Enantioselective extraction of (D)-phenylalanine from racemic (D/L)-phenylalanine using chiral emulsion liquid membranes. *J. Membr. Sci.* 127: 115–130.

Raghuraman, B. and J. Wiencek. 1993. Extraction with emulsion liquid membranes in a hollow-fiber contactor. *AIChE J.* 39: 1885–1889.

Reid, R. C., J. M. Prausnitz, and B. E. Poling. 1987. *The Properties of Gases and Liquids 4th ed.* New York, NY: McGraw-Hill. pp. 52–55 and 598–606.

Reisinger, H. and R. Marr. 1992. Separation of fermented lactic acid and fermented L-leucine by liquid emulsion membranes. *Bioforum* 15: 234–237.

Reisinger, H. and R. Marr. 1993. Comparison of the separation of lactic acid and L-leucine by liquid emulsion membranes. *J. Membr. Sci.* 80: 85–97.

Reschke, M. and K. Schügerl. 1984. Reactive extraction of penicillin II: Distribution coefficients and degrees of extraction. *Chem. Eng. J.* 28: B11–B20.

Rhlalou, T., M. Ferhat, M. A. Frouji, D., Langevin, M. Métayer, and J. F. Verchère. 2000. Facilitated transport of sugars by a resorcinarene through a supported liquid membrane. *J. Membr. Sci.* 168: 63–73.

Rockman, J. T., E. Kehat, and R. Lavie. 1997. Thermally enhanced extraction of citric acid through supported liquid membrane. *AIChE J.* 43: 2376–2380.

Roy, S. C., J. V. Sonawane, N. S. Rathore, A. K. Pabby, P. Janardan, R. D. Changrani, P.

- K. Dey, and S. R. Bharadwaj. 2008. Pseudo-emulsion based hollow fiber strip dispersion technique (PEHFSD): Optimization, modeling and application of PEHFSD for recovery of U(VI) from process effluent. *Sep. Sci. Technol.* 43: 3305–3332.
- Sahoo, G. C., A.C. Ghosh, and N.N. Dutta. 1997. Recovery of Cephalexin from dilute solution in a bulk liquid membrane. *Process Biochem.* 32: 265–272.
- Sahoo, G. C. and N. N. Dutta. 1998. Studies on emulsion liquid membrane extraction of Cephalexin. *J. Membr. Sci.* 145: 15–26.
- Sahoo, G. C., N. N. Dutta, and N. N. Dass. 1999. Liquid membrane extraction of Cephalosporin-C from fermentation broth. *J. Membr. Sci.* 157: 251–261.
- Schaefer, A. and M. M Hossain. 1996. Extraction of organic acids from kiwi fruit juice using a supported liquid membrane process. *Bioprocess Eng.* 16: 25–33.
- Scheper, T., Z. Likidis, K. Makryaleas, C. Nowotny, and K. Schuegerl. 1987. Three different examples of enzymatic bioconversion in liquid membrane reactors. *Enzyme Microb. Technol.* 9: 625–631.
- Schoevaart, R. and T. Kieboom. 2001. Combined catalytic reactions- nature's way. *Chemical Innovation* 31: 33–39.
- Scholler, C., J. B. Chaudhuri, and D. L Pyle. 1993. Emulsion liquid membrane extraction of lactic acid from aqueous solutions and fermentation broth. *Biotechnol. Bioeng.* 42: 50–58.
- Schroën, C. G. P. H., V.A. Nierstrasz, H. M. Moody, M. J. Hoogschagen, P. J. Kroon, R. Bosma, H. H. Beftink, A. E. M. Janssen, and J. Tramper. 2001. Modeling of the enzymatic kinetic synthesis of Cephalexin – Influence of substrate concentration and temperature. *Biotechnol. Bioeng.* 73:171–178.
- Schroën, C. G. P. H., C. B. Fretz, V. H. DeBruin, W. Berendsen, H. M. Moody, E. C. Roos, J. L. VanRoon, P. J. Kroon, M. Strubel, A. E. M. Janssen, and J. Tramper. 2002a. Modeling of the enzymatic kinetically controlled synthesis of Cephalexin, influence of diffusion limitation. *Biotechnol. Bioeng.* 80: 331–340.
- Schroën, C. G. P. H., V. A. Nierstrasz, R. Bosma, P. J. Kroon, P. S. Tjeerdsma, E. DeVroom, J. M VanderLann, H. M. Moody, H. H. Beftink, A. E. M. Janssen, and J. Tramper. 2002b. Integrated reactor concepts for the enzymatic kinetic synthesis of Cephalexin. *Biotechnol. Bioeng.* 80: 144–155.
- Schügerl, K. 2000. Integrated processing of biotechnology products. *Biotechnol. Adv.* 18: 581–599.

- Schügerl, K. 2005. Extraction of primary and secondary metabolites. *Adv. Biochem. Eng. Biotechnol.* 92: 1–48.
- Scovazzo, P., A. E. Visser, J. H. Davis, R. D. Rogers, C. A. Koval, D. L. DuBois, and R. D. Noble. 2002. Supported ionic liquid membranes and facilitated ionic liquid membranes. *ACS Symposium Series* 818: 69–87
- Seki, T., M. Okamoto, O. Hosoya, D. Aiba, K. Morimoto, and K. Juni. 2003. Effect of chondroitin sulfate on the diffusion coefficients of drugs in aqueous solutions. *S.T.P. Pharma Sciences* 13: 215–218.
- Sheldon, R. and F. van Rantwijk. 2004. Biocatalysis for sustainable organic synthesis. *Aust. J. Chem.* 57: 281–289.
- Sirkar, K. K. 1996. Hollow fiber-contained liquid membranes for separations: An overview. *ACS Symposium Series* 642: 222–238.
- Sirman, T., D. L. Pyle, and A. S. Grandison. 1990. Extraction of citric acid using a supported liquid membrane. In *Separation in Biotechnology 2*. London, UK: Elsevier.
- Smolders, G. 2002. DSM Anti-infectives ZOR-f Painting the antibiotics industry green. May, 22.
- Sonawane, J. V, A. K. Pabby, and A. M. Sastre. 2008. Pseudo-emulsion based hollow fiber strip dispersion: A novel methodology for gold recovery. *AIChE J.* 54: 453–463.
- Sonawane, J. V., A. K. Pabby, and A. M. Sastre. 2010. Pseudo-emulsion based hollow fibre strip dispersion (PEHFSD) technique for permeation of Cr(VI) using Cyanex-923 as carrier. *J. Hazard. Mater.* 174: 541–547.
- Stoica-Guzun, A., G. Juncu, and O. Floarea. 1999. Batch extraction of citric acid with liquid surfactant membranes. *Chem. Eng. Technol.* 22: 65–69.
- Tanigaki, M., M. Ueda, and W. Eguchi. 1988. Facilitated transport of zinc chloride through hollow fiber supported liquid membrane. Part 2. Membrane stability. *Sep. Sci. Technol.* 23: 1161–1169.
- Teramoto, M., Y. Sakaida, S. S. Fu, N. Ohnishi, H. Matsuyama, T. Maki, T. Fukui, and K. Arai. 2000. An attempt for the stabilization of supported liquid membrane. *Sep. Purif. Technol.* 21: 137–144.
- Thien, M. P., T. A. Hatton, and D. I. C. Wang. 1988. Separation and concentration of amino acids using liquid emulsion membranes. *Biotechnol. Bioeng.* 32: 604–615.

- Tsikakos, D., E. Kaltsidou-Schottelius, and G. Brunner. 1992. Hollow fiber-supported liquid membranes for the extraction of penicillins and the synthesis of 6-aminopenicillanic acid. *Chemie Ingenieur Technik* 64: 545–548.
- Urtiaga, A., M. J. Abellan, J. A. Irabien, and I. Ortiz. 2006. Use of membrane contactors as an efficient alternative to reduce effluent ecotoxicity. *Desalination* 191: 79–85.
- Urtiaga, A., R. Gutiérrez, and I. Ortiz. 2009. Phenol recovery from phenolic resin manufacturing: Viability of the emulsion pertraction technology. *Desalination* 245: 444–450.
- Vieira, M. F., M. Barboza, and R. de L. C. Giordano. 2003. Adsorption of components of enzymatic Synthesis of ampicillin on different hydrophobic resins. *Appl. Biochem. Biotechnol.* 108: 705–713.
- Vilt, M. E. and W. S. W. Ho. 2009. Supported liquid membranes with strip dispersion for the recovery of Cephalexin. *J. Membr. Sci.* 342: 80–87.
- Vilt, M. E., W. S. W. Ho, and N. N. Li. 2010. Liquid membranes. In *Comprehensive Membrane Science and Engineering*. E. Drioli, Ed. Amsterdam, Netherlands: Elsevier. 4: 79–107.
- Walsh, C. 2003. *Antibiotics: Actions, Origins, Resistance*. Washington DC: ASM Press.
- Wang, K. Y. and T.-S. Chung. 2006. Polybenzimidazole nanofiltration hollow fiber for Cephalexin separation. *AIChE J.* 52: 1363–1377.
- Wang, Y. C., Y. S. Thio, and F. M. Doyle. 1998. Formation of semi-permeable polyamide skin layers on the surface of supported liquid membranes. *J. Membr. Sci.* 147: 109–116.
- Wickramasinghe, S. R., M. J. Semmens, and E. L. Cussler. 1992. Mass transfer in various hollow fiber geometries. *J. Membr. Sci.* 69: 235–250.
- Wijers, M. C., M. Jin, M. Wessling, and H. Strathmann. 1998. Supported liquid membranes modification with sulfonated poly(ether ether ketone). Permeability, selectivity and stability. *J. Membr. Sci.* 147: 117–130.
- Wilke, C. R. and P. Chang. 1955. Correlation of diffusion coefficients in dilute solutions. *AIChE J.* 1: 264–270.
- Yahaya, G. O., B. J. Brisdon, and R. England. 2000. Facilitated transport of lactic acid and its ethyl ester by supported liquid membranes containing functionalized polyorganosiloxanes as carriers. *J. Membr. Sci.* 168: 187–201.

- Yamana, T. and A. Tsuji. 1976. Comparative stability of cephalosporins in aqueous solution: Kinetics and mechanisms of degradation. *J. Pharm. Sci.* 65: 1563–1573.
- Yang, S.-T., S. White, and S.-T. Hsu. 1991. Extraction of carboxylic acids with tertiary and quaternary amines: Effect of pH. *Ind. Eng. Chem. Res.* 30: 1335–1342.
- Yang, Q., T.-S. Chung, Y. Xiao, and K. Wang. 2007. The development of chemically modified P84 Co-polyimide membranes as supported liquid membrane matrix for Cu(II) removal with prolonged stability. *Chem. Eng. Sci.* 62: 1721–1729.
- Yang, Q. and T.-S. Chung. 2007. Modification of the commercial carrier in supported liquid membrane system to enhance lactic acid flux and to separate L,D-lactic acid enantiomers. *J. Membr. Sci.* 294: 127–131.
- Yang, X. J., A. G. Fane, J. Bi, and H. J. Griesser. 2000. Stabilization of supported liquid membranes by plasma polymerization surface coating. *J. Membr. Sci.* 168: 29–37.
- Yordanov, B. and L. Boyadzhiev. 2004. Pertraction of citric acid by means of emulsion liquid membranes. *J. Membr. Sci.* 238: 191–197.
- Yuanli, J., W. Fuan, K. D. Hyun, and L. M. Sook. 2001. Modeling of the permeation swelling of emulsion during lactic acid extraction by liquid surfactant membranes. *J. Membr. Sci.* 191: 215–223.
- Zhu, J. H., D. Z. Wei, X. J. Cao, Y. Q. Liu, and Z. Y. Yuan. 2001. Partitioning Behavior of Cephalixin and 7-ADCA in PEG/ammonium sulfate aqueous two-phase systems. *J. Chem. Technol. Biotechnol.* 76: 1194–1200.
- Zwanenburg, B., G. J. Kemperman, J. Zhu, R. De Gelder, F. J. Dommerholt, G. T. M. Titulaer, R. Keltjens, and A. J. H. Klunder. 2001. Molecular Precision in the chemistry of cephalosporin type antibiotics. In *Synthesis of β -lactam antibiotics*. A. Bruggink, Ed. Dordrecht, The Netherlands: Kluwer Academic Publishers. pp. 57–102.