POROUS POLYMERIC MATERIALS DERIVED FROM BICONTINUOUS MICROEMULSIONS FOR DRUG DELIVERY

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Fen Ye

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POROUS POLYMERIC MATERIALS DERIVED FROM BICONTINUOUS MICROEMULSIONS FOR DRUG DELIVERY

Fen Ye

Thesis

Approved:  

Advisor  
H. Michael Cheung

Accepted:  

Department Chair  
Lu-Kwang Ju

Committee Member  
Stephanie T. Lopina

Dean of the College  
George K. Haritos

Committee Member  
Ernst D. von Meerwall

Dean of the Graduate School  
George R. Newkome

Date
ABSTRACT

During last decades, significant progress has been made in the field of drug delivery with the development in materials synthesis. Polymer drug delivery systems can realize the prolonged release of drugs, enhance effective drug solubility, protect drug from degradation by enzymes, and reduce drug toxicity. Recently porous materials have been developed as the controlled release host of bioactive reagents, and have shown better controlled release of reagents.

Microemulsions are thermodynamically stable, isotropic and transparent dispersions of two normally immiscible fluids stabilized by surfactants and often cosurfactants. Microemulsions have been investigated in a wide range of application including enhanced oil recovery, detergents, bioreactors, drug delivery and to template polymerization. Polymerization of bicontinuous microemulsions can produce materials with defined porous structures. Various surfactants have been reported to form bicontinuous microemulsion for the polymerization of porous materials. Application of biocompatible surfactants eliminate the need for residual surfactant removal after microemulsion polymerization, and the porous polymeric materials obtained can be used in drug delivery to improve the drug diffusion and enhance the deposition of drug within body.
The objective of this study is to develop the biocompatible porous polymeric materials suitable for protein and lipids delivery using methyl methacrylate (MMA) as monomer and 2-hydroxyethyl methacrylate (HEMA) or acrylic acid (AA) as comonomer.

Four biocompatible surfactants were tested for the capacity of forming single phase microemulsion at high aqueous content. The Winsor-IV microemulsions formulated with 3:1 HEMA to MMA and surfactant of L1695, T1307 or F127 were studied for the microstructure by viscosity and conductivity measurements. Conductivity and viscosity measurements confirmed that microstructure of microemulsion was dependent on the aqueous content. With the increase of aqueous content, the structure progressed from W/O droplets, to bicontinuous networks, and finally to O/W droplets.

The structure of polymerized microemulsions formulated with various surfactants was studied by scanning electron microscopy (SEM) and the non-invasive freezing point depression (FPD) method. Under SEM, nanopores were observed from the system formulated with 3:1 HEMA to MMA and surfactant of L1695, T1307 or F127. The FPD results were consistent with SEM morphology examination, and demonstrated that the radiiuses of nanopores presented in these three systems were mostly in the range of 10-50 nm. Moreover, the nanopores had a distribution dependent on aqueous content. Micropores were observed in the SEM image of the stimuli-responsive partially neutralized 3:2 AA/MMA/10 % F77 system. The incorporation of drugs didn’t change the microstructure of polymers.
Polymers derived from microemulsions stabilized by four surfactants were applied to encapsulate drugs, and the drug release profiles were investigated. The nanoporous polymer particle suspension exhibited controlled release of Rhodamine B. The release rate was four times lower than the drug loaded to 10% F127 solution. Nanoporous monoliths derived from L1695 or T1307 stabilize microemulsions could realize the gradual release of $\beta$-galactosidase within 6 hours. The pH of precursor microemulsion stabilized by T1307 was 8, and higher than that of the L1695 stabilized microemulsion. Enzymes released from L1695 system displayed higher apparent activity since lower pH favors enzyme catalysis when pH is larger than 5. The partially neutralized system demonstrated swelling behavior dependent on the pH of aqueous medium. Lipase lost activity in pH=1.2 medium, while lipase released from pH-sensitive system in pH=6.8 buffer showed increased activity over time. The release of lipase became stable after 6 hours. These release profiles suggested that the porous systems could achieve prolonged release of drugs, and all the systems are promising for the application in drug delivery.
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CHAPTER I

INTRODUCTION

1.1 Background

Significant progresses have been made in the field of drug delivery during last decades. Drug delivery systems have evolved from conventional pills to sophisticated programmable controlled delivery systems. Materials of different forms are applied to develop novel drug delivery systems.\(^{[1-3]}\) Among these, polymer systems have been investigated extensively in modern drug delivery, since they can realize the prolonged release of drugs, enhance effective drug solubility, protect drug from degradation by enzymes, and reduce drug toxicity.\(^{[4]}\) Recently nanoporous materials have been developed as the controlled release host of bioactive reagents, and have shown better controlled release of reagents because of the high surface area for drug loading which allows for longer release of drugs.\(^{[5]}\)

Microemulsions are thermodynamically stable, isotropic and transparent liquid microstructured systems composed of both hydrophilic and hydrophobic species that are stabilized by the interfacial film of surface active agents. Due to these unique physical and chemical properties, microemulsions are investigated for a wide range of applications, such as enhanced oil recovery, detergents,
bioreactors, and template polymerization. Microemulsions of different types have also been utilized in drug delivery to incorporate hydrophilic, hydrophobic and/or amphiphilic drugs to enhance their solubility.

Polymerization of bicontinuous microemulsions can generate materials with porous structures. Different porous polymers have been produced by polymerization of bicontinuous microemulsion formulated with surfactants of various types. Application of biocompatible surfactants doesn't require surfactant separation after microemulsion polymerization, and the porous polymeric materials obtained can be used in drug delivery to improve the drug diffusion and enhance deposition of drug within body.

1.2 Surfactant

Since surfactant play a crucial role in the formation and stabilization of microemulsion, an introduction of surfactant is necessary for the better understanding of microemulsion which will be explained in the next section.

By definition, surfactants or surface active agents are materials that do not only accumulate at surfaces, but also facilitate adsorption at surfaces or interfaces at certain concentrations and temperatures. These molecules are amphiphiles with two distinct parts: hydrophilic head group, and the hydrophobic tail group. The accumulation of surfactant at interface is a dynamic process which is the synergic effect of the solubility of hydrophilic head group that tends to keep surfactant staying in water, whereas, the hydrophobic tail group which
ends to disrupt the hydrogen bonding and force surfactant to accumulate in the interface in solution.\[^7\]

1.2.1 Micelle: an Important Feature of Surfactant

A salient feature of surfactant is the self-association to form micelles to avoid the contact of hydrophobic moieties at the concentration beyond the critical micelle concentration.\[^8\] The sizes of micelles can vary from tens to thousands of monomers. With the increase of size, micelle shape can grow from spherical to rod-like one dimensional, or into two dimensional disc-like aggregates. The structure change of micelle with size is illustrated in Figure 1.1.

![Schematic Presentation of Most Occurred Surfactant Associates](image)

Figure 1.1 Schematic Presentation of Most Occurred Surfactant Associates\[^9\]
The driving force for the formation of micelle arises from the attraction of the hydrocarbon chains at the hydrocarbon-water interface, while the opposing forces are hydrophilic, steric and electrostatic repulsion between head groups. The interaction of these effects makes the self-assembly of surfactant depending on a range of factors, such as the length of alkyl tail, the salt concentration, pH, temperature and the nature of counterion.\[4\]

1.2.2 Classification and Property of Surfactant

In the world market, several thousand of surfactant and mixture are available currently. According to the charge type of head groups, surfactants are classified into the following 4 types:\[7, 10, 11\]

**Anionic surfactants**

Anionic surfactants have negatively charged head group when dissociated in water. This type of surfactants is manufactured by far in the largest quantities.\[12\] Fatty acid salts (soaps), sulfates, ether sulphonates carboxylates, and phosphates are the hydrophilic of industrial anionic surfactants. The negatively charged hydrophilic head group can influence: the electrostatic stabilization, the sensitivity of surfactant to pH, the degree of hydrolysis, and the variation of latex stability with time, electrolyte and temperature conditions.

**Cationic surfactants**

Surfactants with positively charged head group when dissociated in water are cationic surfactants. The typical cationic surfactant has amine-containing polar head groups, such as quaternary ammonium, imidazolinium or alkyl
pyridinium groups. This class of surfactants counts the 10% of total surfactant demand. The positive charge leads to the substantial application of cationic surfactants in fabric softening, hair conditioner, skin care and other surface treatments where depositions are assisted with cationic charges. However, the application of cationic surfactants in drug delivery is limited by the fact that cationic surfactants are frequently irritant and some times toxic.[4]

**Zwitterionic surfactants**

Zwitterionic surfactants are less common surfactants with both a negatively charged group and a positively charged group. Surfactants can be anionic, cationic or both depending on the composition and conditions of the medium (such as pH value). In general, the head group of zwitterionic surfactants consists of a quaternary amine group and a sulfonic or carboxylic group. They are commonly used in personal care product because of the low irritating properties.[4, 11]

**Nonionic surfactants**

Surfactants with uncharged head group when dissociated in water are nonionic surfactants. These surfactants have either polyether or polyhydroxyl, for example, glucosides, as the head group. Nonionic surfactants are the largest class of surfactants in terms of volume, and they are frequently used in drug delivery. Due to the uncharged nature of polar group, nonionic surfactants are less sensitive to salt, but quite sensitive to temperature, which may be use as a trigger of drug releasing. Usually, nonionic surfactants are less irritant and better
tolerated than anionic and cationic surfactants because of the much lower critical micellization concentration.\cite{10,13}

Nonionic surfactants are studied in the present study because of their unique nature. In Chapter 2, an elaboration will be provided regarding the property and application of three types of nonionic surfactants, i.e. Sucrose, Pluronic and Tetronic surfactant.

1.3 Microemulsions

In 1943, microemulsion was first reported by Hoar and Schulman. However the term "microemulsion" was only coined till 1958 by Schulman and coworkers to describe the single phase transparent solution of small droplet size formed after addition of alcohol. For the purpose of this work, the microemulsion is defined as 'a system of water, oil and amphiphile which is optically isotropic and thermodynamically stable liquid solution", same definition as provided by Danielsson and Lindman in 1981.\cite{9}

1.3.1 Introduction to Microemulsion

The presence of large quantities of surfactant provides microemulsions with unique physical and chemical properties, including thermodynamic stability, low kinetic barriers to formation, low viscosity and interfacial tension. Given these special properties, microemulsions have attained increasing significance both in research and in industry. \cite{14}
Microemulsion can assume various microstructures depending on the temperature, composition, molecular structure, and hydrophilicity and hydrophobicity of these components. Typically, microemulsions can be classified as oil-in-water (O/W) microemulsion, water-in-oil (W/O) microemulsion, and bicontinuous microemulsion in terms of the internal microstructure. As increase of the oil-to-water ratio, the microstructure of microemulsion progresses from oil-swollen droplets dispersed in water, to bicontinuous structures, and finally to water-swollen micelles dispersed in oil. In the intermediate region, equivalent
amounts of water and oil are present and no droplets are formed. The bicontinuous microemulsions have macroscopic domains of both water and oil which sometimes are described sponge-like.\cite{16} Since the water and oil domains are usually 10 nm in length scale, microemulsions are typically transparent. A diagrammatic representation of these three microstructures is shown in Figure 1.3. Additionally, the oil or water-swollen micelles can adopt various geometries, such as spherical, ellipsoidal, rod-like or disc-like, which make microemulsions a subject of practical interest.

Based on the phase equilibrium of microemulsions, they can be also classified into four different types. Winsor has developed a classification scheme as illustrated in Figure 1.4.\cite{17, 18} This study was concerned exclusively about the Winsor–IV system, which is the one phase microemulsion system as illustrated in Figure 1.4. At a particular composition, the entire system has uniform structure at molecular level. Due to the sponge structure, bicontinuous microemulsions are capable of generating microporous materials, and were of particular interests in this work for the potential application in drug delivery.
**Winsor-I type**

Lower phase microemulsion of oil in water droplets in equilibrium with excess oil phase at the top

**Winsor-II type**

Upper phase microemulsion of water in oil droplets in equilibrium with excess water phase at the bottom

**Winsor-III type**

Middle phase bicontinuous microemulsion in equilibrium with excess oil phase at the top and excess water phase at bottom

**Winsor-IV type**

Macroscopically single phase microemulsion made up of oil in water or water in oil or bicontinuous microemulsion

Figure 1.3 Winsor Classification of Microemulsion
1.3.2 Polymerization in Microemulsions

The concept of polymerization in microemulsion was only introduced around 1980, but this field has developed rapidly due to the interesting features of microemulsions.[19, 20] The thermodynamic stability of microemulsions ensures the duplication of microstructure under the circumstances of the same composition and temperature. The transparency of microemulsions allows photo polymerization by visible light or UV-light. Uniformly heating can be realized due to the easy heat dissipation in low viscosity of microemulsions. The great variety of microstructures and small length scale of microemulsion result in a unique microenvironment to generate polymers with interesting morphology and defined structures.[15, 21]

Due to the large interfacial surface, a large amount of surfactant is needed to stabilize microemulsion system, which is the major difference between emulsions and microemulsions. Since the high solid yield and low surfactant level are desirable for most applications, this drawback severely limits the application of microemulsions. Polymerizable surfactant is one of the possible solutions currently proposed to address this problem. A polymerizable surfactant could be used to create more rigid networks that can entrap molecules within its microstructure.[20]

Polymerization in microemulsion is achieved by the substitution of oil by monomers or addition of water soluble monomers. Over the past two decades, the free polymerizations are mainly in globular microemulsions (both O/W microemulsions and W/O microemulsions) and bicontinuous microemulsions.
Since the potential loci for polymerization in globular microemulsions are the large amount of droplets in a length scale of 5-10 nm, polymeric microlatexes with size <50 nm in diameter are easily produced.\cite{21} Polymerization in bicontinuous microemulsion is another interesting field as a result of the increasing interest in fabricating solid materials with open-cell structure, i.e., an interconnected porous structure with water channels through the polymer.

1.4 Controlled Drug Delivery

The means by which a drug is introduced into the body is almost as important as the drug itself. Conventionally, drugs are administrated in the simple forms, such as oral, topical, inhaled or injection formulations. The drawbacks of these systems include: periodic administration, nonspecific, side effects resulted from high systemic concentration level, and low efficiency.

Controlled drug delivery systems are formulations, devices, and techniques designed to deliver therapeutic agents to targeted physiology and to provide efficacious release of agents. The systems on their own are not therapies, but improve the efficacy and/or safety of the drugs they carry. The advantages of controlled release are\cite{22} (1) maintenance of optimum therapeutic drug concentration in the blood or in a cell, (2) predictable and reproducible release rates for extended periods of time, (3) enhancement of activity duration for short half-life drugs, (4) the elimination of side effects and frequent dosing, (5) better patient compliance. Over the past 30 years, substantial advancements in controlled drug delivery systems have been made. With the developments in the
synthesis of new materials, drug delivery systems are experiencing rapid advancement. Novel drug delivery systems are designed to satisfy the more complicated requirements from pharmaceutical industry.

1.4.1 Rate Control Mechanism

According to the rate control mechanism of drug delivery devices, polymeric controlled delivery systems can be classified into three categories as solvent controlled systems, diffusion controlled systems and chemically controlled systems.[23, 24]

**Solvent Controlled System**

Swelling and osmosis are the two primary solvent controlled mechanisms of drug release. Hydrogels are the most frequently used swelling controlled delivery systems. For the nondegradable hydrogels, drug release is determined by the rate of swelling which is highly dependent on the hydrophilic/hydrophobic balance of the polymeric matrix, and the degree of crosslinking. Osmosis systems are usually composed of a drug reservoir enclosed by a water selective polymeric membrane which only allows the transport of water and drug is released through the cells in membrane when the hydrostatic pressure builds up to the threshold value.

**Diffusion Controlled Systems**

The two fundamentally different diffusion controlled systems are reservoir system and matrix system. In matrix system, drug is distributed uniformly throughout polymer. While in reservoir system, drug is surrounded by polymer
film or membrane, and is released strictly by the rate of drug diffusion through polymeric membrane if polymer is not degradable. In nondegradable matrix system, constant drug release rate is usually not possible due to the decreased rate of drug diffusion as drug being released from the matrix.

**Chemically Controlled Systems**

In chemically controlled systems, the drug release rate is mainly determined by the rate of polymer degradation, the erosion of polymers, or the rate at which drug is chemically cleaved from the polymer backbones.

As the development of novel polymers, these rate controlling mechanisms usually work in a concerted way in modern drug delivery systems.

In this work, nondegradable porous polymers were synthesized for the delivery of pharmacological reagents. The systems are mainly diffusion controlled matrix systems, but there is still possibility of the erosion of the polymers in solutions.

1.4.2 Stimuli-responsive Polymeric Drug Delivery System

The constant or decreasing drug release in most drug delivery system does not always simulate the body’s natural pattern of providing chemicals.\[^{22}\] Stimuli-responsive polymers are defined as polymers that endure relative large and abrupt, physical or chemical changes in response to small external changes in environment conditions.\[^{25}\] These polymers are now widely used in drug delivery for the targeted release of drug at predetermined conditions.
The stimuli can be classified as chemical and physical. Chemical stimuli, such as pH, ionic strength and glucose concentration, will change the interactions between polymer chains and solvents at the molecular level. Temperature, electric or magnetic fields, and mechanic stress belong to physical stimuli, which affect the energy of various sources and change the molecular interaction at a critical point.

Most of the stimuli-responsive polymers focus on change of the drug release by controlling temperature and pH. The majority of these smart polymers are hydrogels. The controlled release can be achieved by the polymer swelling and deswelling at different temperature and pH. In this study, a pH-responsive porous polymer was synthesized for the purpose of protecting enzyme from the harsh gastric environment.

1.4.3 Digestive Enzyme Delivery

It is estimated that 100 million people in American have some forms of digestive disorder. The symptoms of digestive disorders vary from person to person, from irritation and discomfort that may drastically limit people’s lifestyles to frequently miss work. Sometimes, the disorders may be extremely crippling and even fatal.[26]

Digestive enzymes are proteins involved in digestion that stimulate the decomposition of other substances. Enzyme activities are highly dependent on the medium temperature and pH. According to the enzyme source, digestive enzymes can be classified as: animal-derived enzymes, plant-derived enzymes,
and fungal-derived enzymes. In the light of substrates, enzymes can be divided into three functional groups: enzymes to digest protein, enzymes to hydrolyze fat, and enzymes to break down carbohydrates. Proteinase, lipase and amylase are the characteristic representatives of these groups respectively.

In 1897, Pavlov first advanced the concept of digestive enzyme adapted in dietary. Today digestive enzymes are the dominant enzymes used to address digestive disorder in the dietary enzyme supplement industry. While the enzymes are required in biological activity universally, the market for dietary enzyme products is still relatively small. The enzyme products sale was predicted to reach $30 million by 2006. The biggest challenge faced by dietary enzyme supplement is the gastric bypass due to the denature of enzymes by stomach acid. This study investigates novel delivery systems to protect digestive enzymes by encapsulating them within a porous polymer network and to achieve a certain activity level of enzymes which is the uttermost goal in enzyme dietary.

Two important digestive enzymes studied in this work are β-galactosidase and lipase.

**β-galactosidase**

The inherent deficiency of the intestinal enzyme lactase leads to the incapability of consuming lactose, a main carbohydrate in milk and diary products. The data from the National Institute of Diabetics and Digestive and Kidney Diseases (NIDDK) showed 30–50 million American adults lose this ability to digest lactose in their diet. β-galactosidase can convert lactose into the mixture of glucose and galactose. Conventional therapy for this prevalent disorder is the
administration of lactase (β-galactosidase) which is over-the-counter (OTC) as chewable tablet. However, enzyme efficiency is very low since only a portion of the orally administrated enzyme is expected to survive the unfavorable pH and enzymatic conditions of the upper gastrointestinal tract. Encapsulation and delivery of β-galactosidase can help the cleavage of lactose, and the products generated can be absorbed by bloodstream.

**Lipase**

Lipase is the enzyme that catalyzes the hydrolysis of fats and lipids to give di- and mono- glycerides, glycerol and free fatty acids. A shortage of lipase in the body may lead to high cholesterol, difficulty in losing weight, a tendency to diabetes, high urine sugar levels, gall stones, hay fever, prostate problems, heart problems etc. As an important class of enzyme, lipase has been applied to treat digestive disorder together with other classes of enzymes. And these enzyme supplements are also OTC in tablet form. Similar gastric bypass problem are encountered in the administration as mentioned in the previous section. Since extremely low pH results in the completely lost of activity of lipase in stomach, various methods are suggested to improve the efficiency of lipase. Recently, lipase was immobilized on the surface of Chitosan for the controlled release of this enzyme by Alsarra et al.

1.5 Research Objectives

Nanoporous structured polymeric materials have been synthesized in this research group from microemulsions stabilized by various surfactants. This work
is an extension of the application of microemulsion polymerized porous materials formulated with biocompatible surfactants. These porous polymers have the potential for application in controlled release for pharmacological drugs.

This research is to design and evaluate the porous materials as vehicles for drug delivery, especially for the application in controlled delivery of enzyme with a certain activity level. The following parts are included:

1) Selection of biocompatible surfactants and monomers to form bicontinuous microemulsions.

2) Establishment of the ternary diagrams of surfactant solutions and monomers, and determination of bicontinuous region from the tests of phase behavior, conductivity and viscosity.

3) Preparation of porous polymeric materials from photopolymerization of bicontinuous microemulsions.

4) Characterization of porous polymeric materials morphologies by SEM and pore size distributions by freezing point depression method using differential scanning calorimetry (DSC).

5) Evaluation of the potential drug delivery vehicles by the releasing of model drug of Rhodamine B and the therapeutic reagent: β-galactosidase from nanoporous materials. Since the activity of enzyme is the critical indicator of the efficiency of enzyme delivery systems, activity of β-galactosidase was also tested over time.

6) For the pH-sensitive system, the swelling of polymers in different medium were tested. Lipase was delivered by pH-sensitive AA/MMA/10 % F77
system. The activities of lipase released to solutions of different pH were also measured to confirm the effectiveness of pH-sensitive system.

1.6 Significance of the study

Method by which drugs are delivered can be of great significance to the drug efficacy. To minimize the drug loss and elongate the half time of drugs, various efforts are currently making to develop novel drug delivery systems. Porous polymers are of particular interest since high surface area is presented and higher efficiency in drug delivery. Digestive enzymes are used to treat digestive disorder which is estimated to affect half of American. One of the current strategies is to use dietary enzyme to address the disorder. However, the market of dietary enzyme is very much limited by the low efficiency of digestive enzyme. Quite recently, drug delivery systems have been introduced for the enhancement of efficiency of enzyme the administration.

Four biocompatible surfactants with different properties are introduced in this study for the formation of single phase microemulsion in bicontinuous region. Polymerization of these microemulsions resulted in porous materials. Morphology of polymeric materials could be modified by controlling the composition of precursor microemulsions. This enables the design of drug delivery devices for different release requirements of drug release.

This study developed the novel nanoporous polymeric solids and particles to encapsulate model drugs and therapeutic reagents, especially β-galactosidase and lipase, protect drug within polymeric network, and to realize the controlled
release of drugs by varying the porous structure of polymer synthesized from bicontinuous microemulsions.

Lack of stability is the great drawback of colloidal drug delivery systems. The colloids aggregate during storage and couldn’t endure dilution by body fluids. The nanoporous particle suspension synthesized in this system was very stable, and could resist dilution of a thousand times. This makes the materials suitable for delivery of drug administrated by oral routes or I.V. injection.

An advanced pH sensitive systems was also synthesized from bicontinuous microemulsion formulated by monomers of AA and MMA. The porous polymer is of particular interest in enzyme delivery due to deswelling and swelling of materials in mediums with different pH.

This study developed the one-step synthesis of drug delivery systems formulated with biocompatible surfactant, where no separation procedures are required to remove the surfactant residuals. This will also have enormous economical effect on the industrialization of the related drug delivery systems.
CHAPTER II

LITERATURE SURVEY

Microemulsions have been the subject of extensive research over the last two decades due to their distinct properties. The concept of microemulsion polymerization was appeared in 1980 when Stoffer and Bone\[34\] reported a ‘Swiss-like’ polymer by thermal polymerization of the microemulsion formulated with methyl methacrylate (MMA), sodium dodecyl sulfate (SDS), pentanol and ammonium persulfate. Since then, rapid progresses have been made in this field. The large interfacial area of microemulsions (up to 100 m²/ml), the small monomer domain sizes (∼10⁻² μm) and the diversity of microstructure make microemulsion an interesting subject for polymer synthesis.\[20\]

2.1 Polymerization in Bicontinuous Microemulsions

Although the major free radical polymerizations were carried out in globular microemulsion (O/W or W/O microemulsion), increasing interests have been directed to polymerization in bicontinuous microemulsion motivated by the fact that the particular microstructure of bicontinuous microemulsion could be used as template to produce polymer of similar structure.\[21, 35\] Numerous efforts...
have been made to prepare nanostructured materials by polymerization in bicontinuous microemulsion.

Cheung et al.\(^{36-39}\) have compared the polymerization of MMA/acrylic acid (AA)/sodium dodecyl sulphate (SDS) in W/O microemulsions and in bicontinuous microemulsions. They used ethylene glycol dimethacrylate (EGDMA) as cross-linking agent and also used methacrylic acid (MAA)\(^{38}\) as a cosurfactant to formulate the microemulsions. The materials that polymerized in the microemulsions with W/O droplet structures assumed closed-cell type porous structures. However, open-cell type porous structures were observed from the bicontinuous microemulsion systems. The degree of interconnectivity of the pores was demonstrated to increase with aqueous content. The opacity of polymer composites obtained implied that the polymerization process changed the microstructure of the precursor microemulsions. Only when the polymerization took place rapidly enough to minimize the chance of phase separation, could the original microstructure of the precursor microemulsion be preserved during polymerization in microemulsion.

Materials polymerized in non-polymerizable surfactant stabilized bicontinuous microemulsions were usually opaque and experienced phase separation. Stable and transparent polymers with a solid content up to 15 wt % can be obtained with the incorporation of polymerizable cosurfactant. However no microstructure was observed under SEM. Gan and Chew.\(^{40, 41}\) used polymerizable surfactants in microemulsion polymerization firstly to polymerize microemulsions composed of MMA, AA and the polymerizable surfactants,
sodium acrylamido-undecenoate and sodium acrylamindostearate. The opaque polymers were also observed due to the phase separation during polymerization. Although lowering aqueous content (<16 wt %) could avoid phase separation, no microstructures could be detected from SEM micrographs. Gan et al.\textsuperscript{[42]} reported the UV polymerization of transparent solids in bicontinuous microemulsion formulated with MMA (30 wt %), water (35 wt %) and polymerizable AUDMAA (35 wt %). The micrographs of these polymers revealed randomly distributed bicontinuous nanostructures of water and polymer domains with the width of 40-60 nm. Recently, N,N'-dimethyl-N-acryloyloxyundecyl piperazinium bromide (DAOUPB) was synthesized by Gan and coworkers through a two-step procedure, and was used as a surfactant in the bicontinuous microemulsion to produce pH-sensitive hydrogels.\textsuperscript{[43]} Increasing HEMA amount resulted in larger clear phase region in ternary phase diagram DAOUPB/H\textsubscript{2}O/MMA:HEMA system. Both MMA:HEMA and acrylonitrile systems were studied to formulate microemulsions stabilized by DAOUPB. However, only the acrylonitrile system produced transparent materials with nanoporous structures. The average width of water channel was about 33 nm in the dry state. The polymers produced from bicontinuous microemulsions displayed a swelling behavior dependent on pH in acidic media due to protonation of the tertiary amine of the piperazine molecule.

Palani Raj \textit{et al.} also reported the polymerization of bicontinuous microemulsion comprised of MMA, EGDMA and water using potassium undecenoate (PUD) as a polymerizable surfactant.\textsuperscript{[44]} The results indicated the
possibility of controlling the morphology and microcellular structures by the polymerization of microemulsions formulated with PUD.

2.2 Nano/Micro-porous Polymer for Drug Delivery

Nano/Micro-porous polymers are applied in many fields including separation technologies, catalyst surfaces and supports, antireflection coatings, drug delivery systems, tissue engineering, and templates for the growth of various nanoscopic materials. Various techniques have been used to prepare porous polymer systems, including phase separation, the selective dissolution of polymer blends, the degradation of block copolymers, and the polymerization of monomers in sacrificial colloidal or nanoporous silica templates. Polymers of intricate network structure with the presence of extensive surface morphology are perfect candidates for controlled release devices. Micro or nanoporous polymers have been used in the forms of scaffold, film, sphere and tablet in the filed of drug delivery by different administration routes.

Palani Raj et al. synthesized microporous polymer for the potential use in drug delivery from bicontinuous microemulsions with MMA, AA and SDS. These microemulsions were examined by light scattering, conductivity and viscosity measurements. The microstructure of microemulsion was partly reserved during microemulsion polymerization.

Nam et al. reported microporous scaffold prepared by thermal induced phase separation for drug delivery and tissue engineering. Poly(L-lactic acid) and its copolymers with D-lactic and glycolic acid were selected to develop
various porous biodegradable scaffold by thermally induced phase separation, the quenching temperature affected the coarsening effect of pore enlargement. Kovalchuk et al. fabricated microporous biodegradable PGA and PDLLA by solvent extraction after solid-state polymerization. The microporous polymers were shaped by cold uniaxial pressing, and by extrusion at elevated temperature. This polymer as controlled release device demonstrated release of the model drug Phe and of the anti-tumor drug goserelin (an LH-RH agonist) in approximate 2 days. PDLLA or PLLA coating efficiently slowed down the release kinetics. The coated PDLLA showed an almost linear release during 100 days.

Recently an increasingly interest was attributed to nanoporous polymers in drug delivery technology. Several types of controlled release architectures have been developed based on the incorporation of releasing agent in nanoporous hosts. It allows precise control of the host pore size, and various gating strategies have been used to modulate the release characteristics.

Chandran S. reported that nanoporous microparticles could be synthesized in the corresponding transition region of the microemulsion system of HEMA and MMA stabilized by SDS. Quasi-elastic light scattering (QELS) results indicated that the particle size is 5-10 µm in diameter. The diffusion coefficients within specified system were studied in detail by spin-echo nuclear magnetic resonance (PGSE-NMR).

More recently, Vickerman et al. compared the polymerization of nanoporous materials using different surfactants. SDS, polymerizable surfactant TREM LF-40, polymerizable surfactant Adeka Reasoap NE-40 and
biocompatible Royto Sugar D-1216 were studied. All the four systems exhibited the capability of emulsifying bicontinuous microemulsion precursors to synthesize nanoporous microparticles. However, the transition region was highly dependent on the nature of surfactant. The existence of nanopores was confirmed by DSC and SEM measurements. This study also demonstrated the dependency of microstructure on the composition of the precursor microemulsions. The biocompatible surfactant stabilized microemulsions were considered as very promising candidates for synthesizing drug delivery devices.

Wang et al. synthesized nanoporous polymer-based spheres with pores of 5-50 nm via sequential assembly of macromolecules (e.g. polyelectrolytes (PEs), peptides, and proteins) in mesoporous silica (MS) particles.[50] The sequential penetration of PEs in the mesopores for the model system poly-(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) was verified by a series of characterization techniques. Cross-linking between PAA and PAH was found to add structural integrity to the NPS. The solution pH and ionic strength determined the conformation of the PEs in solution and the ability of the subsequent infiltration of PEs in the mesopores. The PAA/PAH NPS exhibited a high capacity for enzyme loading (ca. 470 mg/ml for lysozyme). The nanoparticles were stimuli-responsive, and the release of the protein was triggered by the changes in pH of solution. These nanoporous materials were envisaged to be applied in biosensing, enzyme catalysis, and controlled drug delivery.

Asoh et al.[51] designed the nanoporous linear-polymer penetrating networks (semi-IPNs) which were both temperature-sensitive and pH-sensitive
for the molecule-release in drug delivery. The semi-IPNs were formed by radical polymerization of acrylic acid (AAc) inside nanoporous poly(N-isopropylacrylamide) hydrogels. The porous semi-IPNs showed a rapid deswelling in response to either a change in pH or temperature, presumably due to the nano-tracts through which the water was rapidly released from the hydrogel without any interference by network shrinking.

2.3 Components

A significant number of polymer systems have been proposed as drug carriers. As a successful controlled drug delivery system, the materials should be non-toxic, non-carcinogenic, non-immunogenic, and free of contaminants and leachables. Many biocompatible polymers are considered as well functioned both biologically and physiochemically as drug carriers. The most common polymers that have been reported are poly(2-hydroxy ethyl methacrylate)(p-HEMA), poly(N-vinyl pyrrolidone)(p-NVP), poly(vinyl alcohol) (PVA), poly(acrylic acid) (PAA), polydimethyl siloxanes (PDMS), ethylene-vinyl acetate (EVAc) copolymers, poly(lactic acid) (PLA), poly(glycolic acid (PGA), poly(lactide-co-glycolides) (PGLA), polyanhydrides, poly(ortho esters), collagen and cellulosic derivatives.

2.3.1 Poly(HEMA-co-MMA) in Drug Delivery

This study investigated the porous poly(HEMA-co-MMA) as vehicles in drug delivery. This polymer was frequently used in drug delivery for its
mechanical stability, transparent and hydrophobic nature, and potential biocompatibility. Choudhary et al.\textsuperscript{[52]} investigated the immunological response of poly(HEMA-co-MMA) polymeric implant in humans. The subcutaneous implantation showed an inflammatory response without the presence of mononuclear cells, and the inflammation reduced after a month with fibroblast cells around polymer, which implied that poly(HEMA-co-MMA) was potentially biocompatibility and non-immunogenic.

Challa et al.\textsuperscript{[53]} studied the release of Rhodamine B from nanoporous solids of poly (HEMA-co-MMA), and concluded that increasing aqueous content of precursor microemulsions where the polymers were produced could increase the release rates of Rhodamine B.

Sivakumar et al\textsuperscript{[54]} investigated a new crosslinked poly(HEMA-co-MMA) core–shell hydrogel microsphere incorporated with ibuprofen for potential applications in bone implants. The crosslinked p(HEMA) shell was formed by polymerization of HEMA in a swelling PMMA core microspheres. The core/shell structure was observed under SEM. And these hard core/hydrophilic shell microspheres were more hydrophilic than PMMA microspheres, and exhibited close to zero-order release of ibuprofen.

HEMA was studied as a polymerizable cosurfactant in the emulsion polymerization of MMA stabilized with sodium lauryl sulphate (SLS) by Bhawal et al.\textsuperscript{[55]} The incorporation of HEMA helped the formation of nanoparticles with a diameter less than 500 nm. The properties of nanoparticles were strongly dependent on the HEMA percentage in the emulsion system. And this study
showed that the addition of HEMA decreased the release of carbamazepine, and a zero-order release was demonstrated.

2.3.2 Poly(AA-co-MMA) pH-responsive Drug Delivery System

PAA has been applied widely as a biocompatible synthetic polymer. It has been known as a pH and electrically sensitive material due to the ionization of carboxyl group. PAA/CS complex can be synthesized as pH sensitive hydrogel for controlled release of antibiotics in stomach.\[^{[56]}\] In addition, the PAA are of increasing interest as mucoadhesive hydrogel, i.e. it can be attached to mucus surface.\[^{[57]}\]

The biocompatible copolymer of AA and MMA can be applied as the controlled release devices. Inoue \textit{et al.}\[^{[58]}\] fabricated a block copolymer of PAA and PMMA, which could form micelles when dissolved in aqueous medium. The copolymer micelle demonstrated a prolonged release of doxorubicin. Katime \textit{et al.}\[^{[59]}\] studied the copolymer of poly(AA-co-MMA) hydrogel at different AA/MMA ratio. The study showed the diffusion of water was dependent on the AA/MMA ratio, and the swelling of hydrogel controlled the release of the nafcillin through hydrogel. Katime’s group\[^{[60, 61]}\] also investigated the effect of alky chain length on poly(AA-co n-alky methacrylate) hydrogels. The swelling of hydrogels was highly dependent on the AA composition and alky chain length. Alkyl chain didn’t affect theophylline release, whereas aminophylline release was highly dependent of alkyl chain length. The author concluded that there was a stronger affinity between the latter drug and the hydrogels due to the steric effects.
2.3.3 Biocompatible Surfactant

Biocompatible surfactants have significant effect on the materials to be used as drug delivery devices. For the biocompatible surfactant stabilized microemulsions, no additional procedure is required to extract surfactants which in most case can result in inflammation. Three types of surfactants used in this study are Ryoto Sugar L1695, Pluronics® : F127 and F77, and Tetronics® : T1307. A summarization of these surfactants is presented in Table 2.1. F127 and T1307 are of high molecular weight, and are capable of forming micelles of small size at very low concentration. The detailed properties and applications of these four surfactants are explained in the following paragraphs.

Table 2.1 Properties of Surfactants in the Study

<table>
<thead>
<tr>
<th>Features</th>
<th>L1695</th>
<th>F77</th>
<th>F127</th>
<th>T1307</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>Mitsubishi Chemical</td>
<td>BASF</td>
<td>BASF</td>
<td>BASF</td>
</tr>
<tr>
<td>HLB</td>
<td>16</td>
<td>25</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Molecular Weight</td>
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<td>6600</td>
<td>12600</td>
<td>18000</td>
</tr>
<tr>
<td>pH at 2.5% water</td>
<td>N/A</td>
<td>6-7</td>
<td>6-7</td>
<td>8-10</td>
</tr>
<tr>
<td>Ionic Nature</td>
<td>Non-ionic</td>
<td>Non-ionic</td>
<td>Non-ionic</td>
<td>Non-ionic</td>
</tr>
</tbody>
</table>
**Ryoto Sugar L1695 (L1695)**

Sucrose esters are biodegradable surfactants which can have a wide range of hydrophilic-lyophilic properties using different fatty acids with various lyophilic chain lengths.\(^{[62]}\) Sucrose esters are largely used in Japan, only recently did other countries register sucrose esters as permitted emulsifies that could be used in food process. It is surprising that application of sucrose ester in microemulsion formation is very limited.

Ryoto Sugar L1695 is a polyglycerol ester from the esterification of polymerized glycerol with dodecanoic acid. L1695 is non-irritating on mucous membranes and does not exhibit oral or percutaneous toxicity and, which makes it a particularly interesting surfactant for this study. It does not lower the potency of phenol compounds or antibiotics, in contrast with polyoxyethylene emulsifiers, and has no cloud point.\(^{[63]}\) The structure for sucrose dodecanoate is as follows:

![Figure 2.1 Schematic Presentation of Ryoto Sugar L1695](image)

**Pluronic F127 and F77**

Pluronic surfactants are triblock copolymeric surfactants manufactured by BASF Chemical Company. The triblock structure can be summarized as:
\[(EO)_x(PO)_y(EO)_x\], where \(x\) and \(y\) denote the number of ethylene oxide (EO) and propylene oxide (PO) units per block. Pluronic surfactants with a diversity of HLBS are available by changing the \(x\) and \(y\) in the triblocks. Pluronics\textsuperscript{®} are biocompatible surfactants that have previously been administrated to human patients in large dose without apparent ill effect.\cite{64}

In this study, two Pluronic surfactants were selected for the drug delivery study: F127 and F77, both has EO=70 wt% in the triblock. The molecular formula of F127 is \((EO)_{100}(PO)_{64}(EO)_{100}\), while F77 has a structure as: \((EO)_{53}(PO)_{38}(EO)_{53}\). F127 is FDA approved and has already been used extensively in the pharmaceutical industry as a vehicle for the controlled release of drugs and also as a coating for wound and protection against microbes.\cite{65, 66}

**Tetronic 1307**

Tetronic surfactants are ethylenediamine alkoxylate block copolymer, which is another category of surfactants manufactured by BASF. These surfactants are similar to Pluronics\textsuperscript{®} in that the HLB are dependent on the EO and PO units.

![Figure 2.2 Schematic Presentation of Tetronic Surfactant](image)

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Tetronic 1307 is a solid with 70% of hydrophilic EO groups. The aqueous Tetronic 1307 solutions demonstrate a sol-gel transition as temperature increases. Due to the sol-gel nature, Tetronics® are considered as nontoxic and non-hazardous materials. They are chemically stable under physiological conditions. Tetronic 1307 was reported as a viable drug delivery system and induced minimal skin and eye irritation. Fakes et al. investigated the thermogelling behavior of T1307 for the application in contact lens. Recently Tetronic 1307 was selected by Parisot et al. to form O/W microemulsion with mineral oil for the delivery of immunogens.

A summarization of these surfactants is presented in Table 2.1. F127 and T1307 are of high molecular weight, and are capable of forming micelles of small size at very low concentration.
CHAPTER III

EXPERIMENTAL

The experimental studies were conducted in accordance with the overall research objective to design and evaluate the controlled release drug delivery systems derived from microemulsions. The experimental studies can be categorized as polymer precursor study, polymer formation, polymer characterization, and the release of model drug loaded systems.

The details of the experimental techniques employed throughout this study and the methods of analysis are described in the following sections.

3.1 Materials

The monomers, methyl methacrylate (MMA, 99%), 2-hydroxyethyl methacrylate (HEMA, 97%), acrylic acid (AA, 95%), and cross-linking agent, ethylene glycol dimethacrylate (EDGMA, 98%), were obtained from Aldrich. Ryoto Sugar L1695 was a gift from Mitsubishi Chemical. Pluronic F127, Pluronic F77 and Tetronic T1307 were kindly provided by BASF and used without further purification. The photoinitiator, 2, 2'-dimethoxy, 2-phenyl acetophenone (DMPA) was obtained from Aldrich and used as received. The water used in this study was double deionized. In the release study, Rhodamine-B, Lipase from porcine
pancreas and β-galactosidase from *Aspergillus oryzae* were purchased from Sigma-Aldrich. In enzyme activity analysis, the substrates used were 2-Nitrophenyl β-D-galactopyranoside (ONPG) and p-nitro phenyl valerate (pNP-Valerate) from Sigma-Aldrich. Micro BCA Kit for protein assay was from Pierce. All other reagents except specified were ACS grade.

3.2 Characterization of Precursor Microemulsion

Characterization of microemulsion precursors was designed to determine the range of compositions that could be used to form monolithic systems by polymerization of Winsor-IV microemulsion precursors. The phase behavior study was to select the compositions that generate single phase microemulsions. The single phase Winsor IV microemulsion samples were characterized by electrical conductivity and viscosity measurement techniques to trace the boundaries between different microstructures of microemulsion.

3.2.1 Phase Behavior Study

The formation and thermodynamic stability of the microemulsions were studied by performing a ternary phase behavior studies with various surfactants at a 10 wt % active surfactant concentration.

Ternary phase diagrams were constructed by observing the phase behavior of microemulsions at various concentrations of water, monomer and surfactant at room temperature. Samples for the phase behavior studies were prepared by pipetting the required mass of the various components into clean
and dry test tubes. The mass was measured by a Mettler AT200 electronic balance. The test tubes were sealed with cap and taped to prevent leakage, and then mixed for 10 seconds by Scientific Instruments Vortex Genie-2 to ensure complete incorporation. After equilibration for 24 hours in dark, samples were observed under visible light for phase separation. The macroscopically uniphase regions would show no presence of a phase boundary. The multiphase regions, on the contrary, would show the presence of a phase boundary or fine droplets. The single and multiphase domains thus observed were then marked on the phase behavior diagram by taking entire plot of 36 points at the room temperature. Initial results were reconfirmed after further mixing and equilibration for another 24 hours.

3.2.2 Electrical Conductivity Measurements

The samples for the conductivity measurements are similar to those in phase behavior study except 4 wt % of ethylene glycol dimethacrylate (EDGMA), based on the weight of the monomers and 2 wt % of DMPA based on total monomer content. An Omega Digital Conductivity Meter, PHH-80BMS was used to measure the conductivity. Conductivity measurement was performed at 22 ± 0.5 °C.

3.2.3 Viscosity Measurements

Right after the conductivity measurement, viscosity measurements were conducted on each sample. Viscosity measurements was carried out using a
Brookfield LVT digital viscometer modified with a small sample adaptor having an 8 ml sample holder with the capability to control temperature by circulating coolant. Viscosity measurement was performed at 22 ± 0.5 °C.

3.3 Polymerization of Microemulsion

Free radical polymerization of microemulsion formulations was carried out using a photoinitiator, 2, '2', dimethoxy, 2-phenyl acetophenone (DMPA). Preparation of samples was identical to that in conductivity and viscosity measurements. In the pH-sensitive system, 0.12 wt % sodium hydroxide (based on the total weight of sample) was added to polymer precursor before polymerization for the purpose of producing partially neutralized system.

After the appropriate amounts of each component were added to the test tube, the material was capped and vortex mixed for 10 to 15 seconds. Then the samples were purged with nitrogen for 5 minutes. After a 24 hour-equilibration in dark, the sealed test tubes were polymerized by visible light for 2 hours in a reaction cell as show in Figure 3.1. The photopolymerization reactor consists of two 300W halogen lights placed facing each other, the sample rack placed between the lights, and a fan to cool samples. The samples were visually inspected to ensure that polymerization had taken place and then stored at room temperature for further examination.
Figure 3.1: Reaction Cell Used For Photo-Polymerization

Seen in the figure are: (1 and 2): Halogen lamps, (3): Fan for cooling, (4): Test tubes on stand, (5) Sample rack with vents

3.4 Characterization of Polymerized Microemulsions

The experiments in this category involved characterization steps designed to reflect the physical nature of the polymer and also in some cases the precursor microemulsion. The monoliths and nanoporous microparticles were analyzed to determine the morphology and pore size distributions. The details of the characterization techniques employed are described in the next few subsections.
3.4.1 Scanning Electron Microscopy (SEM)

The morphology of the polymer samples was examined by SEM. Since the pore structure of the aqueous samples collapses at dry state, the SEM result was only assumed as an indication of their structure and as a support of other characterization techniques.

A FEI (formerly Philips) Quanta 200 Environmental Scanning Electron Microscope was employed for this study. For solid monoliths, samples were freeze fractured by immersion into liquid nitrogen after drying in room temperature for a week. A fragment of the polymer was then placed onto an SEM stub such that the freshly cleaved surface was facing upwards. For nanoporous particles, samples were diluted with deionized water with a dilution factor of 100 in order to prevent particle aggregation. A drop of these solutions was placed on an aluminum covered stub and dried at room temperature for one week. The stub was then mounted in the SEM and scanned at various magnifications to study the morphology of the sample.

3.4.2 Freezing Point Depression Study

The pore size distributions (PSD) of the polymer substrates were determined from freezing point depression (FPD) analysis of the water trapped within the pores. This method has been widely used by researchers in this field to quantify the pore size of materials using Differential Scanning Calorimetry (DSC).\[70-72\]
The high curvature of the interfaces leads to the depressed freezing point of the liquid present in the nanoporous material.\textsuperscript{[70, 73-76]} Laplace equation describes the relation of pore diameter with depression in melting temperature of water in the pores compared to its normal freezing point\textsuperscript{[70]} This phenomenon was utilized in this study to obtain the distribution of pore sizes for the polymeric materials synthesized.

For the case of the ice-water interface, ice is the non-wetting phase compared to water and exists on the concave side of the interface as presented in Figure 3.2. It was also shown that the lowering in melting temperature could be related to the capillary radius by the relation.\textsuperscript{[70]}

\[ T_0 - T_m = \frac{2\gamma T_0}{\rho_w \lambda R + 0.5T_0} \quad (3.1) \]

where, \( T_0 \) is the normal melting point of ice; \( T_m \) is the depressed melting point of ice; \( \gamma \) is the ice-water interfacial tension; \( \lambda \) is the molar heat of fusion of ice; \( \rho_w \) is the density of water; \( R \) is the radius of the capillary. This relation provides a method to determine the pore diameter by determining the depression in freezing point.

Samples for FPD were prepared by placing 10 – 20 mg of sample into a hermetic aluminum pan. TA Instruments Q100 DSC instrument was used for PSD analysis. The samples were first equilibrate to -40 °C, and then heated to 10 °C at a ramp rate of 0.5 °C per minute. Enthalpy vs. temperature was plotted. The running integral by TA Instruments Universal Analysis software gives the
cumulative fraction of the pores that melt at a corresponding temperature. Microsoft Excel spreadsheet was applied to evaluate the pore size at each temperature point according to equation 3.1. Then the pore size distribution of the sample in consideration was generated by plotting number fraction of the pores versus pore diameter.

![Figure 3.2 Schematic Representation of Ice Melting in a Capillary. The capillary is assumed to be spherical with a uniform radius of R.](image)

3.4.3 Swelling Behavior of pH-sensitive p(AA-co-MMA)

The swelling characteristics of samples used in release studies were considered to be very important. To determine the swelling ratio of p(AA-co-MMA), sodium hydroxide of 0.12 wt % precursor was added to the polymer precursor identical to the samples in conductivity measurement, and 3 g sample
was pipetted in to glass tube for polymerization. After polymerization, samples were taken out from glass tube. Each sample was put into a tea bag of known weight ($W_b$). The initial weight ($W_0$) was the total weight of sample and tea bag. Then samples together with tea bags were placed into 100 ml pH=1.2 (0.2 M, HCl-KCl) and pH=6.8 (0.2 M, sodium phosphate) buffer respectively. At predetermined time intervals, weights of tea bags were measured ($W_t$), and the swelling ratio was defined as:

$$S(\%) = \frac{W_t - W_0}{W_0 - W_b} \times 100 \% \quad (3.2)$$

3.5 Release Profile Study

The fundamental question this work seeks to answer is the utility of polymerized microemulsion as controlled release system. Four different systems formulated with different surfactants were fabricated for the potential use as drug delivery vehicles. The release of a model drug, rhodamine B, and therapeutic reagents, β-galactosidase and lipase, were determined.

3.5.1 Rhodamine B Loading and Release

5 ml of drug loaded fluid were added to a cellulose membrane tubing (molecular weight cutoff 5000 Da), and dialyzed against 250 ml of 0.01 M phosphate buffer (pH 7.4) in sink condition at 35 °C. Aliquots of 3 ml of the dialysis solution were withdrawn at appropriate intervals, absorbance was
measured for each at 554 nm by UV-1601 UV-Visible spectrophotometer (Shimadzu, Japan), and same amount of fresh buffer was added into the sink.

3.5.2 Release of β-galactosidase

Two nanoporous monoliths were studied in β-galactosidase release. The precursors of these two systems were stabilized by 10 wt % L1695 and 10 wt % T1307 respectively. Both systems have an organic content of 15 wt %, which was comprised of 3:1 HEMA/MMA, 4 wt % of EDGMA and 2 wt % of DMPA, based on total monomer content. β-galactosidase was mixed with precursor microemulsions at an amount of 3mg/g precursor. After polymerization, samples were stored at 4°C for further study.

β-galactosidase loaded polymers (about 3 g) were suspended in 100 ml buffer (0.01 M, pH=7.4) in a water bath (35°C) shaking at 100 oscillations/min. Aliquots of 2 ml were collected at various times and replaced with preheated fresh buffer. All the collected samples were stored at 4°C for additional study.

Enzyme Amount Assay

The amount of β-galactosidase release into buffer was determined by Micro BCA Protein Assay Kit from Pierce (Rockford, IL). The detailed procedure is described as below: A working reagent was mixed by 25 parts of reagent A, 24 parts of reagent B and 1 part of reagent C. For a typical measurement, 0.5 ml of sample and 0.5 ml of working reagent was mixed well and incubate at 60°C for 60 min. After cooling down back to room temperature, the absorbance at 562 nm was measured by UV-1601 UV-Visible spectrophotometer (Shimadzu, Japan).
The standard curve was measured by using β-galactosidase of known concentration as standard.

Activity of β-galactosidase released

The activity analysis of β-galactosidase released into buffer was conducted using 2-Nitrophenyl β-D-galactopyranoside (ONPG) as substrate to measure the apparent activity of β-galactosidase in releasing medium. 0.9 ml of ONPG (15 mM) was mixed with 0.1 ml of free enzyme solution in a 1 ml UV-spectrum cuvette, and absorbance at 405 nm was immediately monitored for 2 min at room temperature by UV-1601 UV-Visible spectrophotometer (Shimadzu, Japan). ONPG was dissolved in 0.1 M sodium acetate buffer with a pH=4.5. The activity of enzymes was calculated as:

\[
\text{Activity (Units/ml)} = \frac{\Delta \text{ABS/min} \times 1 \times \text{DF}}{\varepsilon \times 0.1}
\]

where, \(\Delta \text{ABS/min}\) is the change of absorbance per minute reading from UV kinetic analysis, 1 is the total volume (in milliliters) of the assay, DF is the dilution factor, \(\varepsilon\) is the extinction coefficient of o-Nitrophenol at 405 nm, and 0.1 is the total volume of enzyme.

One unit will hydrolyze 1.0 μM ONPG to o-nitrophenol and D-galactose per minute at pH 4.5 at 25 °C.

3.5.3 Determination of Lipase Releasing

The pH-sensitive monolith was studied in lipase release. The precursor microemulsion was stabilized by 10 wt % F77, with an organic content of 15 wt %,
which comprised of 3:2 AA/MMA, 4 wt % of EDGMA and 2 wt % of DMPA, based on total monomer content. Sodium hydroxide of 0.12 wt % precursor was added to partially neutralize the precursor. Lipase was mixed with precursor microemulsions at an amount of 3 mg/g precursor. After polymerization, samples were stored at 4°C for the ensuing study.

Similar to the procedures in section 3.5.2, samples were suspended in 100 ml pH=1.2 (0.2 M, HCl-KCl) and pH=6.8 (0.2 M, sodium phosphate) buffer respectively in a water bath (37°C) shaking at 100 oscillations/min. These releasing buffers were selected to simulate the environment in intestine and stomach. Samples (2 ml) were collected at various times and replaced with fresh buffer. The collected samples were stored are 4°C for the succeeding activity test.

Activity of Lipase

0.4 ml of samples with enzyme was incubated at 40°C for 2 minutes, 0.5 ml p-nitro phenol valerate substrate was then mixed with samples. The substrate was dissolve in pH=7.7 Tris-buffer, with a concentration of (0.132×10^{-3} g/ml, 0.59 mM). After 25 minutes, 1ml cold water (4°C) was added to stop the reaction. Absorbance of sample was measured at 405nm by UV-1601 UV-Visible spectrophotometer (Shimadzu, Japan). The activity was calculated as:

\[
\text{Activity(Units/ml)} = \frac{(A_{405}^{\text{Test}} - A_{405}^{\text{Blank}}) \times 1.9 \times \text{DF}}{25 \times \varepsilon \times 0.4}
\] (3.4)
where, 1.9 is the total volume (in milliliters) of the assay, DF is the dilution factor, 25 is the time of assay, \( \varepsilon \) is the extinction coefficient of p-Nitrophenol at 405 nm, and 0.4 is the total volume of enzyme.

One unit will hydrolyze 1.0 \( \mu \)M substrate of pNP-Valerate per minute at pH 7.7 at 25 °C.
CHAPTER IV

RESULT AND DISCUSSION

The objective of this study is to develop polymers derived from bicontinuous microemulsions. Polymers produced in highly aqueous region (>80%) are of particular interested for the application in drug delivery. It has been demonstrated in the previous research of this group that materials polymerized from bicontinuous microemulsion systems exhibited intricate network structures with the presence of extensive surface morphology in the form of pores. These porous polymers with large surface area are potential candidates for controlled release.

Four different porous polymer systems were synthesized in this study, and would be applied for different purposes of drug delivery according to the characteristics of the related systems. The nanoporous polymer in the form of free-flowing particle suspension is envisaged as delivery vehicles of proteins and lipids which can be administrated by IV injection or by oral route since they can resist the dilution of a thousand times. And the porous polymers could be potentially applied as drug delivery vehicles of enzymes administrated by oral route in the form of tablet. An especially interesting pH-sensitive porous polymeric system was also developed in this study, the deswelling and swelling
of polymer can control the pore size of network wherein, and thereby determine
the release of proteins encapsulated. The formulations of these four systems are
summarized in Table 4.1 as follows. All the microemulsions precursors were
photo polymerized. 4 wt % (based on monomer content) EGDMA and 2 wt %
DMPA (based on monomer content) were used as crosslinking agent and
photoinitiator respectively.

Table 4.1 Systems Synthesized in this Study

<table>
<thead>
<tr>
<th>Features</th>
<th>System I</th>
<th>System II</th>
<th>System III</th>
<th>System IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomers</td>
<td>3:1 HEMA/MMA</td>
<td>3:1 HEMA/MMA</td>
<td>3:1 HEMA/MMA</td>
<td>3:2 AA/MMA</td>
</tr>
<tr>
<td>Surfactant</td>
<td>L1695</td>
<td>T1307</td>
<td>F127</td>
<td>F77</td>
</tr>
<tr>
<td>Aqueous Content</td>
<td>85%</td>
<td>85%</td>
<td>90%</td>
<td>85%</td>
</tr>
<tr>
<td>Shape of Polymer</td>
<td>Soft Solid</td>
<td>Soft Solid</td>
<td>Particle</td>
<td>Soft Solid</td>
</tr>
<tr>
<td>Pore Nature</td>
<td>Nanoporous</td>
<td>Nanoporous</td>
<td>Nanoporous</td>
<td>Microporous</td>
</tr>
<tr>
<td>Drug Delivered</td>
<td>β-galactosidase</td>
<td>β-galactosidase</td>
<td>Rhodamine B</td>
<td>Lipase</td>
</tr>
</tbody>
</table>

Prior to the release profile study, systems I, II and III were examined to
determine the single bicontinuous phase region by the pseudo ternary phase
diagram, viscosity and conductivity studies. Samples were then polymerized, and
the morphologies were examined by SEM. Freezing point depression method
was applied to study the pore size and distribution of related porous polymeric
systems. System IV was partially neutralized since there was phase separation
and water was squeezed out when p(AA-co-MMA) formed without neutralization.

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The swelling behavior was determined for the partially neutralized system since it is the dominant factor for drug release from pH-sensitive system. All the four systems were examined for *in vitro* drug release characteristics. The results obtained from different characterization methods of each system are presented and interpreted in the following sections.

4.1 Characterization of Precursor Microemulsion

It is necessary to determine precursor composition that can result in Winsor IV microemulsion before the sample preparation. The phase behavior examination was designed for this purpose. Since we are highly interested in the bicontinuous region to produce porous polymers for drug delivery, investigation of microemulsion precursor structure was conducted by conductivity and viscosity measurements. Three systems analyzed in this section were L1695 solution/HEMA/MMA, T1307 solution/HEMA/MMA and F127 solution/HEMA/MMA.

4.1.1 Phase Behavior Studies

This study investigated the phase behavior of microemulsions formed by surfactant solutions and monomers of HEMA and MMA. All surfactants were evaluated at a 10 wt % active surfactant concentration. Figure 4.1, 4.2 and 4.3 shows the pseudo ternary phase behavior of microemulsion system of L1695 solution/HEMA/MMA, T1307 solution/HEMA/MMA and F127 solution/
HEMA/MMA respectively. The phase diagrams were used as a guide for the preparation of desired microemulsions.

The phase diagram L1695 solution/HEMA/MMA in Figure 4.1 indicates the presence of a small single phase region. L1695 could provide sufficient emulsification in the highly aqueous region, but would experience phase separation on the 3:1 HEMA to MMA tie line from 30 – 80 w/w aqueous surfactant solution.

Figure 4.2 shows the phase behavior of the microemulsion system formulated with MMA, HEMA and a 10 wt % aqueous solution of T1307. A slightly larger single phase was presented in the ternary diagram. Non-uniform phase behavior was also experienced with this system from 30 - 70 w/w aqueous surfactant solution on the 3:1 HEMA to MMA tie line.

The phase behavior of the microemulsion system formulated with MMA, HEMA and a 10 wt % aqueous solution of F127 is presented in Figure 4.3. The phase behavior ternary diagram is similar to that of L1695 with similar single phase region. This system also displayed non-uniform phase behavior along the 3:1 HEMA to MMA tie line when aqueous surfactant solution was between 30 – 70 wt % of total sample.

In all the three systems, single phase region is observed where HEMA content is high and MMA content is relatively low, while the non-uniform phase is observed in the region with low HEMA and high. This phenomenon is ascribed to the difference in water solubility between these two monomers. MMA is known as having poor solubility in water, whereas, HEMA has very good solubility in water
and has been used as hydrogels in many medical applications. HEMA performs dual functions as a cosurfactant and a comonomer as well in all the three microemulsion systems. It was reported by Chew et al.\cite{77} that the HEMA content could greatly influence the microstructure of polymer formed whereby.

Isotropic single phase microemulsions and turbid two phase microemulsions were observed among the microemulsions prepared by all the three surfactants. Three-phase microemulsions were only observed in microemulsions stabilized by L1695. All the three surfactants can provide efficient emulsification in the highly aqueous content region that we are interested in for synthesis of vehicles for drug delivery. Since the surfactants are not polymerizable, their molecules can diffuse from the network. However, all these surfactants are biocompatible and some have already been approved by FDA for the application in food and drugs, they would be acceptable for use in a controlled delivery device. In addition, the surfactants are commercialized which ensures the low cost in production of produce novel biocompatible drug delivery devices formulated with these economical surfactants. Therefore, the surfactants were continued to be used for the succeeding studies of microemulsions despite of the relatively small single phase region.
Figure 4.1 Pseudo-Ternary Phase Diagram of 10% L1695/HEMA/MMA at 22°C
Figure 4.2 Pseudo-Ternary Phase Diagram of 10 % T1307/HEMA/MMA at 22 °C
Figure 4.3 Pseudo-Ternary Phase Diagram of 10 % F127/HEMA/MMA at 22 °C
4.1.2 Conductivity Measurement

The electrical conductivity of microemulsion is sensitive to the structure of microemulsion and is frequently used to investigate the change of structures in microemulsion. Conductivity measurements of the microemulsion samples formulated by the three surfactants of L1695, T1307 and F127 were carried out at $22 \pm 0.5 ^\circ C$. This measurement was completed by changing the aqueous content of the microemulsions, keeping the relative amounts of the other components constant.

Figure 4.4 displays the results of the conductivity measurements for the three surfactant systems. The result is almost similar to what Davis\textsuperscript{[78]} and Vickerman\textsuperscript{[79]} observed in the conductivity study of AA/MMA/SDS and HEMA/MMA/SDS microemulsion systems. Since all the microemulsions formulated with these three surfactants experienced phase separation, no conductivity data were displayed in the region between 30-70 wt%, only discontinuous conductivity curves are presented.

As shown in the figure, all the systems examined demonstrated a general trend in conductivity measurements as a function of aqueous content. When aqueous content is low, micelles formed are discrete W/O droplets. The conductivity is negligible when aqueous content is lower than 20 wt %. At this low aqueous content, all the water in W/O microemulsion is confined in the interfacial region. The low mobility of water significantly affects the conductivity of microemulsion. Therefore, the conductivity at this region resembles that of the oil phase which is very low initially. As the aqueous content increases, a majority of
water goes to the interface to form W/O micelles, whereas the remaining water is presented in the form of free-water. Consequently, there is a slight increase in the conductivity with the increase of aqueous content.

When the aqueous content increases to a threshold, a sharp increase of conductivity is usually observed and the microemulsion suddenly becomes conductive. This phenomenon is known as the percolation transition, and has been used for interpreting the conductivity of disordered media such as microemulsion. The percolation transition signifies the formation of bicontinuous structure. In such systems, conductivity is governed by a universal law independent of the physical properties of the medium. Conductivity of system near the percolation threshold can be expressed by the following equation:

$$\sigma = (\Phi_W - \Phi_p)^t$$  \hspace{1cm} (4.1)

where $\sigma$ is the conductivity, $\Phi_W$ is the dispersed aqueous volume fraction, and $\Phi_p$ is the dispersed volume fraction at percolation threshold, and $t$ depends on the dimensionality of system.

As all the plots are discontinuous, the percolation threshold could not be identified. In bicontinuous region, conductivity increases with the increase of aqueous content. Due to the formation of bicontinuous structure, numerous water conductive channels are presented within the bicontinuous microemulsion. The addition of aqueous content leads to the augmentation in the number of water channels, which gives rise to the increased conductivity. However, the conductivity does not vary significantly, and remains almost stable as the further
addition of water, which implies the formation of o/w droplets. Given that oil is locked in the interface, the conductivity depends mostly on the aqueous phase. As a result of the nonionic nature of the three surfactants in aqueous phase, the conductivity is not much dependent on the addition of surfactants.

Although these three surfactants are all nonionic, there still exist difference between the three systems stabilized by the L1695, T1307 and 77. As shown in Figure 4.4, the conductivity is dependent on the structure of surfactant. F77 and T1307 demonstrated lower conductivity, and the conductivity of L1695 is higher. More associates form in the surfactant solutions of F77 and T1307 due to the higher molecular weight (18000 and 12600 respectively). The entanglement of molecules makes the transport of electrons difficult, which leads to the lower conductivity. Since T1307 has a branched structure, the steric effect facilitates the transport of electrons, and hence the conductivity is higher than that of F77. The L1695 is of low molecular weight (Mw=561.1). The fewer associations in the surfactant solution make the transport of electron easier, which is the reason for the higher conductivity of the solution. The conductivity results are consistent with the viscosity results that will be displayed later.
4.1.3 Viscosity Measurements

Similar to conductivity measurement, viscosity measurement can also reflect the structure change of microemulsion, and be served as guidance for locating the bicontinuous region of the single phase Winsor-IV microemulsion. Viscosity measurements of the microemulsion samples were performed at as a
function of increasing aqueous content of the microemulsions. All the measurements were made at 22 ± 0.5 °C.

The viscosity data is plotted as a function of aqueous phase weight fraction in Figure 4.5. The trend of viscosity change is similar for all the three surfactant stabilized microemulsions.

It is well documented that viscosity of microemulsion is structure dependent. Discrete W/O droplets are formed at low aqueous content. The viscosity of W/O microemulsion is close to the oil phase. As show in the figures, when aqueous content is below 20 wt %, the viscosity increase slightly as the increase of aqueous content. This suggests that addition of water increases the droplet number, which results in less mobility in the system and higher viscosity of microemulsions. When the aqueous content increases to a critical value, abrupt increase of viscosity is always observed for most of the microemulsion. At this region, bicontinuous structure begins to form, and the structure of reverse micelle changes to bicontinuous network. The interconnected network leads to the significant decrease of mobility of molecules, which displays as the sharp increase of viscosity in macroscopic level. On account of the non-uniform phase behavior, all the three systems are discontinuous. But the viscosity of microemulsion changes greatly within the data range, and it can still be concluded from the graphs that bicontinuous microstructure is presented in the microemulsions formulated with the three surfactants. However, when the aqueous content reaches around 80 wt % in T1307 and F127 systems, the addition of water causes the collapse of interconnected structure, which is
reflected as a drastic decrease of viscosity with the increase of aqueous content. The viscosity continues to decrease as the water domains per volume formulation increase in number, decrease in size, and become more and more diluted with water. After the bicontinuous structures are all replaced by o/w droplets, the viscosity still undergoes a decrease of viscosity as a result from the dilution effect of adding water into microemulsions. Similar results are obtained for L1695 stabilized microemulsion, except the transition of bicontinuous to O/W microemulsion is observed around 95 wt %.

It is important to note that the viscosity is highly dependent on the nature of surfactant. Disparity in the viscosity of microemulsions formulated with these surfactants was detected. The MW of T1307 and F127 are 18000 and 12600 respectively. The entanglement of long molecular chain makes the surfactant solution much more viscous than the low molecular weight L1695 (MW=561.1) solution. L1695 systems display the lowest viscosity and F127 systems have the highest viscosity. The lower viscosity of T1307 is attributed to steric effect from the branched structure of its molecules. This phenomenon is consistent with that in the conductivity measurement.
The discrepancy between viscosity and conductivity measurements is easily perceivable since the viscosity reflects the interdroplet structure, the amount and strength of the interface, while measurement of conductivity traces structure transitions and is a measure of the size and structure of the aqueous domain. Both viscosity and conductivity results show similar trends to those reported in the literature.\cite{80-83}
4.2 Structure Characterization of Porous Polymers

Bicontinuous region was determined for the synthesis of porous polymers, after the examination of microemulsion precursors. Polymerized samples that were examined by SEM and freezing point depression were 3:1 HEMA/MMA/10% L1695, 3:1 HEMA/MMA/10% T1307 and 3:1 HEMA/MMA/10% F127, and partially neutralized 3:2 AA/MMA/10 % F77 systems.

4.2.1 Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used as a qualitative method to examine the gross morphology of the polymers. During the SEM sample preparation, drying the specimen was always necessary, and the network structure of aqueous sample usually collapsed. The observation of morphology by SEM is only representative of the original material.

The morphology of the polymer microparticles created in the bicontinuous region of all the four systems were examined by SEM. Parts A through D of Figure 4.6 show the characteristic morphology of each system. All the images shown here are from the 4 systems that are used for drug delivery. Part A has a composition, before drying, of 85 wt % aq. solution of 10 % L1695 with a ratio of 3:1 HEMA to MMA. Part B has a composition of 85 wt % aq. solution of 10 % T1307 with ratio of 3:1 HEMA to MMA. Both systems were soft solids before SEM examination. Nanoporous structure was observed from these two images. Part C has a composition of 90 wt % aq. solution of 10 % F127 with ratio of 3:1 HEMA to MMA. Particles were synthesized from this system with an average
particle size of 30 μm. Nano pores were presented in the SEM image. And part D has a composition of 85 wt % aq. solution of 10 % F77 with ratio of 3:2 AA to MMA. Pores with larger scaled pores were formed in this system from the SEM examination.

The polymerization of microemulsions from the systems with W/O structure or bicontinuous structure resulted in the formation of solids. As the aqueous content of the system is increased, the precursor microemulsion transits from a bicontinuous to an oil-in-water structure and the resulting polymeric materials shift from nanoporous solids to dispersed particles.

It is demonstrated in the images that the scale of polymer is significantly larger than that of microemulsion micelles. This result has been explained in our group by the phase separation and droplet coalescence during polymerization.

The morphologies of drug loaded samples were also examined under SEM. Two representatives were selected from the 4 systems that were proposed as drug delivery devices in this study. The morphologies of drug loaded polymeric materials are presented in Figure 4.7. Part A is the 85 wt % aq. 3:1 HEMA/MMA/10 % T1307 system loaded with rhodamine B. Part B is the 90 wt % aq. 3:1 HEMA/MMA/10 % F127 system loaded with β-galactosidase. Both systems maintained the nanoporous structure under SEM, and loading of drug into polymer network didn’t change the morphology of polymeric materials severely.
Figure 4.6 Representative SEM images of Blank Polymers

(A) 3:1 HEMA/MMA/ 10 % L1695 at 4737x
(B) 3:1 HEMA/MMA/ 10 % T1307 at 9467x
(C) 3:1 HEMA/MMA/ 10 % F127 at 4955x
(D) 3:2 AA/MMA/ 10 % F77 at 947x
4.2.2 Pore Size Distribution by Freezing Point Depression

The examination of freezing point depression of water entrapped in the porous systems allows for a quantitative determination of the pore size and distribution. Unlike SEM, this method can keep the integrity of the highly aqueous polymeric samples since it is not necessary to dry the polymer composite. Samples from all four surfactant systems except the partially neutralized system were examined by the freezing point depression method as described in section 3.4.2. The DSC data was converted into a pore size distribution by integrating the area under the DSC curve. The area fraction of the DSC curve at any given temperature represents the fraction of pores that melt at that temperature using
Laplace Equation as expressed in Equation 3.1. The calculations were rounded off to the nearest nanometer size.

*Pore Size Distribution of System II*

The pore size distributions of system I at different aqueous content are displayed in Figure 4.8 - 4.10. The distribution pattern is closely related to the aqueous content, and most of the pore radiiuses are less than 30 nm. Larger pores were observed at the highest aq. (95 wt %). As the aqueous content increase, more flattened distribution patterns were assumed, which indicates higher percentage of large pores.

![Figure 4.8 Pore Size Distributions of 3:1 HEMA/MMA/10 % L1695, Aq=85%](image)
Figure 4.9  Pore Size Distributions of 3:1 HEMA/MMA/10 % L1695, Aq=90%

Figure 4.10  Pore Size Distributions of 3:1 HEMA/MMA/10 % L1695, Aq=95%
Pore Size Distribution of System II

Figure 4.11, 4.12 and 4.13 displays the effect of aqueous content on the pore size distribution of system II. The distributions of pore size at different aqueous contents assume similar pattern as system I. The freezing point depression study indicates that the majority of the pores are in the range of 5-30 nm, and the pore size distribution is highly dependent on the aqueous content in precursor microemulsion. The pore sizes are more uniform at higher aqueous content. A trend of more narrow distribution of pores is observed with increasing aqueous content in the precursor microemulsion.

Figure 4.11  Pore Size Distributions of 3:1 HEMA/MMA/10 % T1307, Aq=85%
Figure 4.12  Pore Size Distributions of 3:1 HEMA/MMA/10 % T1307, Aq=90%

Figure 4.13  Pore Size Distributions of 3:1 HEMA/MMA/10 % T1307, Aq=95%
Figure 4.14, 4.15 and 4.16 show the pore size distribution of microemulsions stabilized by F127 at different aqueous content. The most commonly observed pore size is in the range of approximately 10 - 40 nm. However, some larger pore sizes were also observed. With the increasing of aqueous content, wider distribution of pore radius is observed. This indicates that larger pores formed with increasing of aqueous content. It is interesting that the larger tail in the distribution was observed when the aqueous content increased. This indicates that larger pores begin to form once the system begins to reach the end of the bicontinuous region at the water rich end.

Figure 4.14  Pore Size Distributions of 3:1 HEMA/MMA/10 % F127, Aq=85%
Figure 4.15  Pore Size Distributions of 3:1 HEMA/MMA/10 % F127, Aq=90%

Figure 4.16  Pore Size Distributions of 3:1 HEMA/MMA/10 % F127, Aq=95%
The study of pore size distribution by freezing point depression is significant mostly in the confirmation of the presence of pores rather than the measurement of the exact pore sizes. And the qualitative information from SEM examination was consistent with the FPD study. Both methods verified the existence of nanopores. The presence of nanopores suggests that the polymers derived from microemulsions could have potential pharmacological applications as diffusion controlled drug delivery systems.

4.2.3 Swelling Ratio of System IV

PAA is a pH and electrically sensitive material due to the ionization of carboxyl group. The swelling of PAA could have considerable influence on the release of drugs from the microemulsion system IV based on AA in the precursor.

AA was selected as a comonomer due to its stimuli-responsive nature. F77 was applied as surfactant in the pH-sensitive system. L1695, F127, and T1307 were initially tried to form pH-sensitive system. However, L1695 could not form single phase Winsor-IV microemulsion with the monomer mixture of AA and MMA. Soft solid could not be produced by polymerization of microemulsion of F127, AA and MMA at bicontinuous region. Although polymer monoliths were produced from the microemulsion of T1307, AA and MMA at various compositions, but the monolith did not swell in solution due to the highly protonation of PAA in the basic solution of T1307. The finally proposed pH-sensitive system is System IV formulated with 3:2 AA/MMA and 10% F77 with an aqueous content of 85 wt %. After polymerization, a large amount of water
was observed as squeezed out from the bulk polymer. The possible reasons could be the phase separation during polymerization and the deswelling of PAA in the acidic monomer situation. This system was then partially neutralized to eliminate the possibility of deswelling of PAA. Sodium hydroxide was added to microemulsion precursor at an amount of 0.12 wt % of precursor.

The swelling of partially neutralized system was examined and presented in Figure 4.17. 0.2 M buffer was selected because of the acidity of polymers. Two buffer solutions with different pH were selected to simulate the pH conditions of stomach and intestine.

![Swelling Behavior of System IV in Different Buffer Solution](image)

Figure 4.17 Swelling Behavior of System IV in Different Buffer Solution

Figure 4.21 shows that the swelling of p(AA-co-MMA) is highly dependent on the swelling medium. At low pH (pH=1.2), the polymer did not swell, while the
polymer weighted as 3 times of initial polymer at high pH (pH=6.8). The pKa of PAA is known as 4.5 \cite{84}, therefore PAA could not be ionized at pH=1.2, when pH=6.8, the acrylic acid portion of he polymer chain was ionized which resulted in the chain-chain electrostatic repulsion, and the subsequent swelling of polymer.

This result suggests that system IV displayed swelling behavior dependent on the pH of medium, and this system could be used as drug delivery device for the controlled release of drugs by means of the predetermined swelling of materials in different medium conditions.

4.3 Release Profile Study

All the four systems were polymerized to produce porous polymers for drug delivery. System I and system II were highly aqueous soft-solid, and were envisaged as tablet to deliver enzymes; system III was free-flowing particle suspension for the application in the delivery of lipid or proteins by oral administration or I.V. injection. System IV is an intelligent system to address the challenge in enzyme delivery that only a small portion of enzyme can survive the acidic gastric bypass. By the swelling and deswelling at different medium, the device can realize the release of enzymes in a controlled way. All the systems are formulated with biocompatible components except the crosslinking agent and photo initiator, which are of very small amounts in the precursor.
4.3.1 Drug Release from System III

System III is produced from microemulsion formulated with 3:1 HEMA/MMA and 10 % F127 with an aqueous content of 90%. The polymerized nanoporous particle was stable during storage and could resist dilution to a ratio of 1000. The polymeric surfactant of F127 could form micelles at very low concentration (CMC ~10^{-6} M) with small size (<100 nm), which could be used to explain the superior stability of System III. Most importantly, the system is biocompatible since all the major components are reported to have good biocompatibility, and there is no need to eliminate the large amount of surfactant residuals from reaction matrix. This system is planned to deliver drugs by oral administration. In addition, the suspension is in the form of free-flowing fluid, it is also possible to be applied in drug delivery by I.V. injection.

Rhodamine B was used as a model drug to test the capacity of nanoporous particle suspension as a controlled drug delivery device. Before polymerization, Rhodamine B was dissolved in microemulsion precursor with an amount of $2 \times 10^{-5}$ mol/g precursor. Polymeric particle suspension was formed after photo polymerization. The 10 % F127 surfactant solution with same amount of Rhodamine B in polymer suspension was used here as a control, since F127 solutions are FDA approved for pharmaceutical application.

Figure 4.18 compares the release profiles of Rhodamine released from the blank surfactant solution and from the polymeric drug device. Both systems initially showed a burst release of about 20 % of total Rhodamine B. The blank surfactant solution exhibited faster release of Rhodamine B, and the release of
Rhodamine B reaches 100 % after 20 hours. The high viscosity of F127 solution may be a barrier of the transportation of Rhodamine B, thus the drug is gradually released within 20 hours. However, a lower release level of Rhodamine B was demonstrated by nanoporous polymeric suspension derived from microemulsion. The release rate of Rhodamine B was approximately 4 times lower in the polymeric microemulsion system than in 10 % F127. The nanopores presented in the polymeric materials could provide more surface area than the F127 micelles, and resulted in a more efficient encapsulation of Rhodamine B. At the same time, interconnected nanopores could be acted as a barrier to limit the diffusion of drug.

![Graph showing the comparison of Rhodamine B release from polymer suspension and surfactant solution.](image)

**Figure 4.18 Comparison of Rhodamine B Release from Polymer Suspension and from Surfactant Solution**
The release study indicates that the nanoporous particle suspension can realize the prolonged release of model drug. This nanoporous polymeric suspension is proposed as an attractive device for the delivery of proteins and lipids via oral route given the prolonged release and superb stability of the nanoporous polymer suspension. Moreover, the loading efficiency of hydrophilic drugs is known to be very low in O/W microemulsions, since a majority of drugs is dissolved in the aqueous phase instead of the interior of o/w micelles. Application of nanoporous polymeric suspension for the delivery of lyophilic drugs can protect oil soluble drug within o/w micelles. The lyophilic drugs are not soluble in the bulky aqueous phase, which ensures a higher efficiency of drug loading. Indomethacin was loaded successfully into the polymer suspension as hydrophobic drug. Adding indomethacin at an amount of 0.14 wt % of microemulsion precursor didn’t change the phase behavior during photo polymerization. The characterization of the release of indomethacin from polymerized microemulsion is under the way. Finally, F127 surfactant solution has been recently used as polymeric micelles for the site-specific targeted drug delivery especially for anticancer agents due to its low CMC to resist the dilution of blood stream, the small size and nonionic nature to avoid the rapid elimination from the bloodstream by the reticulum endoplasmic system (RES). This nanoporous polymeric suspension that formulated with F127 and p(HEMA-co-MMA) demonstrated higher efficiency in prolonged release than F127 solution, and is considered to be more efficient in long-circulating site-specific drug delivery.
4.3.2 Release of β-galactosidase from System I and II

β-galactosidase is a very important enzyme in the treatment of digestive disorder. It can help the catalysis of lactose into glucose and galactose. The conventional administration of enzyme tablets provides low efficiency since large amount of enzymes lose activity in the gastric acid.\cite{31} This study developed two nanoporous systems for the protection of encapsulated enzymes. The compositions of system I was similar to that of system II except the surfactants used. Both systems have 85 wt % aqueous content and 3:1 HEMA to MMA. 10 % L1695 was used in system I, and 10 % T1307 was used in system II. The enzyme concentration in releasing medium was measured by Micro BCA. ONPG was used as the substrate in enzyme activity analysis.

The colorimetric mechanism of Micro BCA in protein content analysis is presented in Appendix A. BCA protein analysis is reported to be compatible with surfactants. However, the Micro BCA assay leads to the accumulative release of β-galactosidase close to twice of the initial amount in this study. It may be explained by the interference from the surfactant diffused from polymer network. Keeping other factors the same, polymers without enzyme were tested as blank to identify the effect of surfactant on the accuracy of Micro BCA. Blank and enzyme loaded polymers were suspended in releasing buffer. Aliquots of 2 ml were taken out from these two systems at predetermined time. After reacting with Micro BCA reagent, the absorbance of each sample was measured by UV-vis at 562 nm.
Figure 4.19 Comparison of Micro BCA Blank and Enzyme Loaded Samples for System I

Figure 4.20 Comparison of Micro BCA Blank and Enzyme Loaded Samples for System II
Figure 4.21 compares the absorbance of reacted samples of the blank polymer and enzyme loaded polymer for the systems stabilized by L1695. The absorbance comparison of enzyme loaded T1307 system and blank T1307 system is presented in Figure 4.20. The samples from blank polymers of system I and system II demonstrated increased absorbance over time. This confirms our initial proposition that surfactant is an interference in the Micro BCA assay of protein concentration. Figure 4.21 is the adjusted accumulative release when the absorbance from blank was subtracted from the enzyme loaded system. The final enzyme amount is still higher than 100% of the initial, which suggest the protein and surfactant have synergic effects on the reactions in Micro BCA test.

The activity of enzyme in releasing buffer at different time was tested using ONPG as substrate. The apparent activity of enzyme from system I and system II as function of time released is compared in figure 4.22.
As shown in Figure 4.22, both systems exhibited increasing enzyme activity in releasing medium over time. And the gradual increase of enzyme activity eliminates the possibility that enzymes were initially mostly absorbed onto the surface of polymers, while it suggests that the enzyme is encapsulated within porous polymer systems instead. The relatively low activity level indicates there is no severe initial burst in system II, and the initial burst is higher for system I. For both systems, enzyme activity becomes relatively stable after 6 hours. Enzymes released from system I exhibited a higher activity level than those from system II. This could be attributed to the basic nature of T1307 solution. The enzyme we used in the study is β-galactosidase from Aspergillus oryzae. The activity of this enzyme is highly dependent on pH of medium. It is known in
literature that the enzyme displays maximum activity at pH around 5, and, after pH=5, the activity decreases as the pH increases.\cite{87} Since the pH of T1307 is around 8, the basic microemulsion precursor resulted in the decreased enzyme activity.

Since the Micro BCA test could not provide a reliable measurement of how much enzyme was released at different time intervals, the specific activity (units/gram) of enzyme could not be calculated. However, the activity test could be also used as an indicator to confirm that enzymes are gradually released from these two systems and the release reaches a plateau after six hours. Both systems demonstrated the capacity of controlled release of drugs encapsulated. Although the enzyme accumulative amount from system II is at slightly higher level than system I, system II could not be concluded as more efficient for drug release than system I, considering the inaccuracy of enzyme concentration measurement. However, system I displayed higher enzyme activity level and is considered more suitable for the delivery of enzymes.

4.3.3 Lipase Release from System IV

The most difficulty in enzyme delivery is the acidic stomach environment that denatures a large portion of enzymes. A lot of efforts have been made to address this problem. The above mentioned system I and system II can protect enzyme to a certain degree. In this study, a more advanced stimuli responsive system is developed especially as enzyme delivery device that can protect the enzyme at acidic environment and can facilitate the enzyme release to intestine
at neutral pH environment. To this end, AA/MMA/10 % F77 with an aqueous content of 85 wt % was synthesized and characterized as the pH-sensitive systems. From the swelling behavior in section 4.2.3, this system displayed capacity of changing swelling ratio at different medium. In this section, the lipase was loaded into polymer network by mixing enzyme with microemulsion precursor. The enzyme encapsulation formed directly after photo polymerization. Lipase release study was conducted at pH=1.2 KCl-HCl buffer (0.2M) and pH=6.8 phosphate buffer (0.2M).

The current protein content colorimetric assays could not eliminate the interference caused by surfactants diffused from polymer matrix. Although the enzyme amount released from protein over time is very important, the lipase amount measurement in was also not reliable, and isn’t displayed in this section. Enzyme activity is the most import indicator of enzyme efficiency, and it is not interfered by the presence of a very small amount of surfactant. The apparent activity released from system IV into different buffers was measured using pNP-Valerate as substrate. The comparison of enzyme activity at two pH conditions is presented in Figure 4.23.

Since lipase was denatured at pH=1.2, no activity was detected from the enzyme in releasing buffer. When pH was 6.8, the activity of lipase in releasing buffer was stepwisely increased over time. The relatively high activity level of lipase released into phosphate buffer at 1 hour implied the presence of initial burst. The increase of activity over time suggests the continuous releasing of lipase from polymer matrix. Lipase activity was stable after 6 hours, and this time
scale matches with the length of digestion. And this system could be used as pH controlled drug delivery device.

![Graph](image)

**Figure 4.23 Comparison of Activity of Lipase Release from System IV at Medium of Different pH.**

Due to the denature of enzymes, no information was provided regarding the releasing of enzymes into medium at pH=1.2. The results in the releasing study are of great significance for the application of the pH sensitive system for the treatment of digestive disorder by digestive enzymes. The totally lost of activity of lipase in pH=1.2 medium indicates that this type of enzymes couldn’t survive the gastric bypass if they are administrated directly. The porous system showed increased activity over time in pH=6.8, that is to say the enzymes can maintain some level of activities in small intestine. This experiment method is undergoing an improvement to make it more close to the way in which digestion
system works. The enzyme loaded sample will be suspended in pH=1.2 medium for two hours and then transferred to the medium with a pH of 6.8 for four hours. This method is more efficient in determining how much enzyme could survive the acidic bypass, and what the activity level of the remaining enzyme is. Besides, this method offers a direct way to estimate the capability of pH-sensitive in protecting enzymes from acid bypass.
CHAPTER V

SUMMARY

5.1 Summary

The three biocompatible surfactants: L1695, T1307 and F77, could be used to form Winsor-IV microemulsions. Each surfactant displayed the capacity to emulsify highly aqueous systems. Increasing HEMA content and decreasing MMA content facilitated the formation of single phase microemulsion. Conductivity and viscosity measurements could be applied to give an insight into the structure of microemulsion. And these measurements confirmed that W/O micelles were formed at low aqueous content, O/W microemulsions were formed at highly aqueous content. Bicontinuous microemulsions were formed in the intermediate region. They displayed very different conductivity and viscosity properties from O/W and W/O microemulsions.

The microemulsion in bicontinuous region located by precursor microemulsion study could be photo polymerized to form porous structured materials. SEM was a qualitative method to investigate the morphology of polymers derived from microemulsions. Nanopores were observed from the system formulated with 3:1 HEMA to MMA and surfactants of L1695, T 1307 as well as F127. The non-invasive freezing point depression method was extremely
useful in the study of highly aqueous system. The SEM morphology examination was consistent with FPD results. For all these three systems, the radiiuses of nanopores presented were mostly in the range of 10-50 nm. And the aqueous content could significantly change the size distribution of nanopores. The stimuli-responsive exhibited micropores under SEM. Additionally, the incorporation of drug didn’t change polymer microstructure.

The nanoporous polymer particle suspension was very stable and could resist the dilution to 1000 times. This system exhibited controlled release profile of Rhodamine B. The release rate was four times lower than the drug loaded to 10 % F127 solution.

Nanoporous monoliths derived from L1695 and T1307 stabilize microemulsions could realize the gradual release of β-galactosidase within 6 hours, and, after 6 hours, the release became stable. Enzymes released from L1695 system had higher apparent activity due to the favorable pH to enzyme.

The partially neutralized system demonstrated swelling behavior dependent on the pH of aqueous medium. Lipase lost activity in pH=1.2 medium, while lipase released from system IV in pH=6.8 buffer showed increased activity over time. The release of lipase became stable after 6 hours.

5.2 Recommendations for Future Work

The following suggestions are proposed for further research related:
1 Polymer Structure Characterization by Cryo-TEM

SEM always requires the drying of samples which destroys the structure of sample. Cryo-TEM is the fast growing technique involves the frozen of hydrated samples with high resolution, and it works at extremely low electron illumination condition to avoid the damage from water. Cryo-TEM can be applied to observe the structure of our aqueous samples without drying. The polymer size and structure are whereby maintained, more precise information regarding the precursor and polymer microstructure could be provided.

2 Isothermal Reactor

The microstructure of partially neutralized system is different from the other three systems. Addition of sodium hydroxide is to produce uniform structure without water phase separation. However, the micelle structure may have undergone changes as a consequence. Temperature induced separation could be a possible reason for the water that was squeezed out from polymer matrix during polymerization. Isothermal reactor could maintain the reaction temperature throughout the reaction, and may eliminate the phase separation induced by temperature. Isothermal reactor could also minimize the loss of enzyme activity by the reason of increased temperature during polymerization, which has significant effect on the efficiency of enzyme delivery.

3 New Methods to Analyze Protein Content

Enzyme content in releasing medium is very important for the rate of enzyme releasing and the later modification of polymeric carriers. However, the colorimetric methods of Bradford, Better Bradford, BCA and Micro BCA couldn’t
eliminate the interference from surfactants that diffused from polymer matrix. High performance liquid chromatography (HPLC) can use the UV detector to measure the absorbance at 280 nm (protein shows absorbance at 280 nm). By identifying the absorbance peak at different elution time, the concentration of corresponding substances could be calculated according to the standards. Since enzymes and surfactants have different molecular weights, their elution times are different. For this reason, the interference from surfactant could be avoided.

4 Multifunctional Enzyme Delivery

A combination of enzymes is usually used for the treatment of digestive disorder due to need for digesting different substances. Enzymes that can digest protein, fat and carbohydrate can be delivered at the same time to satisfy the different demands.

5 Poly(ethylene glycol) dimethyl acrylate (PEGDMA) as crosslinker

The crosslinker used in this study is not biocompatible. The residual of EGDMA could have adverse effect on drug delivery devices. PEGDMA is a biocompatible crosslinker. In addition, the long PEG chain can control the diffusion of monomers, and accordingly control the morphology of polymers. The release of drugs within polymer matrix could consequently be adjusted by changing the morphology.
BIBLIOGRAPHY


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APPENDIX

MECHANISM OF PROTEIN ASSAY

In this study, the enzyme concentration was measured based on the colorimetric methods, including Bradford, Better Bradford, BCA, and Micro BCA. Among these, Bradford and Better Bradford are Coomassie Dye-based Protein Assays with different working range. BCA and Micro BCA are also different in the working range. The mechanism of Coomassie Dye-based Protein Assays and BCA based Protein Assays will be illustrated in the following section in accordance with the information provided by Pierce.

1. Coomassie Dye-based Protein Assays

Dr. Marion Bradford in 1976 first reported the use of Coomassie G-250 Dye in a colorimetric reagent for the detection and quantization of total protein. Since protein with the presence of certain basic amino acids (primarily arginine, lysine and histidine) can binds to the Coomassie dye in the acidic environment of the reagent, which results in a spectral shift from the reddish/brown form of the dye (absorbance maximum at 465 nm) to the blue form of the dye (absorbance maximum at 610 nm) as shown in Figure A.1. The blue color Coomassie dye-protein complex is measured at 595 nm which is the greatest difference between
these two forms. The blue color can be also measured at any wavelength between 575 nm and 615 nm. At the two extremes (575 nm and 615 nm) there is a loss of about 10% in the measured amount of color (absorbance) compared to that obtained at 595 nm. Free amino acids, peptides, and low molecular weight proteins (Mw < 3,000 Daltons) do not produce color with Coomassie dye reagents. The number of Coomassie dye ligands bound to each protein molecule is approximately proportional to the number of positive charges found on the protein.

Figure A.1 Reaction Schematic for the Coomassie Dye-based Protein Assays[89]

Coomassie dye binding assays are the fastest and easiest protein assays. The assay is performed at room temperature and no special equipment is required. However, Coomassie based protein assays is incompatible with surfactants at concentrations routinely used to solubilize membrane proteins, which is the major disadvantage of these assays. The presence of a surfactant in
the sample, even at low concentrations, causes precipitation of the reagent. Additionally, a small number of proteins can not be assayed with this reagent due to their poor solubility in the highly acidic medium.

2. BCA-based Protein Assays

BCA is a highly sensitive and selective colorimetric detection reagent reacts. It has become the most popular method for colorimetric detection and quantization of total protein since its introduction by Paul K. Smith, et al.\textsuperscript{[90]} in 1985.

The BCA Protein Assay is comprised of two steps. In the first step, blue colored complex is formed due to the chelation of copper with protein in an alkaline environment, as shown in Figure A.2. The second step is known as the biuret reaction. In this reaction, peptides containing three or more amino acid residues form a colored chelated complex with cupric ions in an alkaline environment containing sodium potassium tartrate. In biuret reaction, BCA reacts with the cuprous cation (\(\text{Cu}^{1+}\)) formed in step 1, and the purple colored reaction product is developed by the chelation of two molecules of BCA with one cuprous ion. The water-soluble BCA/copper complex exhibits a strong linear absorbance at 562 nm with increasing protein concentrations. The rate of BCA color formation is dependent on the incubation temperature, the types of protein present in the sample and the relative amounts of reactive amino acids contained in the proteins.
The BCA Protein Assay is compatible with most surfactants (even if present in the sample at concentrations up to 5%), which is the primary
advantage of BCA over other protein assays. The BCA Protein Assay, as a simple protein assay, provides one of the most accurate measurements of protein concentration in biological samples. BCA Protein Assay formulations have less protein to protein variation than the Coomassie based assays.

Substances that reduce or chelate copper will also reduce color produced in the BCA assay, thus interfere with the accuracy of the protein quantization. Certain single amino acids (cysteine or cystine, tyrosine and tryptophan) will also produce color and interfere in BCA assays.