INCLUSION FORMATION IN POLY(LATIC-CO-GLYCOLIC ACID) MICROSPHERES

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ABSTRACT

ELIZABETH LAUREANO: Inclusion Formation in Poly(lactic-co-glycolic acid) Microspheres
(Under the direction of David Needham)

In polymer encapsulated drug delivery systems, degradation and drug release is
defined by the material and process parameters used to make the microsphere carrier.
Poly(lactic-co-glycolic acid) (PLGA) microspheres were systematically prepared by solvent
extraction/evaporation and micropipette manipulation with different polymer concentrations,
solvents, and surfactants to define the material systems that developed water inclusions.
Acidity profiles were then modeled based on inclusion formation within the microsphere.
The experiments revealed more inclusions were formed with greater polymer-in-solvent
solution concentration as well as higher mutual solubility of solvent and water. Unlike
previous works in literature, the results describe explicitly the material conditions when
inclusions form within a size distribution and during formation.
ACKNOWLEDGEMENTS

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<tr>
<td>A</td>
<td>area</td>
</tr>
<tr>
<td>c</td>
<td>concentration</td>
</tr>
<tr>
<td>$C_s$</td>
<td>concentration of droplet material considered to be saturated</td>
</tr>
<tr>
<td>CSA</td>
<td>cross sectional area</td>
</tr>
<tr>
<td>$C_0$</td>
<td>initial concentration</td>
</tr>
<tr>
<td>CMC</td>
<td>critical micelle concentration</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>d</td>
<td>diameter</td>
</tr>
<tr>
<td>D</td>
<td>diffusion coefficient of the solute</td>
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<td>EtOAc</td>
<td>ethyl acetate</td>
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<tr>
<td>$f$</td>
<td>concentration ratio of initial to saturate solute</td>
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<tr>
<td>G</td>
<td>Gibbs free energy</td>
</tr>
<tr>
<td>[G]</td>
<td>glycolic acid concentration</td>
</tr>
<tr>
<td>[H$^+$]</td>
<td>hydrogen ion concentration</td>
</tr>
<tr>
<td>[HA]</td>
<td>hydronium concentration</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Hildebrand solubility parameter</td>
</tr>
<tr>
<td>$\Delta$ H</td>
<td>heat of evaporation</td>
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<tr>
<td>HFIP</td>
<td>Hexafluoroisopropanol</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>surface free energy</td>
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<tr>
<td>$\kappa$</td>
<td>diffusion coefficient</td>
</tr>
<tr>
<td>$K_A$</td>
<td>acid ionization constant</td>
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<tr>
<td>[L]</td>
<td>lactic acid concentration</td>
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<tr>
<td>m</td>
<td>mass</td>
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<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>n</td>
<td>number of molecules</td>
</tr>
<tr>
<td>$N_i$</td>
<td>number of particles composing the $i$th chemical component</td>
</tr>
<tr>
<td>o/w</td>
<td>oil-in-water emulsion</td>
</tr>
<tr>
<td>$\phi$</td>
<td>solubility</td>
</tr>
<tr>
<td>$\phi_w$</td>
<td>solubility of water in solvent</td>
</tr>
<tr>
<td>$\phi_s$</td>
<td>solubility of solvent in water</td>
</tr>
<tr>
<td>p</td>
<td>pressure</td>
</tr>
<tr>
<td>$\rho$</td>
<td>density of the solute</td>
</tr>
<tr>
<td>PLGA</td>
<td>poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PVA</td>
<td>poly(vinyl alcohol)</td>
</tr>
<tr>
<td>Q</td>
<td>heat</td>
</tr>
<tr>
<td>r</td>
<td>radius</td>
</tr>
<tr>
<td>R</td>
<td>ideal gas constant</td>
</tr>
<tr>
<td>S</td>
<td>entropy</td>
</tr>
<tr>
<td>$SA$</td>
<td>surface area</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
</tr>
</tbody>
</table>
\( U \) \hspace{1cm} \text{internal energy}
\( \mu_i \) \hspace{1cm} \text{chemical potential of the } i\text{th chemical component}
\( \nu \) \hspace{1cm} \text{volume fraction}
\( V \) \hspace{1cm} \text{volume}
\( V_m \) \hspace{1cm} \text{molar volume}
\( V_s \) \hspace{1cm} \text{volume of solvent}
\( V_1 \) \hspace{1cm} \text{volume of droplet}
\( V_2 \) \hspace{1cm} \text{volume of solution}
\( V_m \) \hspace{1cm} \text{volume of microsphere}
\( V_w \) \hspace{1cm} \text{volume of water}
\( W \) \hspace{1cm} \text{work}
CHAPTER 1
INTRODUCTION

Drug delivery design is based on the interactions that exist between the material components, structures, and properties during formation. The objective of this thesis is to define the material systems which water inclusions form in a polymer based delivery system prior to encapsulation of drug. Once injected in the body, drug encapsulated polymer microspheres aims to control the release of a drug by maintaining a rate of constant degradation. Uncontrolled inclusion formation arbitrarily increases internal water surface area and complicates hydrolytic degradation. Meanwhile, defining the material without a drug gives a baseline understanding of the system.

In this study, 50:50 poly(D,L-lactic-co-glycolic acid) (PLGA) microspheres were systematically formed by solvent evaporation and micropipette manipulation to assess the effects of polymer concentration, solvent solubility, and surfactant on water inclusion formation. First, microspheres were prepared in bulk by dissolving PLGA in either ethyl acetate (EtOAc) or dichloromethane (DCM) then emulsified and hardened with sodium dodecyl sulfate (SDS) or poly(vinyl alcohol) (PVA) to define water inclusion formation within a microsphere size distribution. Similarly, single PLGA droplets were formed by micropipette manipulation then observed during formation and hardening into microspheres as a function of overall diameter and time.
Relating inclusions to increased internal surface area at the water and polymer interface, an internal acidity theoretical model was made and compared to experimental results by Langer et al. where the author illustrated an acidity gradient inside a microsphere using pH sensitive fluorescent dyes viewed on a confocal fluorescence microscope. The study showed visual evidence of an acidic environment within a degrading PLGA microsphere, specifically the pH inside a 40µm PLGA microspheres was established to be a minimum 1.5 pH (Fu 2000).

Greater polymer-in-solvent concentration, higher mutual solubility of water and solvent, plus use of surfactants were predicted to increase the number of inclusions within a size range of microspheres based on studies resulting in porous formation. Siepmann et al. pointed out pores, the outcome of inclusions, altered the mass transport mechanisms in a 50:50 PLGA microsphere and increased drug release (Klose 2006). Park et al. demonstrated that pores formed in PLGA microspheres during rapid solvent removal in formation (Crotts 1995). Discussed in later in detail, the rate of solvent removal can be altered by changing polymer concentration, solvent solubility, and critical micelle concentrations of a surfactant used during microsphere formation. In addition, factors including solvent removal rate, volume ratio of oil phase to internal water phase, and polymer concentration influenced release profiles in a study by Chen et al. PLGA microspheres encapsulated with bovine serum albumin (BSA) were made by double emulsion solvent extraction/evaporation. Slow mixing resulted in no pores whereas faster mixing resulted in the opposite. Microspheres made with low volume ratio of oil phase to internal water phase and a low polymer concentration were more likely to have a large surface area and lower density resulting in a high initial burst and a faster release of BSA (Yang 2000).
1.1 Drug Delivery Systems

When a drug is administered into a patient, there is a therapeutic interval between toxic and minimal effectiveness levels. In a single dose, the level can be administered high to prolong effect or low in repeated dosage for the same result. Controlled release drug delivery systems are designed to release a pharmaceutical agent over a set time period after a single dose into the body for a therapeutic effect. They allow dosage within a safe therapeutic range while improving effectiveness by targeting higher levels of release against specific areas and minimizing cytotoxicity on normal tissue (Willmott 1994). Furthermore, patient comfort and compliance are improved with less frequent and overall dosages (Ratner 2004, Langer 1998). Spansule®, the first commercial drug delivery system, was introduced in the late 1940s to extend the effectiveness of an orally administered drug (Burness 1955). Drug microspheres were covered with a range of soluble coating thicknesses to vary dissolution time and release (Allen 2005). While oral intake has become the most common type of drug delivery system, new proteins and peptides being developed as drugs are highly sensitive to the conditions in the gastrointestinal system. Trehan et al. lists examples of protein based formulations including leuprolide acetate to stimulate the production of progesterone in men with prostate cancer and recombinant human growth hormone for idiopathic short stature (Sinha 2003). Release kinetics must now consider large molecular mass, protein stability, and the high acidic pH and proteolytic enzymes in the body’s enzymatic digestive environment to the design (Langer 1998). One solution is subcutaneous injection. Subcutaneous delivery avoids the biological barriers of the digestive system and is easier to administer to the patient. Drug is injected under the skin and moves from the blood vessels into the bloodstream whereas other methods involve potential enzymatic breakdown.
of the drug by the digestive system. Upon subcutaneous injection, biodegradable polymer hydrolysis results in sustained drug release with end products that can be processed naturally by the body as metabolites (Ratner 2004). The combination of subcutaneous injection and polymer encapsulation of drug meets many of the requirements of protein and peptide based drug delivery systems.

Numerous works on the release profile and activity level of protein encapsulated polymer microspheres have been published (Alonso 1994, Capan 1999, Crotts 1998, Yan 1994, Yang 2001). It has been shown that polymer encapsulation protects protein from loss of activity, and results depend not only on the drug but the encapsulation system. Langer et al. showed us horseradish peroxidase at 37°C decreased 80% activity within a few days whereas PLGA microsphere encapsulated horseradish peroxidase retained greater than 55% activity (Cohen 1991). A study by Venkatraman et al. detailed deviations from expected biphasic diffusion-controlled and degradation-controlled drug release. PLGA chemistry and crystallinity were shown to govern drug release based on the rate of degradation and water absorption (Frank 2005). Schwendeman et al. visualized pores using scanning electron microscopy and laser scanning confocal microscopy giving evidence that pores can open and close during formation and within physiological conditions (Kang 2006). These studies show the potential for polymer drug encapsulation and the challenges faced in predicating drug release. By focusing on the system initially without drug, this thesis creates a baseline for understanding the formation of pores prior to addition of a drug.
1.2 Microsphere Formation

Microparticles, defined to be in the range of 1 – 1000 micrometers, contain drug molecules in solid or liquid form as microspheres or microcapsules. Microspheres have drug dispersed throughout a microparticle whereas a microcapsule has a core surrounded by a different material (Birnbaum 2003). Microspheres, the focus of this study, need to be a magnitude larger than the encapsulated drug and less than 100 microns (0.1mm) to pass through a 25-gauge (0.26mm OD) needle for subcutaneous injection (Pope 2002).

Techniques are continually being developed for microsphere formation including polymerization, phase separation, solvent extraction/evaporation, and mechanical processing with nozzles or spray dryers. The ideal protocol is repeatable in ambient room conditions at atmosphere pressure. Polymerization is a solution of monomers solidified by chemical reactions to form a polymer chain and entrap drug. Alternatively, phase separation is polymer precipitated around a drug (Willmott 1993). The most cited protocol is solvent evaporation, introduced over 40 years ago (Jain 2000, O’Donnell 1997, Rosilio 1991, Yeo 2004). First, a biodegradable polymer is dissolved in an organic solvent to make an oil phase. The polymer-in-solvent solution is added to water with a surfactant above its critical micelle concentration to make an oil-in-water (o/w) emulsion. This emulsion is now transferred to a large quantity of water with or without surfactant causing volume extraction of the solvent. Combined with stirring, the solvent evaporates into the air and water interface hardening the polymer-in-solvent droplets into solid polymer microspheres. Changes in the preparation method result in different polymer microsphere morphologies including works by Chien et al on sheer force in the primary emulsion, Yaszemski et al on

1.3 Polymers

Originated by Swedish chemist Jons Jakob Berzelius in 1833, the name polymer was derived from the Greek terms ‘poly’ and ‘meros’, meaning ‘many’ and ‘parts’ respectively. There are two types of polymer, natural and synthetic. Natural polymers include rubber, protein, and cellulose (Ratner 2004). In 1920, German chemist, Hermann Staudinger, had a novel idea that combining multiple small molecules would make polymer macromolecules, and this began the rapid progress of synthetic polymers (Gillespie 1994). Numerous polymers have been used for protein and peptide delivery. Now, the most common biodegradable material in medicine is the synthetic polymer, poly(lactic-co-glycolic acid) (Gombotz 1995). Poly(lactic-co-glycolic acid) (PLGA) is a synthetic copolymer, defined as a macromolecule made of repeating structural units of lactic and glycolic acid connected by covalent bonds as shown in Figure 1.1.

![Chemical structure of poly(lactic-co-glycolic acid).](image)

Figure 1.1 Chemical structure of poly(lactic-co-glycolic acid).

The structure is based upon the physical arrangement of the units including monomer identity, chain linearity, and chain length. The angles between single bonded carbon (C-C) atoms are 109° and 0.154 nm in length, forming the zigzag pattern seen in Figure 1-1. These bonds are able to rotate and bend, forming various ‘bends, twists, and kinks’ (Callister 1991).
Lactic acid has a chiral center, not superimposable on its mirror image, and exists in one of four stereoisomer forms, poly(L-lactic acid), poly(D-lactic acid), meso-poly(D,L-lactic acid), and a racemic mixture of poly(L-lactic acid) and poly(D-lactic acid) (Ratner 2004). The structure of PLGA can be changed based on molecular weight, lactic to glycolic ratio, and sequence of constitutional units. For a single polymerization process, the average molecular weight is defined by the distribution of chain lengths and determined based on physical properties such as viscosity (Callister 1991). Lactic to glycolic ratio is the percentage of each monomer unit whereas constitutional units can be arranged in alternating, periodic, random, or block patterns (Ratner 2004). The structure of PLGA lends to its strength and biodegradable properties for use in drug delivery.

Poly(lactic-co-glycolic acid)(PLGA) degrades by hydrolysis of its ester linkage into lactic and glycolic acids. Both lactic and glycolic acids are produced naturally by cells during anaerobic metabolism and are removed by the liver making PLGA an ideal choice in terms of biocompatibility. The erosion rate of PLGA is controlled by the ratio of poly(lactic acid)(PLA) to poly(glycolic acid)(PGA).

![Poly(lactic acid) and poly(glycolic acid) monomer units.](image)

Degradation is faster with greater PGA content because PLA has an extra chiral methyl group making it more hydrophobic, but there is normally no more than fifty percent PGA because higher concentrations increase toxicity (Birnbaum 2003). On the other hand, PLA
erosion is difficult to control. A purely PLA microsphere will initially erode on the surface then inward. The inside of the microsphere will then become more acidic, causing greater internal degradation and an outside shell to form. As the shell becomes thinner, the inside oligomers diffuse through, and the entire microsphere becomes soluble within the external aqueous solution (Ratner 2004). This study only used 50:50 PLGA, but future work can be expanded to include other lactic to glycolic acid ratios.

Previous studies have shown changes in polymer modified morphology, size, and burst release, specifically variations in the polymer type (Capan 1999), polymer concentration (Brodbeck 1999, Frank 2005, Mao 2007, Yeo 2004), and lowering the molecular weight (Alonso 1994, Viswanathan 2001). Yaszemski et al determined microsphere surface morphology was affected primarily by polymer viscosity (Kempen 2004). Consequently, a higher viscosity polymer requires greater solvent volume ratios in solvent evaporation (Godbee 2003).

1.4 Surfactant

Surface tension results from intermolecular attractive forces and reflects the energy necessary to increase the area of a surface. When PLGA microspheres are made in an emulsion, surfactants prevent polymer microspheres from coalescing by lowering the surface tension (Birnbaum 2003). Surfactants are amphipathic compounds, meaning they contain both hydrophobic and hydrophilic groups. The concentration which surfactants form micelles is known as the critical micelle concentration (CMC) and is dependent on the surfactant. Below the CMC, surfactant molecules are loose monomers in the solution. At concentrations above the CMC, the hydrophilic ends of a surfactant
create an interface with water. The hydrophobic ends attract one another and result in a micelle structure. A common example is laundry detergent. Oil and dirt are trapped in the inside of the micelle and are easier to wash away. For an oil-based solution, the opposite occurs and the hydrophobic ends are attracted to the outside surface resulting in a reverse micelle (Hiemenz 1997).

Surfactants and their effects on surface tension can be described by the Gibbs free energy equations. Beginning with the first law of thermodynamics,

“During an interaction between a system and its surroundings, the amount of energy gained by the system must be exactly equal to the amount of energy lost by the surroundings” (Cengel 1998).

It is the conservation of energy principle. For a closed system, this is defined by

\[ \delta U = \delta Q + \delta W + \sum \mu_i dN_i \]

where \( \delta U \) is the change in internal energy, \( \delta Q \) is the amount of heat added to the system, \( \delta W \) is the amount of work done on the system by the surroundings, \( \mu_i \) is the chemical potential of the \( i \)th chemical component, and \( dN_i \) is the number of particles composing the \( i \)th chemical component. For a reversible process,

\[
\begin{align*}
\delta Q &= TdS \\
\delta W &= pdV
\end{align*}
\]
\[ \delta U = TdS + pdV + \sum \mu_i dN_i \]

where \( T \) is temperature, \( dS \) is the change in entropy, \( p \) is pressure, and \( dV \) is the change in volume. Per definition, the Gibbs (\( G \)) free energy is the maximum amount of work by a closed system at constant pressure and temperature whose fundamental equation is:

\[ G = U + pV - TS \]

\[ dG = dU + pdV + VdP - TdS - SdT \]

where \( dG \) is Gibbs free energy change. Surface tension is surface free energy for a liquid. It is equal to the work required to produce a new surface (and represented by)

\[ \gamma = \left( \frac{\delta G}{\delta A} \right)_{T,P,n} \]

where \( A \) is area. In terms of surface tension and area, the Gibbs free energy change is

\[ dG = \gamma dA + Ad\gamma \]

A Gibbs free energy change less than zero will cause the surface area to spontaneously get smaller, and mechanical energy such as mixing is combined to break apart the liquid into droplets. For a liquid polymer and solvent droplet in an external aqueous phase, the change in surface area \((dA)\) is defined as

\[ A = 4\pi r^2 \]

\[ dA = 8\pi r dr \]

where \( r \) is radius, and \( dr \) is the change in radius.

Figure 1.4 The cross section of a polymer microsphere (Duncan 2005).
When a surfactant above critical micelle concentration is included in the external aqueous phase, the surface free energy of a polymer and solvent solution decreases to prevent coalescing of the droplets. Various surfactants have been shown to affect polymer microsphere size and morphology (Rosa 2000). Re and coworkers revealed varying PVA concentration from 0.5 to 4 % (w/v) changes the average particle size from an average of 389.20 µm to 39.54 µm (Maia 2004).

Quaglia et al determined that the choice of surfactant affected distribution and morphology (Rosa 2000). In this study, single and bulk polymer droplets were made with surfactants, poly(vinyl alcohol) (PVA) and sodium dodecyl sulfate (SDS), below and above the critical micelle concentration. Shown in Figure 2.5a, PVA is composed of an OH group and carbon chain.

![Chemical structure of poly(vinyl alcohol) and sodium dodecyl sulfate.](image)

The carbon chains are hydrophobic. In PVA, the hydrophilic OH groups bind with water resulting in a structure parallel along an interface potentially creating a thin film. On the other hand, SDS creates a micelle structure. Illustrated in Figure 1.5b, SDS has a twelve carbon atom chain attached to a sulfate group with a negative charge, making it anionic. The hydrophilic sulfate group binds with water, and the carbon chains attract to one another creating a micelle. Walle et al concluded use of SDS increased water absorption of polymer microspheres (Bouissou 2006). At room temperature, SDS has a critical micelle value of 8mM (Quina 1995). The CMC of poly(lactic acid) depends on its molecular weight and percentage hydrolyzed (Blackley 1997).
1.5 Organic Solvent

A solvent is a liquid that can dissolve a solute to make a solution. Solutions are chemical interactions between materials resulting in a homogenous mixture (Gillespie 1994). For polymer microspheres made by emulsion solvent evaporation, solid PLGA solute is first dissolved in a liquid organic solvent. This primary solution is then added to a larger aqueous phase where the solvent is leached out of the polymer and into air. The degree of polymer and solvent interaction can be estimated with the Hildebrand solubility parameter

$$\delta = \sqrt{\frac{\Delta H - RT}{V_m}}$$

where $\Delta H$ is heat of evaporation, $R$ is the ideal gas constant, $T$ is absolute temperature, and $V_m$ is the molar volume. Non-polar materials with similar Hildebrand solubility parameters are likely to be miscible (Gedde 1995).

The organic solvents, dichloromethane (DCM) and ethyl acetate (EtOAc), are ideal options to make polymer microspheres by emulsion solvent evaporation for their solvent properties in PLGA and immiscibility in water.

![Figure 1.6 Chemical structure of organic solvents (a) ethyl acetate and (b) dichloromethane.](image)

<table>
<thead>
<tr>
<th></th>
<th>EtoAc</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility of solvent in water (w/v)</td>
<td>8.7%, slightly soluble</td>
<td>1.60%, immiscible</td>
</tr>
<tr>
<td>Solubility of water in solvent (w/v)</td>
<td>3.3%</td>
<td>0.24%</td>
</tr>
<tr>
<td>Solubility of PLGA in solvent</td>
<td>good solvent</td>
<td>excellent solvent</td>
</tr>
<tr>
<td>Density (g/cm$^3$)</td>
<td>0.897</td>
<td>1.3255</td>
</tr>
</tbody>
</table>

Table 1.1 Solubility and densities of organic solvents in water and PLGA at 20°C
DCM is widely used due to higher polymer solubility and minimal water miscibility, but it has been shown to cause carbon monoxide poisoning when inhaled and metabolized by the body (Fagin 1980). EtOAc is a safe alternative although slightly more soluble in water and not an equivalent solvent for PLGA. Various studies have used ethyl acetate, including multiple works by Sah et al showing the effects on primary size distributions and microsphere morphology (Bahl 2000, Sah 1997). To further understand the conditions under which inclusions form, the relationship between solvent and water is initially defined and later expanded with the polymer system. For nanoparticles, Choi et al determined use solvents with higher solubility with water and PLGA increased mean particle size when formed by solvent evaporation (Song 2005). The rate of solvent removal and ultimately microspheres being formed is controlled by the combined material characteristics of the polymer, solvent, and surfactants used during preparation (Jain 2000).

Equilibrium occurs when there is a uniform distribution of molecules throughout a system. Diffusion of liquid organic solvent molecules into solution can be described by the microparticle dissolution theory (Duncan 2005). The second law of thermodynamics states, "...processes occur in a certain direction, not any direction" (Cengel 1998). Randomly distributed molecules or particles always move from higher to lower concentrations. The derivation of random walk process and Fick’s laws of diffusion describe diffusion (Duncan 2005). For a symmetrical volume of unit passing through a cross sectional area A, and length, Δx, Fick’s second law of diffusion in spherical polar coordinates for a symmetrical system is

$$\frac{\partial c}{\partial t} = \kappa \left( \frac{2 \partial c}{r \partial r} + \frac{\partial^2 c}{\partial r^2} \right)$$
where $dc$ is the concentration gradient, $dt$ is the change in time, $\kappa$ is the diffusion coefficient,
and $r$ is radius. The Epstein-Plesset model description of single gas microsphere dissolution
in an infinite liquid was modified for a liquid droplet with the initial conditions that molecule
velocity can be neglected compared to diffusion and concentration effects. The result is the
microparticle dissolution theory,

$$\frac{dR}{dt} = -\frac{DC_s(1-C_o/C_s)}{\rho \frac{1}{R} + \frac{1}{\sqrt{\pi Dt}}}$$

where $R$ is the radius, $t$ is time, $D$ is the diffusion coefficient of the solute, $C_s$ is the
concentration of droplet material considered to be saturated, $C_o$ is the initial concentration,
and $\rho$ is density of the solute. The initial dissolved solute concentration ratio to the
concentration at saturation can be defined as $f$ where

$$f = \frac{C_o}{C_s}$$

The following graph compares the theoretical dissolution times of DCM and EtOAc per the
model (Duncan 2005).

Figure 1.7 Epstein-Plesset model of DCM and EtOAc dissolution time.
When polymer and solvent droplets are made, organic solvents, ethyl acetate (EtOAc) and dichloromethane (DCM), diffuse out to the external aqueous phase and evaporate through the water-air interface (Birnbaum 2003). The solubility of each solvent controls the extraction rate and thus the morphology of the resulting microsphere. As the solubility of a solvent increases in water, it will leave faster and result in highly porous polymer microspheres whose degradation and thus mechanism become difficult to control. DeLuca et al creates a mathematical model based on material composition to simulate the solvent extraction/evaporation method and predict microsphere morphology (Li 1995).
CHAPTER 2
MATERIALS & METHODS

2.1 Materials

Polymer, 50:50 poly(DL-lactic-co-glycolic acid) by Lactel Absorbable Polymers (7400kD MW, B6010-4P, 0.17 dL/g IV in HFIP) was dissolved in organic solvents, ethyl acetate (99.8% HPLC grade, Aldrich) or dichloromethane (Fluka 66740), and emulsified with poly(vinyl alcohol) (86-89% hydrolyzed, 57-66kD MW, Alfa Aesar, 41239) or sodium dodecyl sulfate (Fluka, 71725). The manufacturer supplied the molecular weight based on lot number using gel permeation chromatography. Each organic solvent molecular weight and density was based on company specifications whereas solubility values were found in the literature at 20°C (Knovel 2003).
Table 2.1  Summary of the material compositions used to form polymer microspheres.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Organic Solvent</th>
<th>Surfactant</th>
<th>CMC Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg PLGA</td>
<td>Dichloromethane</td>
<td>Sodium dodecyl sulfate</td>
<td>1 mM (&lt;&lt; CMC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 mM (&lt; CMC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 mM (&gt; CMC)</td>
</tr>
<tr>
<td>100 mg PLGA</td>
<td>Dichloromethane</td>
<td>Poly(vinyl alcohol)</td>
<td>1% PVA</td>
</tr>
<tr>
<td>100 mg PLGA</td>
<td>Ethyl Acetate</td>
<td>Sodium dodecyl sulfate</td>
<td>1 mM (&lt;&lt; CMC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5 mM (&lt; CMC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 mM (&gt; CMC)</td>
</tr>
<tr>
<td>100 mg PLGA</td>
<td>Ethyl Acetate</td>
<td>Poly(vinyl alcohol)</td>
<td>1% PVA</td>
</tr>
<tr>
<td>200 mg PLGA</td>
<td>Dichloromethane</td>
<td>Sodium dodecyl sulfate</td>
<td>1 mM (&lt;&lt; CMC)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>10 mM (&gt; CMC)</td>
</tr>
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<tr>
<td>200 mg PLGA</td>
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</tr>
<tr>
<td>400 mg PLGA</td>
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<td>Sodium dodecyl sulfate</td>
<td>1 mM (&lt;&lt; CMC)</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 mM (&gt; CMC)</td>
</tr>
<tr>
<td>400 mg PLGA</td>
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<td>1% PVA</td>
</tr>
<tr>
<td>400 mg PLGA</td>
<td>Ethyl Acetate</td>
<td>Sodium dodecyl sulfate</td>
<td>1 mM (&lt;&lt; CMC)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td>10 mM (&gt; CMC)</td>
</tr>
<tr>
<td>400 mg PLGA</td>
<td>Ethyl Acetate</td>
<td>Poly(vinyl alcohol)</td>
<td>1% PVA</td>
</tr>
</tbody>
</table>

2.2 Organic and Water Phase Solution Preparation

Organic (o) phase solutions were made by dissolving 100, 200, and 400 mg of poly(lactic-co-glycolic acid) with 2.0 mL organic solvents, dichloromethane and ethyl acetate, in a 3.8mL glass vial (Qorpack, GLC-09098). When not in use, organic phase solutions were capped and wrapped with Parafilm (PM-996) to prevent solvent evaporation. Next, surfactant based water (w) phase solutions of sodium dodecyl sulfate and poly(vinyl alcohol) were prepared with deionized water. At ambient temperature, SDS was made at 1, 5, and 10mM concentrations whereas PVA at 1% (w/v) was prepared at elevated temperatures to aid solute dissolution into water. Specifically, 250 mL of deionized water
in a 500 mL volumetric flask was heated to near boiling point on a Corning Stirrer/Hotplate
(PC-420). While continuously mixing at stir rate of ‘6’ and heat setting of ‘3’, 5 mg of PVA
was slowly added and mixed until homogenous. The remaining 250 mL of deionized water
was then added to the flask and cooled at ambient temperature before being transferred to a
closed container. To examine effects of solubility, polymer-in-solvent and surfactant
solutions were also made pre-saturated with organic solvent. All solutions were stored at
5°C and used at ambient temperature.

2.3 Micropipette Preparation

Glass pipettes were made by pulling glass capillary tubes (0.75mm x 0.4 mm x 6 in,
A-M Systems, Inc. 62550) with a vertical pipette puller (David Kopf Instruments Model
700C/730) of adjustable heater and solenoid, set at 55 and 55 respectively. The microneedle
formed by the vertical pipet puller was mounted in a micromanipulator and viewed under a
light microscope (Bausch & Lomb). The microneedle was positioned near a glass bead
which was heated using a foot pedal, and the tip of the microneedle was inserted into the
molten glass. The glass bead was then allowed to cool for a 5 seconds before being pulled
away to produce an opening at the tip of the microneedle (Duncan 2005). The opening made
in this way is still not perfect. The opening in the pipet is then re-inserted into the molten
glass bead, the glass is allowed to flow up the inside of the pipet for a few microns and then
the bead is cooled. Slight movement of the cooling wires as they contract or a slight pull
back on the holding manipulator then breaks the pipet glass right at the point where the
molten glass column in the micropipette tip solidified producing a perfectly flat tip.
2.4 Micropipette Manipulation System

The micropipette was mounted on a chuck (Research Instruments, Inc.) connected to a pressure control system controlled by a 5ml syringe (Becton Dickinson).

![Figure 2.1 Photograph of the micropipette manipulation system.](image)

2.5 Microsphere Formation by Microsphere Manipulation

A 2 mm microchamber cuvette (Nova Biotech) was filled with surfactant solution and placed onto the microscope stage. A glass pipette mounted on a chuck was then front-filled by dipping the pipette into the glass vial of polymer and solvent solution then applying negative pressure. The filled pipette and corresponding chuck were mounted on the micromanipulator. Once the tip of the pipette was focused within the field of view, video recording began as positive pressure was applied onto the pipette to release material and form polymer and solvent droplets. Pressure was no longer applied when the maximum predetermined microsphere diameter was reached, and recording continued until no size or inclusion formation changes were observed on the microsphere. Times were recorded with details on the material system for examination on the video measurement system.
2.6 Microchamber Assembly

To view bulk prepared microspheres, a microchamber was made with a 3 x 1 inch, 1mm thick glass microslide (VWR, 48300-047) and two No. 1 22 x 30 mm micro cover glass slips (VWR, 48393-026). The two glass cover slips were adhered onto opposite ends of one side of the microscope slide using optical cement leaving approximately 20 mm space for sample placement in the middle. A disposable No.1 22 x 40mm micro cover glass slip (VWR, 48393-048) was adhered to the top with high vacuum grease (Dow Corning, 2021846-0799).
2.7 Bulk Microsphere Preparation by Solvent Extraction/Evaporation

Polymer microspheres were prepared by solvent extraction/evaporation. In a 20 mL scintillation vial (Wheaton, 986546), 200 μL water (W) phase was added to 100 μL organic (O) phase. Holding the vial upright, the contents were vortexed for 30 seconds at speed 10 with a VWR Vortex Mixer to make an oil-in-water (O/W) emulsion (Scientific Industries, Model K-550-G). To harden the microspheres, an additional 10mL water phase was added to the emulsion and mixed with a magnetic stir bar using a Corning mixer (PC-420) on stir rate 10 for 0.5 and 8-hours.
2.8 Bulk Microsphere Sampling

Microspheres prepared in bulk were examined after both vortexing and mixing steps. To determine the effects of vortexing, 0.5mL of emulsion was transferred to a microchamber, and 200 μL of surfactant, pre-saturated with solvent, was added to the sample in the microchamber and viewed on an inverted microscope (Nikon Diaphot TMD). To sample effects once mixed, 1mL of microsphere suspension was transferred into a 1.7mL Posi-Click™ centrifuge tube (Denville, C-2170) and spun at 6000 rpm for 1 minute using a Glaxy Mini Centrifuge (VWR C1213). Because optical microscopes are limited to one micrometer resolution, 850 μL supernatant was removed and analyzed by light particle measurement for submicron-sized microspheres. The remaining concentrated sample of microspheres was re-suspended by gently pipetting the solution and transferred to a microchamber for observation on the inverted microscope. For each different material system, a minimum of 300 polymer microspheres was measured on the video measurement system for diameter, number of inclusions, and shape.

2.9 Light Particle Measurement

Light scattering measurements were taken to determine the sub-micron distributions of polymer microspheres in solution. In a glass cuvette, 1mL of sample and 1 mL of surfactant solution was slowly mixed for even dispersion without creating air bubbles, and then placed into the Zeta Plus Quasi-Electric Light Scatterer. Parameters were set per Table 2.2. Lognormal and MDS data summaries were saved for distribution analysis.
Table 2.2 Equipment parameters for Zeta Plus Quasi-Electric Light Scatterer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Duration</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Temperature</td>
<td>20º C</td>
</tr>
<tr>
<td>Angle</td>
<td>90º</td>
</tr>
<tr>
<td>Wavelength</td>
<td>676.1 nm</td>
</tr>
<tr>
<td>Real</td>
<td>1.6</td>
</tr>
<tr>
<td>Imaginary</td>
<td>0.0</td>
</tr>
</tbody>
</table>

2.10 Video Acquisition & Measurement System

Samples were viewed on an inverted microscope (Nikon Diaphot 200) with a Nikon 10X 0.3 NA objective in air and Nikon 60X 1.4 NA oil immersion. The image was captured onto video with an in-line CCD camera (Hamamatsu, XC-77) and videocassette recorder (Panasonic Omnivision PV-4511) onto a video monitor (Sony Trinitron). The videocassette was transferred to another videocassette recorder (JVC, SR-V101US) and analyzed using a video caliper system (Vista Electronics, 305v).

Figure 2.5 Video Acquisition System
Video calibration was required before each measurement to standardize dimensions. A graticule with 10 μm unit markings was placed onto the microscope stage and adjusted to focus. Recording of the graticule was made on the videocassette recorder and transferred to the video measuring system. When both horizontal and vertical lines were superimposed, the ‘zero’ knob was dialed to establish the baseline at ‘000’. To calibrate for 60X magnification, the left and right ‘vertical’ knob was aligned to the respective top edge 10-μm unit markings per Figure 4-5 then set to ‘010’ on the video caliper system with the ‘calibrate’ knob. For 10x calibration, the video calibration system was set to ‘001’. The calibration of 10 μm for the desired magnification is now completed.
Figure 2.7 Video calibration with 40X magnification graticle.
CHAPTER 3
RESULTS & DISCUSSION

3.1 Effects of Vortexing and Mixing

The solvent extraction/evaporation process is divided into two steps, vortexing and mixing. Illustrated in the following figures, no inclusions were found after the vortex step in droplets prepared with poly(lactic-co-glycolic acid) (PLGA) dissolved in ethyl acetate (EtoAc) and 1% (w/v) poly(vinyl alcohol) (PVA). In comparison, inclusions were observed upon subsequent mixing and dilution of droplets into hardened polymer microspheres.

![Figure 3.1](image)

Figure 3.1 Microspheres prepared by solvent evaporation with 100 mg of PLGA and EtOAc in 1% (w/v) PVA after [a] vortexing and [b] mixing at 10X magnification.
An oil-in-water (o/w) emulsion with polymer-in-solvent droplets was made by vortexing 100 μL organic and 200 μL water phases. The mechanical energy of the vortex step broke apart the organic phase, which the surfactant solution prevented from aggregation and recoalescence to re-form droplets. Due to the solubility of each component, water molecules moved into the droplet and saturated the solvent. Similarly, solvent molecules exited the droplet and dissolved into the external aqueous solution as depicted in Figure 3.4.
Figure 3.4 Schematic diagram of solvent and water molecule diffusion.

The movement of molecules is a function of volume fraction ($v$) and solubility ($\phi$) of both the organic and water phases can be represented by the following set of equations

\[
\begin{align*}
V_s &= v_{s1} + v_{s2} = 100\mu L \\
V_w &= v_{w1} + v_{w2} = 200\mu L \\
\phi_1 &= 1 = \phi_{s1} + \phi_{w1} \\
\phi_2 &= 1 = \phi_{s2} + \phi_{w2}
\end{align*}
\]  

(3-1)

where $V_s$ is the volume of solvent, $V_w$ is the volume of water, $\phi_w$ is the solubility of water in solvent and $\phi_s$ is the solubility of solvent in water. Solving these equations simultaneously, the total volume of water was established in terms of solvent volumes in the microsphere phase ($v_{s1}$) and solution phase ($v_{s2}$).

\[
V_w = 200 = v_{w1} + v_{w2} = v_{s1} \left( \frac{\phi_{w1}}{\phi_{s1}} \right) + v_{s2} \left( \frac{\phi_{w2}}{\phi_{s2}} \right)
\]  

(3-2)

This was used to determine the volume of water ($V_{w1}$ and $V_{w2}$) in each phase.

\[
\begin{align*}
v_{w1} &= v_{s1} \left( \frac{\phi_{w1}}{\phi_{s1}} \right) \\
v_{w2} &= v_{s2} \left( \frac{\phi_{w2}}{\phi_{s2}} \right)
\end{align*}
\]  

(3-3)

The droplet ($V_I$) and surrounding solution ($V_J$) volume was then computed by fulfilling the original equation.
\[ V_1 = v_{st} + v_{wl} \]
\[ V_2 = v_{st} + v_{wl} \]  
(3-4)

The solvent and water volume change in the droplet after vortexing increased polymer concentration by 19.08% and 3.07% for organic solvents, ethyl acetate and dichloromethane respectively. No inclusions formed because the droplet was still in a liquid phase.

After creating the initial emulsion by vortex, an additional 10mL of water phase was added to the system driving further extraction of solvent out of the droplet. Concurrently, mixing of the emulsion facilitates evaporation of solvent at the water and air interface. This removal of solvent from the droplets produced a polymer microsphere suspension.

### 3.2 Surfactant Effect

Microspheres prepared in bulk by solvent extraction/evaporation with surfactants, sodium dodecyl sulfate (SDS) and poly(vinyl alcohol) (PVA), demonstrated identical effects on water inclusion formation. Below critical micelle concentration, the surface tension of the organic phase solution was not adequately lowered to prevent aggregation and produced large, irregular shaped clump masses of polymer. Above the critical micelle concentration, aggregation and coalescence of droplets was prevented and dispersions of microspheres were formed. Inclusions were found in both 10mM SDS and 1% (w/v) PVA prepared microspheres using 100 mg of PLGA dissolved in ethyl acetate as shown in Figure 3.5.
Similarly, Figure 3.6 illustrates the results using dichloromethane as a solvent where little or no inclusions develop in polymer microspheres formed with either 10mM SDS or 1% (w/v) PVA.

The appearance of water inclusions was alike for either surfactant, but the resulting overall suspension differed after centrifugation. Microspheres made with 10 mM sodium dodecyl sulfate in all compositions clumped and could not be resuspended, even with addition of surfactant. As seen in Figure 3.7 for a 100 mg PLGA in ethyl acetate solution emulsified with 10mM SDS, an additional mixing of eight hours showed no effect in re-suspension.
Figure 3.7 Microspheres prepared by solvent evaporation with 100 mg of PLGA to 2 mL EtOAc in 10mM SDS at [a] 0.5 and [b] 8 hour intervals at 10X.

In theory, the 10mM sodium dodecyl sulfate concentration used in the bulk preparation of microspheres is greater than both values and should effectively create a size distribution of polymer microspheres. The number of sodium dodecyl sulfate molecules required to cover the interface of all microspheres in a single batch was significantly less than the amount and concentration of surfactant used to make the emulsion. Based on experimental values, the average microsphere size for any material composition is approximately 10 micrometers.

\[ d_{avg} = 10 \mu M \]
\[ V_{microsphere} = \frac{4}{3} \pi \left( \frac{d_{avg}}{2} \right)^3 \]
\[ V_{microsphere} = 1.258 \times 10^{-9} \text{cm}^3 \quad (3-5) \]
\[ m_{PLGA} = \rho_{PLGA} \times V_{microsphere} \]
\[ m_{PLGA} = 1.535 \times 10^{-9} \text{g} \]

There is 0.05 g of polymer inside a 100 mL solution of 100 mg polymer in 2 mL solvent. Hence, approximately 52 million microspheres were formed in the final emulsion. To effectively prevent aggregation, the surface of each microsphere had to be covered with
sodium dodecyl sulfate molecules. The number of sodium dodecyl sulfate molecules \( n \) for a single microsphere was

\[
S_{A_{\text{microsphere}}} = 4\pi \left( \frac{d_{\text{avg}}}{2} \right)^2
\]

\[
n_{\text{SDS}} = \frac{CSA_{\text{SDS}}}{S_{A_{\text{microsphere}}}}
\]

where \( CSA_{\text{SDS}} \) is the cross sectional area of a sodium dodecyl sulfate molecule. Therefore, approximately \( 1.4 \times 10^{-10} \) mM sodium dodecyl sulfate was required to completely coat all the microspheres with surfactant molecule while 8 mM is critical micelle concentration.

### 3.3 Solubility Effect

The solubility of solvent molecules into water and the reverse, water molecules into solvent, were characterized with organic solvents, ethyl acetate (EtOAc) and dichloromethane (DCM), designated ‘good’ and ‘excellent’ solvents for PLGA respectively (Song 1996). For microspheres made with ethyl acetate, the dynamic movement of solvent and water resulted in our hypothesis that water molecules were trapped inside the polymer microsphere as it hardened generating inclusions. On the other hand, no inclusions were formed with PLGA dissolved in DCM solution emulsified in 1% (w/v) PVA. The following video micrographs illustrate these results at 100, 200, and 400 mg PLGA concentrations.
Figure 3.8 Microspheres prepared by solvent evaporation with 100 mg of PLGA and [a] DCM and [b] EtOAc in 1% (w/v) PVA at 10X magnification.

Figure 3.9 Microspheres prepared by solvent evaporation with 400 mg of PLGA and [a] DCM and [b] EtOAc in 1% (w/v) PVA at 10X magnification.

The same effects can be seen when comparing dichloromethane and ethyl acetate made microspheres emulsified with 10mM sodium dodecyl sulfate in Figure 3.10.
Figure 3.10 Microspheres prepared by solvent evaporation with 100 mg of PLGA and
[a] DCM and [b] EtOAc in 10mM SDS at 10X magnification.

Again, there is little to no water inclusions in dichloromethane while there are more in ethyl acetate. The choice of solvent varied the solubility with either surfactant solution and changed the rate of solvent removal.

Further evaluation of microsphere formation by micropipette manipulation produced equivalent results. Initially, droplets of ethyl acetate and dichloromethane were made in deionized water to evaluate solubility of the pure solvents without polymer. For each solvent, 50 μm diameter droplets were made and measured in triplicate then compared to theoretical values. While being formed by micropipette, the droplet diameter was measured by calibration of video micrographs. To generate the Epstein-Plesset model for dissolution, the diffusion coefficient found in literature were $2.9 \times 10^{-5}$ cm$^2$/s for dichloromethane and $1.4 \times 10$ cm$^2$/s for ethyl acetate (Duncan 2005). In Figure 3.11 and Figure 3.12, the experimental droplet diameters for ethyl acetate and dichloromethane are plotted alongside the Epstein-Plesset based model for liquid droplet dissolution as a function of time respectively.
Figure 3.11 Diameter of a single 50 micron microdroplet of dichloromethane in deionized water dissolving as a function of time at the tip of a micropipette in comparison to the Epstein-Plesset model.

Figure 3.12 Diameter of a single 50 micron microdroplet of ethyl acetate in deionized water droplet diameter as a function of time by micropipette compared to the Epstein-Plesset model.

In both plots, the experimental droplet diameters diverged from Epstein-Plesset theory with greater time. The inaccuracy is caused by error made in the theoretical model and is low when comparing the magnitude of dissolution time between dichloromethane and ethyl
acetate. The dissolution lifetime of dichloromethane and ethyl acetate is approximately 15 and 4 seconds respectively for a 50 μm droplet. As predicted, the dissolution rate of ethyl acetate is faster than that of dichloromethane, in water. The mutual solubility of ethyl acetate into water and water into ethyl acetate is greater than dichloromethane in the same arrangement (Knovel 2003). The dissolution of solvent in water provides baseline properties that can now compared to a system with polymer.

Similarly, polymer and solvent organic phase solutions were blown out of a micropipette into a water-based surfactant solution. Unlike bulk preparations, surfactant below critical micelle concentration was simply used to help detach and isolate the droplet from the column of liquid in the micropipette after being made and was not necessarily required to emulsify a suspension. Distinctive from other works, the concentration change and solvent extraction was observed during formation rather than just the final product. Video micrographs taken of 100 mg poly(lactic-co-glycolic acid) and 2 mL solvent solutions in 2 mM sodium dodecyl sulfate are illustrated in Figure 3.13 and 3.14.
Analogous to bulk solvent evaporation prepared microspheres, water inclusions were then formed with ethyl acetate but not dichloromethane Figure 3.14. The formation of ethyl acetate and dichloromethane microspheres of equal diameter, 96 μm, is shown in Figure 3.15.
Figure 3.15 Diameter of droplet formed by micropipette manipulation with 100 mg PLGA and organic solvents, EtOAc and DCM, solution in 2mM SDS.

Direct comparison to the pure solvent systems cannot be made as the maximum droplet diameters of the pure solvent system are not equal to the polymer system. The relative volumes ($V_{\text{microsphere}}$) can be calculated based on droplet diameter ($d$) measurements,

$$V_{\text{microsphere}} = \frac{4}{3} \pi \left(\frac{d}{2}\right)^3$$  \hspace{1cm} (3-7)

In Figure 3.16, the relative volume of ethyl acetate and dichloromethane based polymer microspheres were plotted as a function of time.
The linear decrease in volume before reaching final volume fraction was in the region of 9 and 52 seconds for ethyl acetate and dichloromethane respectively. The ethyl acetate dissolved into solution at a greater rate thus forming the microsphere within a shorter period of time compared to dichloromethane. This magnitude difference corresponds to the pure solvent system, and inclusion formation is analogous to microspheres prepared in bulk by solvent extraction/evaporation.

**3.4 Polymer Concentration Effects**

A magnified look at PLGA microspheres formed with ethyl acetate in 1% (w/v) PVA by solvent extraction/evaporation, showed that, increasing polymer concentration results in more water inclusions inside a polymer microsphere. The following micrographs illustrate the number of inclusions increased with increasing polymer concentrations (100 mg/ml, 200 mg/ml and 300 mg/ml) within two size ranges.
The number of inclusions appeared to be proportional to the increase in the polymer concentration. In addition, smaller microspheres had less or no inclusions indicating formation of inclusions was somehow attributable to initial and final microsphere size. A
size distribution of solvent evaporation-prepared PLGA microspheres was then completed for varying polymer concentrations by bulk solvent extraction/evaporation. Light scattering particle measurements established no microspheres under one micrometer for any polymer concentration. The size distribution of 350 microspheres for each polymer concentration using ethyl acetate and 1% (w/v) PVA is shown in Figure 3.20.

The percentage of microspheres for each size range varied relative to polymer concentration. The number of microspheres within the 1 to 5 μm range increased 77% from 118 to 209 as the polymer concentration doubled from 100 to 200 mg of polymer. It continued to increase another 5% at 400 mg of polymer concentration in solvent. Generally, the size distribution of microsphere diameter begins to distribute more uniformly with decreasing polymer concentration. Further examination of water inclusion content within each size distribution shows that the polymer concentration affected the percentage of inclusion within a specific size range. Shown in Figure 3.21, inclusions were first seen in 100 mg PLGA concentration at the 6 to 10 μm range. Out of 46 droplets within the size range, one had an inclusion.
Figure 3.21 Size distributions of microspheres with and without inclusions prepared by solvent evaporation with 100 mg PLGA and EtOAc in 1% (w/v) PVA.

For 200 mg PLGA concentration prepared microspheres, inclusion began to form at the 11 to 15 μm range. Shown in Figure 3.22, two microspheres out of 25 within the size range had inclusions.

Figure 3.22 Size distributions of microspheres with and without inclusions prepared by solvent evaporation with 200 mg PLGA and EtOAc in 1% (w/v) PVA.
The size distribution of microspheres made with 400 mg PLGA in ethyl acetate is shown in Figure 3.23. At this concentration, two out of 18 polymer microspheres had inclusions within the 11 to 15 μm size range.

Figure 3.23 Size distributions of microspheres with and without inclusions prepared by solvent evaporation with 400 mg PLGA and EtOAc in 1% (w/v) PVA.

Microspheres formed with higher organic phase concentrations by identical bulk solvent extraction/evaporation protocol resulted in more water inclusions within a defined size range. For microspheres prepared by solvent extraction/evaporation, the percentage of inclusions within a specific size range at various polymer concentrations is summarized in Figure 3.24.
Figure 3.24 The percentage of microspheres with inclusions prepared by solvent evaporation with 100, 200, and 400 mg PLGA and EtOAc emulsified in 1% (w/v) PVA.

At 200 and 400 mg PLGA concentration with ethyl acetate, all microspheres 31 μm and above contained inclusions. On the contrary, all microspheres greater than 46 μm in diameter had inclusions when prepared with 100 mg PLGA concentration and ethyl acetate.

Evaluation on a single droplet using micropipette manipulation gave better insight in microsphere and to when inclusion form. Shown in Figure 3.25, greater polymer concentration increased the final relative microsphere volume of a polymer droplet made with dichloromethane.
Figure 3.25 Relative volume of microspheres formed with 100 mg and 400 mg PLGA and 2mL DCM solution in 2mM SDS.

The final relative volume of a microsphere made with 100 mg PLGA in dichloromethane is 0.07 whereas it is 0.18 when prepared with 400 mg PLGA in dichloromethane. No inclusions formed in microspheres made with dichloromethane in any polymer concentration.

Further assessment with ethyl acetate prepared droplets at various concentrations of PLGA in solvent result in microspheres with inclusions and a comparable relative volume increase with greater polymer concentration. As listed in Table 3.1, three microspheres were formed with 100 mg poly(lactic-co-glycolic acid) in 2 mL ethyl acetate.

Table 3.1 Dimensions of 100 mg PLGA and EtOAc prepared microspheres.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Initial Diameter (μm)</th>
<th>Final Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>83.0</td>
<td>31.0</td>
</tr>
<tr>
<td>2</td>
<td>94.0</td>
<td>36.0</td>
</tr>
<tr>
<td>3</td>
<td>96.0</td>
<td>36.0</td>
</tr>
</tbody>
</table>

As the microspheres were being formed, the relative volume as a function of time is plotted in Figure 3.26 based on diameter measurement.
Figure 3.26 Relative volume of microspheres prepared by micropipette manipulation with 100 mg PLGA and EtOAc solution in 2mM SDS.

For all three trials of 100 mg PLGA and ethyl acetate prepared microspheres, inclusions were first observed at 7.5 seconds. The average microspheres diameter was initially $91.0 \pm 7.0 \mu m$ where the final relative volume was $0.054 \pm 0.002$. Next, Table 3.2 lists microspheres formed with 200 mg PLGA and ethyl acetate by micropipette manipulation.

Table 3.2 Dimensions of 200 mg PLGA and EtOAc prepared microspheres.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Initial Diameter (µm)</th>
<th>Final Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81.1</td>
<td>37.3</td>
</tr>
<tr>
<td>2</td>
<td>75.8</td>
<td>37.1</td>
</tr>
<tr>
<td>3</td>
<td>79.6</td>
<td>39.7</td>
</tr>
</tbody>
</table>

A plot of relative volume for this system is illustrated in Figure 3.27.
Figure 3.27 Relative volume of microspheres prepared by micropipette manipulation with 200 mg PLGA and EtOAc solution in 2mM SDS.

The final relative volume for microspheres prepared with 200 mg PLGA in ethyl acetate solution was $0.113 \pm 0.014$. For this system, the average maximum diameter of the microspheres was $78.8 \pm 2.7 \, \mu m$, and inclusions began to form after $3.96 \pm 0.35$ seconds.

Microspheres prepared with 400 mg PLGA and ethyl acetate are listed in Table 3.3.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Initial Diameter ($\mu m$)</th>
<th>Final Diameter ($\mu m$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60.3</td>
<td>37.2</td>
</tr>
<tr>
<td>2</td>
<td>64.9</td>
<td>40.2</td>
</tr>
<tr>
<td>3</td>
<td>61.1</td>
<td>37.7</td>
</tr>
</tbody>
</table>

Shown in Figure 3-28, the final relative microsphere volume is $0.236 \pm 0.002$ for microspheres made with 400 mg PLGA in ethyl acetate.
Figure 3.28 Relative volume of microspheres prepared by micropipette manipulation with 400 mg PLGA and EtOAc solution in 2mM SDS.

Inclusions begin to form at $3.61 \pm 0.59$ seconds. Greater polymer concentration consistently increased the final relative microsphere volume and decreased the time it took for water inclusions to form. The specific rate of volume change cannot be compared between polymer concentrations as the initial diameter of each varied per composition.

Subsequently, the droplet density was calculated for each material system as it solidifies into a microsphere. Based on microsphere diameter ($d$) and time interval ($dt$), the solvent mass change ($\Delta m_{\text{solvent}}$) of the polymer-in-solvent solution while being released from the micropipette was determined per Epstein-Plesset microparticle dissolution theory,

$$\Delta m_{\text{solvent}} = 4\pi R^2 D(C_o - C_s) v_f \left[ \frac{1}{R} + \frac{1}{\sqrt{\pi D t}} \right] dt$$

where $R$ is radius, $D$ is the diffusion coefficient, $C_o$ is the initial concentration of solvent, $C_s$ is final concentration of solvent, $v_f$ is volume fraction of solvent, and $t$ is time in seconds.
Assuming all the solvent has been removed from the microsphere at final volume, the remaining amount is purely PLGA. To determine the volume fraction of solvent in the microsphere, the PLGA volume was subtracted from the microsphere volume. Thereafter, the volume fraction was calculated at each time step and used to determine the microsphere density by partial volumes listed in Table 3.4.

<table>
<thead>
<tr>
<th>Polymer-in-Solvent Concentration</th>
<th>Microsphere Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg PLGA in 2mL ethyl acetate</td>
<td>1.0987 ± 0.0262 g/cm³</td>
</tr>
<tr>
<td>200 mg PLGA in 2mL ethyl acetate</td>
<td>1.0772 ± 0.0958 g/cm³</td>
</tr>
<tr>
<td>400 mg PLGA in 2mL ethyl acetate</td>
<td>1.2637 ± 0.0416 g/cm³</td>
</tr>
</tbody>
</table>

Based on these results, inclusion formation is not based solely on microsphere density as there is no trend. Polymer dissolved in organic solvent defines the concentration and thus viscosity of the initial solution.

### 3.5 Acidity Model

A model was created to describe the high acidity profile of inclusions during formation and degradation of the polymer microspheres. Based on measurements of micropipette experiments and several assumptions on degradation properties, the model formed conclusions analogous to those found in experimental results by Langer et al [REF]. Using confocal fluorescence microscopy and pH-sensitive fluorescent dyes, the acidity inside a polymer microsphere was shown to be 1.4 – 1.5 (Fu 2000).

Water inclusions enlarge the internal surface area contact for the breakdown of PLGA. Once formed, the slow increase in water inclusion diameter is due to hydrolysis.
For a single polymer microsphere, the microsphere diameter ($D_s$) and inclusion diameters ($d_i$) are observed over a period of time and shown in Figure 3.30 for a microsphere made by micropipette with 100 mg PLGA and ethyl acetate in 1% (w/v) poly(vinyl alcohol).
\( t = 10 \text{ minutes} \)

\( t = 20 \text{ minutes} \)

\( t = 30 \text{ minutes} \)
Figure 3.30 Video micrograph of microsphere formed by micropipette manipulation at time intervals 0, 9, 10, 12 seconds, and 5, 10, 20, 30, 40, 50, and 60 minutes with 100 mg PLGA and ethyl acetate in 1% (w/v) PVA at 20x magnification.
The diameter of a microsphere prepared with 100 mg PLGA in EtOAc in 1% (w/v) PVA is illustrated by Figure 3.31.

![Figure 3.31 Diameter of microsphere made by micropipette manipulation with 100 mg PLGA and ethyl acetate solution in 1% (w/v) PVA.](image)

This data was used to calculate the volume of the sphere ($V_s$) and inclusion ($V_i$) respectively

$$V_s = \frac{4\pi}{3} \left( \frac{d_s}{2} \right)^3$$

$$V_i = \frac{4\pi}{3} \left( \frac{d_i}{2} \right)^3$$

and plotted as a function of time in Figure 3.32 and Figure 3.33.
The volume of PLGA ($V_p$) is the overall microsphere volume $V_s$ subtracted by the inclusion volume. From this value, the total mass of PLGA ($m_p$) and number of PLGA molecules ($n_p$) were calculated as follows.

$$V_p = V_s - V_i = \frac{4\pi}{3} \left[ \left( \frac{D_s}{2} \right)^3 - \left( \frac{d_i}{2} \right)^3 \right]$$

$$m_p = \rho_p \times V_p$$
\[ n_p = m_p \times \frac{N_A}{MW_p} \]

where \( \rho_p \) is the density of PLGA and \( MW_p \) is the molecular weight of the PLGA. The inclusion was hypothesized to be water based on the components of the system; the increase of inclusion volume was thought to be due to degradation of the polymer. Based on these assumptions and the known properties of PLGA, the change in inclusion volume is correlated to the number of PLGA molecules being degraded over time \( (m_{p-hydrolyzed}) \)

\[ m_{p-hydrolyzed} = \rho_p (V_i - V_o) \]

where \( V_o \) is the initial inclusion volume based on measurement at the previous time step.

![Figure 3.34 Polymer mass of microsphere made by micropipette manipulation with 100 mg PLGA and ethyl acetate solution in 1% (w/v) PVA.](image)

With the assumption that all PLGA loss led to degradation into equal parts of lactic and glycolic acid, the number of PLGA molecules being hydrolyzed \( (n_{p-hydrolyzed}) \) is specific to the molecular weight of the polymer and mass of polymer being degraded due to hydrolysis of the water inclusion interface.
\[ n_{p-hydrolyzed} = \frac{m_{p-hydrolyzed}}{MW_p} X \frac{N_A}{n_{G}} \]

\[ n_L = n_G = \frac{MW_p}{m_{ap}} \]

where \( n_L \) and \( n_G \) is the number of lactic and glycolic acid molecules formed respectively and \( m_{ap} \) is the atomic mass of PLGA. The concentration of lactic [L] and glycolic [G] acid was then calculated upon the number of resulting lactic and glycolic acid molecules.

\[ [L] = \frac{n_L}{N_A} x V_i \]

\[ [G] = \frac{n_G}{N_A} x V_i \]

Figure 3.35 Lactic and glycolic concentration of microsphere made by micropipette manipulation with 100 mg PLGA and ethyl acetate solution in 1% (w/v) PVA.

These concentrations were then correlated into the hydronium concentration for the glycolic and lactic acid components.
\[ [HA] \Leftrightarrow [A^-] + [H^+] \]
\[ [LA] - x \Leftrightarrow x + x \]
\[ K_d = \frac{[H^+][A^-]}{[HA]} = \frac{x \cdot x}{[LA] - x} \]
\[ K_d([LA]x) = x^2 \]
\[ K_d[LA] - K_dx = x^2 \]
\[ x^2 + K_dx - K_d[LA] = 0 \]
\[ a = 1, b = K_d, c = K_d[HA] \]
\[ x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \]
\[ x = [H_3O^+] \]
\[ [GA] = [H^+] + [GA^-] \]
\[ [GA] - y = y + y \]
\[ [H^+] = [H_3O^+] = x + y \]

The pK\textsubscript{A} is converted to K\textsubscript{A}

\[ K_d = 10^{-pK_d} \]

and y is found based on the K\textsubscript{A} of glycolic acid.

\[ K_{A-gly} = \frac{(x + y)y}{[GA] - x} \]
\[ K_{A-gly}([GA] - x) = (x + y)y \]
\[ K_{A-gly}[GA] - x[GA] = xy + y^2 \]
\[ y^2 + xy + x[GA] - K_{A-gly}[GA] = 0 \]
Assumption, \( x \ll y \)

\[ K_{A-gly} = \frac{y^2}{[GA] - 0} \]
\[ y = \sqrt{K_{A-gly} \times [GA]} \]

Using calculated y, the value for x is solved.
\[
K_{A-lac} = \frac{(x + y)x}{[LA] - x} \\
K_{A-lac}([LA] - x) = (x + y)x \\
[LA]K_{A-lac} - xK_{A-lac} = x^2 + yx \\
x^2 + yx + xK_{A-lac} - [LA]K_{A-lac} = 0 \\
a = 1, b = y + K_{A-lac}, c = -K_{A-lac}[LA] \\
x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}
\]

The effects of both lactic and glycolic units are combined,

\[
[H_3O^+] = x + y \\
pH = -\log[H_3O^+]
\]

and the result is the internal pH of the microsphere.

Figure 3.36 pH of microsphere made by micropipette manipulation with 100 mg PLGA and ethyl acetate solution in 1% (w/v) PVA.

Although several assumptions were made in polymer degradation, the resulting 1.40 – 1.45 pH proved to be analogous to literature values found in Robert Langer’s study using confocal fluorescence microscopy (Fu 2000).
CHAPTER 4
CONCLUSION

Mechanics of materials and material science was used to investigate the relationship between composition, structure, and manufacturing process of the materials used to form polymer microspheres to predict the formation of water inclusions under certain conditions. This study demonstrated the effectiveness of the micropipette method to better understand the formation of inclusions in poly(lactic-co-glycolic) acid. Based on experimental results, polymer concentration and organic solvent choice made noteworthy differences in the formation of water inclusions in polymer microspheres using the solvent evaporation method and micropipette manipulation. Lower concentrations of polymer and solvent solubility in water decreased the number of water inclusions formed in a microsphere. Neither surfactants, sodium dodecyl sulfate or poly(vinyl alcohol), made a significant difference in water inclusion formation. Water becomes trapped during microsphere development due to both increased polymer viscosity and variations of extraction rate. Equivalent observations were made on the final product after bulk preparation and throughout formation by micropipette manipulation.

The results of this work define the effects of polymer concentration, solvent solubility, and surfactant use to provide underlying information for the improvement polymer drug delivery systems. Polymer-based drug delivery systems have been developed with an interest in controlled protein and peptide release. To create polymer microspheres without
inclusions and have better control of drug release, a manufacturer should use lower polymer concentrations and solvent with higher mutual water solubility.

Future studies will consider different polymer, surfactant, and solvent material systems. Endless variations in bulk microsphere preparation can include temperature, vortex and mixing time. Venkatraman et al has done work to characterize the differences of water intake of polymer microspheres loaded and unloaded with drug (Frank 2005). In the end, the baseline characteristics of polymer microspheres developed in these studies will be used for drug delivery systems that include a pharmaceutical agent.
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