COMPARISON OF NON-TOXIC METHODS FOR CREATING BETA-CAROTENE ENCAPSULATED IN PMMA NANOPARTICLES

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ABSTRACT OF THE THESIS

Comparison of Non-toxic Methods for Creating Beta-Carotene Encapsulated in PMMA Nanoparticles

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Nano/microcapsules are becoming more prevalent in various industries such as drug delivery, cosmetics, etc. Current methods of particle formation often use toxic or carcinogenic/mutagenic/reprotoxic (CMR) chemicals. This study intends to improve upon existing methods of particle formation and compare their effectiveness in terms of entrapment efficiency, mean particle size, and yield utilizing only non-toxic chemicals.

In this study, the solvent evaporation (SE), spontaneous emulsification, and spontaneous emulsion solvent diffusion (SESD) methods were compared in systems containing green solvents ethyl acetate, dimethyl carbonate or acetone. PMMA particles containing encapsulated beta carotene, an ultraviolet sensitive substance, were synthesized. It was desired to produce particles with minimum mean size and maximum yield and entrapment of beta carotene. The mass of the water phase, the mass of the polymer and the pumping or blending rate were varied for each synthesis method. The smallest particle sizes for SE and SESD both were obtained from the middle water phase sizes, 200 g and 100 g respectively. The particles obtained from the larger water phase in SESD were much bigger, about 5 microns in diameter, even larger than the ones obtained

from SE. When varying the mass of PMMA used in each synthesis method, as expected, more PMMA led to larger particles. Increasing the blending rate in SE from 6,500 to 13,500 rpm had a minimal effect on average particle size, but the higher shear resulted in highly polydisperse particles (PDI = 0.87). By decreasing the pump rate in SESD, particles became smaller and had lower entrapment efficiency.

The entrapment efficiencies of the particles were generally higher for the larger particles within a mode. Therefore, we found that minimizing the particle size while maximizing entrapment were somewhat contradictory goals. The solvent evaporation method was very consistent in terms of the values of mean particle size, yield, and entrapment efficiency. Comparing the synthesis methods, the smallest particles with the highest yield and entrapment efficiency were generated by the spontaneous emulsification method.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.1 Motivation

Nanoparticles and microparticles have become a large area of interest in the scientific research community. They have applications in almost every industry including catalysis, cosmetics, paints, pharmaceuticals, and many more. Many applications involve encapsulating materials that are prone to degradation from the sun, air, and or heat.

Prolonged exposure to the sun, specifically ultraviolet (UV) radiation, has been shown to cause damage to materials including human skin, paint, pharmaceuticals, and textiles. UV radiation is a type of electromagnetic radiation consisting of wavelengths ranging from approximately 100 nm to 400 nm. Some of the ways to protect UV sensitive materials from UV radiation are physical blockers that scatter light and chemical absorbers that absorb the radiation and emit it at longer wavelengths.

Encapsulating a UV sensitive material in a polymer matrix can increase protection from UV radiation by the effects of light scattering[1,2] and movement restriction.[3] Incorporating UV absorbers, such as those found in sunscreen lotions, into a formulation can also increase UV protection.[4] It has also been shown that using antioxidants in addition to UV absorbers enhanced the photostability of both protective materials.[5] To optimally protect a UV sensitive material, it would be best to incorporate multiple protective methods.

There are concerns about safety of the chemicals used in making particles. Many of the most popular solvents, that have the most desirable characteristics for nanoparticle creation such as dichloromethane and chloroform, are known or suspected toxic or carcinogenic/mutagenic/reprotoxic (CMR) chemicals. Since there are always trace

amounts of solvent in the final particle formulation,[6] it is prudent to strive to use safer chemicals for nanoparticle formation and developing and enhancing alternative methods of particle formation utilizing non-toxic chemicals.

1.2 Synthesis/Encapsulation

There are various methods of synthesizing nanoparticles. In this thesis, we examine three methods for the formation of PMMA nanoparticles containing encapsulated beta-carotene. One of the methods, known simply as solvent evaporation, involves applying a large amount of energy in the form of high-shear mixing to create an emulsion that yields particles. The other methods emulsify without high-shear mixing simply by pouring one solution in to the other either rapidly (spontaneous emulsification) or slowly (spontaneous emulsion solvent diffusion).

The three methods discussed in this thesis each utilize two solutions, one of which is an aqueous solution that contains stabilizer and/or surfactant and water. The other solution is referred to as the organic phase or dispersed phase and it contains a dissolved polymer and other materials dissolved in a solvent.

One of the simplest, most common methods is solvent evaporation. Solvent evaporation utilizes high shear mixing to force two distinct liquid phases, one of the phases contains a polymer dissolved in a solvent, to form an emulsion. Typically, a nonpolar (hydrophobic) organic solvent is used which is insoluble or only slightly soluble in the aqueous continuous phase. After the high shear mixing, the solvent is allowed to evaporate off which then yields polymeric nanoparticles.

The spontaneous emulsification method, also known as nanoprecipitation, involves using a solvent that is more hydrophilic. In this method, the solvent solution is

poured into the aqueous phase very quickly under slight agitation, an oil-in-water (O/W) emulsion forms very rapidly. The solvent is again allowed to evaporate and the particles are collected.

The spontaneous emulsion solvent diffusion (SESD) technique, involves using a device such as a syringe pump or peristaltic pump to slowly deliver the organic phase into the aqueous phase under moderate stirring. The organic phase is usually made up of a polymer dissolved in two solvents of the following characteristics: one highly hydrophobic solvent (or with low water solubility), such as dichloromethane, and one highly hydrophilic (or water miscible) solvent, such as acetone. The hydrophilic solvent diffuses into the aqueous phase and eventually forms an emulsion. The prevailing mechanisms for the particle formation is not definitively known, but current ideas in the literature are presented in the following sections of this chapter. The solvent is again allowed to evaporate and the polymeric nanoparticles remain in the aqueous phase.

1.2.1 Solvent Evaporation

The solvent evaporation encapsulation method has been widely used for creating nanoparticles and microparticles. There are many articles on both the solvent evaporation process,[7,8] and its various uses. [9,10,11] There are many techniques and additives used to fine tune this process. The most basic form of the solvent evaporation method involves a single oil-in-water emulsion. It consists of dissolving a polymer and any substance being encapsulated, such as a dye, in a solvent to create an oil phase. Agitation is then used to disperse the oil phase into an aqueous phase containing water and surfactant creating an oil-in water-emulsion. After emulsification, the solvent is allowed to migrate to the

aqueous phase and eventually evaporate all while under stirring to yield solid polymeric nanoparticles and/or microparticles.

This method requires a careful choice of polymer and solvent in order to be successfully attempted. The polymer should be able to dissolve in the solvent, but not in the aqueous phase. The choice of polymer, its molecular weight, and its concentration can each affect the particle quality.[7] The solvent can affect much of the process including the rate of particle formation, porosity of the particles, and the encapsulation efficiency of the particles.[7,12] A solvent that is partially miscible in water can cause the particles to have irregular shapes and sizes and sometimes even high porosity resulting in bursts of the encapsulated material from the particles.[6,13]

1.2.2 Spontaneous Emulsification

Emulsions are thermodynamically unstable systems that are formed by at least two immiscible liquid phases. When emulsions separate into their bulk phases, the free energy of the system goes down due to the decrease of interfacial area. Generally, mechanical means must be supplied to liquid phases that are at equilibrium for them to form an emulsion. The mechanical work (W) needed to increase the interfacial area is:

$$W = \Delta A * \gamma$$

where ΔA is the increase in interfacial area and γ is the interfacial tension. This would suggest that a higher amount of work is needed to generate a larger interfacial area, or make smaller emulsion droplets. Nevertheless, there are many systems known to spontaneously form thermodynamically stable emulsions.

Davies and Rideal (1961) proposed three possible mechanisms for the phenomenon involved in spontaneous emulsification. Two of them involve mechanical

breakup of the interface due to either “interfacial turbulence” or the existence of negative values of interfacial tension. The negative interfacial tension mechanism is oversimplified because there are other factors that can have an effect on the stability when the interfacial tension is very low such as electrical forces in double layers. Interfacial turbulence, which is governed by the well-known Marangoni effect,[14] can arise at the interface of two miscible or partially miscible liquid phases. A concentration gradient can cause longitudinal variations of interfacial tension at the interface, which can induce interfacial turbulence or spontaneous agitation of the interface between the two phases that are not in equilibrium. The rapid diffusion of solvent across the interface between the organic and aqueous phase spontaneously produces an increased surface area leading to smaller droplets and therefore smaller particles.[15]

The third proposed mechanism involves a chemical instability and is called “diffusion and stranding”. The idea for this mechanism is that due to the diffusion of the solvent from the organic phase to the aqueous phase, regions of local supersaturation occur and emulsion droplets form due to the phase transformations in these regions.[16,17]

These mechanisms are still the main ones used to describe spontaneous emulsification, though many other mechanisms have been proposed such as phase transitions due to temperature changes, osmotic pressure gradient effects, and myelinic figures at the water-oil interface. It is noted that spontaneous emulsification can happen in certain systems even without surfactant, at higher surface tensions.[18] The spontaneity of the emulsion is affected largely by the following: interfacial tension, interfacial and bulk viscosity, phase transition region, and surfactant structure and concentration.[19] It is possible to make inferences into which mechanism is functioning because mechanisms

governing spontaneous emulsification are affected by the physiochemical characteristics and compositions of the systems.[19]

1.2.3 Spontaneous Emulsification Solvent Diffusion

Solvents in general are unique and have varying properties pertinent to liquid-based nanoparticles formation such as viscosity, vapor pressure, solubility in water, polymer solubility, etc. Solvent evaporation's success relies on the solvent and water to have low affinity toward each other. Many solvents, particularly the less toxic solvents, tend to be partially soluble with water. The degree of solubility with water of a solvent can directly influence the diffusion rate of solvent from the organic phase to the aqueous phase. This factor has a major role in the nanoparticle formation. Greater water solubility often makes a poorer solvent for solvent evaporation. On the other hand, the quality of being soluble or partially soluble with water can be beneficial for other nanoparticle formation techniques.

SESD is conventionally accomplished by using a water-soluble solvent such as methanol or acetone along with a water-insoluble solvent such as dichloromethane or toluene as the organic phase. A polymer is dissolved in the organic phase and this polymeric solution is slowly poured into the aqueous phase consisting of water and surfactant under light stirring. Nanoparticles are formed when the organic phase is introduced into water. Some suspect that an interfacial turbulence is created resulting from the diffusion of the water-soluble solvent into the aqueous phase, and therefore resulting in the formation of smaller emulsion particles.[20] The solvent is subsequently evaporated. It was also found that increasing the ratio of water-miscible solvent in the organic phase lead to a decrease in particle size.[15] There have also been modifications to

this method using two water soluble solvents for the organic phase which had similar results for poly(DL-lactide-co-glycolic acid) (PLGA) nanoparticles.[21]

This thesis investigates the nanoparticle synthesis process by making a further modification to the SESD method. The original and modified methods were designed for PLGA nanoparticle formation. PLGA has very different characteristics compared to PMMA, and most notably is the solubility or dissolution characteristics. PLGA is much more hydrophilic since it can dissolve in a solution of a water miscible and immiscible solvents such as acetone and dichloromethane, or even two water miscible solvents such as acetone and methanol. PMMA, on the other hand, does not dissolve in a 50:50 solution of acetone and methanol. We propose a further modification to the method that is less toxic than methods that use chemicals such as toluene or dichloromethane in order to dissolve PMMA. We are using a single solvent with the following characteristics: non-toxic, dissolves PMMA, is partially soluble in water. The solvent being partially soluble in water is hypothesized to behave similarly to a mixture of water-miscible and water-immiscible solvents.

1.3 Solvent Selection

The solvent plays an important role in the particle formation process. Qualities such as the ability to dissolve a polymer, affinity to water, interaction with surfactants, evaporation rate, and viscosity are all crucial for making particles. The polymer must be able to dissolve in the solvent to be able to form nanoparticles that can encapsulate a material. The solvent and water dissolution properties can vary greatly between solvents and are crucial in particles synthesis.

Many solvents currently used in particles synthesis are not considered safe regarding long-term toxicity. Chemicals such as dichloromethane or chloroform have been suspected of causing cancer. In addition to effectiveness of making particles, solvents should be chosen that are safer for humans and the environment.

1.4 Beta-Carotene Stability

Beta-carotene was chosen as the model chemical to be encapsulated. Beta-carotene is an orange pigment found abundantly in various plants. It can be used as a natural dye and it also received interest due to its potential health benefits.[22] Since beta-carotene is prone to degradation, it is an excellent model for developing steps to enhance stability from UV exposure. It is suspected that one way to do so is to incorporate anti-oxidant stabilizers and UV absorbers such as α-tocopherol in formulations with beta-carotene. Also, polymeric encapsulation has been shown to improve stability of beta-carotene.[2]

1.5 Objective of Thesis

There are three main aims for this thesis. The first is to describe and compare three nanoparticles synthesis methods in terms of particle sizes, yields, and entrapment efficiencies. We also want to investigate how these qualities are affected by varying the mass of the aqueous phase, the mass of the polymer, and the blend or pump rate (if applicable) of the formulations. We also intend to optimize the formulations from after each trial to minimize particle size, maximize yield, and maximize entrapment with each quality having equal weight.

The contribution of this work is developing a comparison of three nanoparticle creation methods utilizing only green, non-toxic chemicals. Within this comparison, we

developed a modified version of the spontaneous emulsion solvent diffusion method of particle synthesis using green solvent, dimethyl carbonate for PMMA particles.

Chapter 2 will discuss the solvents and their characteristics such as solubility and toxicity. Chapter 3 is the bulk of the work which describes the preparation methods for making nanoparticles and compares their effectiveness. Chapter 4 discusses beta-carotene and its degradation in various solvents and the effect of α-tocopherol on the stability.

Chapter 2: Toxicity and Solubility

2.1 Introduction

The techniques of solvent evaporation and spontaneous emulsification rely on the choice of a solvent with particular characteristics. The miscibility of the solvent with water can affect many aspects of particle synthesis such as the rate of particle formation. For the solvent to evaporate, it must first migrate from the oil phase to the aqueous phase. A higher miscibility leads to shorter particle creation times, therefore, a solvent with too high of a miscibility in water can cause particles to deform or have large pores due to the rapid migration of solvent from the emulsion droplet to the aqueous phase.[12]

Many solvents, particularly the less toxic solvents, tend to be partially soluble with water. This often makes a poor solvent choice for solvent evaporation. Conversely, this quality of being soluble with water is beneficial for spontaneous emulsification processes.[20]

2.2 Toxicity

Median lethal dose (LD_{50}) is a commonly reported as the dose that would kill one-half of a population. The LD_{50} results are classified into the Hodge and Sterner scale (I_{tox}), which has 6 levels. Level 1 contains the most dangerous substances ($LD_{50} < 1$ mg/kg; oral; rat) and level 6 for the most harmless substances ($LD_{50} > 15000$ mg/kg; oral; rat).[23]

LD_{50} and I_{tox} are good indicators of short-term toxicity of various chemicals, but they do not necessarily represent how harmful certain chemicals can be regarding the long-term effects of exposure. Many chemicals that have low risk of immediate lethal

toxicity from exposure can still have chronic or even lethal consequences later on in terms of carcinogenic, mutagenic, or reprotoxic (CMR) effects. Although the United States regulatory agencies are fairly lenient on CMR chemicals, the EU has compiled a system to much more rigorously classify the CMR risks of various chemicals.[24] The highest and most dangerous classification is the Category 1A substance: CMR potential for humans, based largely on human evidence. A Category 1B substance is presumed to have CMR potential for humans, based largely on experimental evidence on animals and a Category 2 substance is suspected to have CMR potential for humans (table 1).

Table 1: EU CMR Toxicity Classifications

Classification	Details
Category 1A	Known CMR
Category 1B	Presumed CMR
Category 2	Suspected CMR

There is also a separate classification for substances that show evidence of adverse effects on offspring due to transfer in the milk and/or quality of milk and/or potentially toxic levels of substance in the milk. Unclassified substances are presently considered to be safe.[25]

In tables 2 and 3, we compiled a list of potential solvents to compare their most pertinent characteristics for particle synthesis including toxicity, solubility, and volatility. Some of these solvents are well known to be good for particle synthesis, but are more toxic such as dichloromethane and chloroform. Others are less tested in particle creation and are less toxic such as dimethyl carbonate.

Table 2: Relative Evaporation, Boiling point, Vapor pressure, Solubility, and Viscosity of various solvents[26]

Solvent	Relative Evap. Rate	Boiling Point at 760 mmHg [°C]	Vapor Pressure at Room Temp. [kPa]	Solubility in H_2O at Room Temp. [g/dL]	Viscosity at 25 °C [mPa]
n-Butyl Acetate	1	125	2	-	0.685
Dichloromethane	7.5	39.8	47.1	1.75	0.413
Dimethyl Carbonate	0.22	90	2.4	13.9	0.589
Ethyl Acetate	4.1	77.1	9.73	8.3	0.423
Toluene	2	110	2.93	0.052	0.56
Acetone	5.6	56.5	28.6	Miscible	0.306
Water	0.3	100	-	N/A	0.89
Limonene	0.05	-	0.2	0.0018	0.923
Chloroform	-	61.2	25.9	0.809	0.537
Ethanol	1.4	-	5.95	Miscible	1.074
Methanol	-	-	13.02	Miscible	0.544
Iso-Octane	-	99.1	5.5	0.00007	0.478

Table 3: Hansen Solubility Parameters, Carcinogenic, Mutagenic, Reporotoxic (CMR) Rating, and HS Scale for toxicity (ITox) for various solvents[2827,28]

Solvent	Hansen Dispersion [$MPa^{1/2}$]	Hansen Polar [$MPa^{1/2}$]	Hansen H-bonding [$MPa^{1/2}$]	Molar Volume [mL/mol]	CMR	Itox
n-Butyl Acetate	15.8	3.7	6.3	132.5	-	2
Dichloromethane	18.2	6.3	6.1	63.9	2	4
Dimethyl Carbonate	15.5	3.9	9.7	84.2	-	5
Ethyl Acetate	15.8	5.3	7.2	98.5	-	5
Toluene	18	1.4	2	106.8	2	4
Acetone	15.5	10.4	7	74	-	5
Water	15.5	16	42.3	18	-	6
Limonene	-	-	-	-	-	-
Chloroform	17.8	3.1	5.7	80.7	2	4
Ethanol	15.8	8.8	19.4	58.5	-	5
Methanol	15.1	12.3	22.3	40.7	-	5
Iso-Octane	-	-	-	-	-	-

2.3 Solubility

For many of the commonly used solvents, there is precise solubility data. However, many green solvents do not have solubility data reported. Without solubility

data, one can predict solubility using solubility parameters in order to determine a good choice of solvent, in terms of solubility.

Solubility parameters have found great use in many industries. Solubility parameters are used to assist in selecting solvents for coatings materials and in other industries to predict compatibility of polymers, chemical resistance, and permeation rates. They have also been used to characterize the surfaces of pigments, fibers, and fillers.[28] For this research, solubility parameters predict the compatibility between solvents and polymers in the absence of experimental data.

Hildebrand and Scott, along with contribution from previous work by Scatchard, developed the first solubility parameter.[29] The Hildebrand solubility parameter is defined as the square root of the cohesive energy density given as:

$$\delta = \sqrt{\frac{E}{V}}$$

Where V is the molar volume of pure solvent and E is the measurable energy of vaporization. For a solution process to occur spontaneously, the thermodynamics requires that the free energy of mixing be zero or negative by the relation:

$$\Delta G^M = \Delta H^M - \Delta T S^M$$

Where ΔG_M is the free energy of mixing, ΔH_M is the enthalpy change of mixing, T is the absolute temperature and ΔS_M is the entropy change due to mixing. Hidlebrand and Scott proposed that the heat of mixing is given by:

$$\Delta H^M = \varphi_1 \varphi_2 V_M (\delta_1 - \delta_2)^2$$

Where φ_1 and φ_2 are volume fractions of the solvent and polymer, and V_M is the volume of the mixture. There have been further improvements and corrections to

this relation to allow for negative heats of mixing. But, this approach was limited to regular solutions, as defined by Hildebrand and Scott, and does not account for polar and hydrogen-bonding interaction between molecules. The problem seems to have been mostly solved by the use of multicomponent solubility parameters.

The Hansen solubility parameter (HSP) is a three-dimensional solubility parameter that is useful for predicting whether a material will dissolve in a solvent to form a solution. The HSP consists of three components or partial parameters: dispersion force component (δ_D), polar force component (δ_P), and hydrogen bonding component (δ_H). The equation governing the assignment of the HSP components is that the total cohesive energy, *E*, must be the sum of the individual energies that make it up.

$$E = E_D + E_P + E_H$$

Dividing this equation by the molar volume give the square of the total or Hildebrand solubility parameter as a sum of the Hansen dispersion, polar, and H-bonding components.

Originally, the best method to determine the individual components of HSP depended largely on the data available. The dispersion and polar parameters can be found according to a procedure outline by Blanks and Prausnitz,[30] which depended on whether the molecule is aliphatic, cycloaliphatic, or aromatic. The hydrogen-bonding parameter was originally found by subtracting the other two parameters from the total parameter. But, more recently there have been developed statistical thermodynamic methods for determining each parameter quite accurately.[31]

These components may be used to create a three-dimensional sphere system based on a polymer's own HSP. Solvents within the sphere are likely to dissolve the polymer.

Conversely, solvents outside of the sphere are likely to not dissolve the polymer. The radius of the sphere also known as the interaction radius, *R*, is calculated empirically for each polymer.

A Bagley graph is one way to visualize the three-dimensional sphere on a two-dimensional surface. Bagley determined the polar and dispersion force components of HSP are highly correlated and can be combined into a single parameter, δ_V, without losing much accuracy or precision.[32] A graph of the solubility circle, now that is two-dimensional, shows the hydrogen bonding component, δ_H, on one axis and the combined dispersion and polar parameter, δ_V, on the other axis. Lu Li et al have created a Bagley Graph for PMMA with various solvents shown in Figure 1 along with dimethyl carbonate superimposed. Table 3 has HSP values for various solvents.

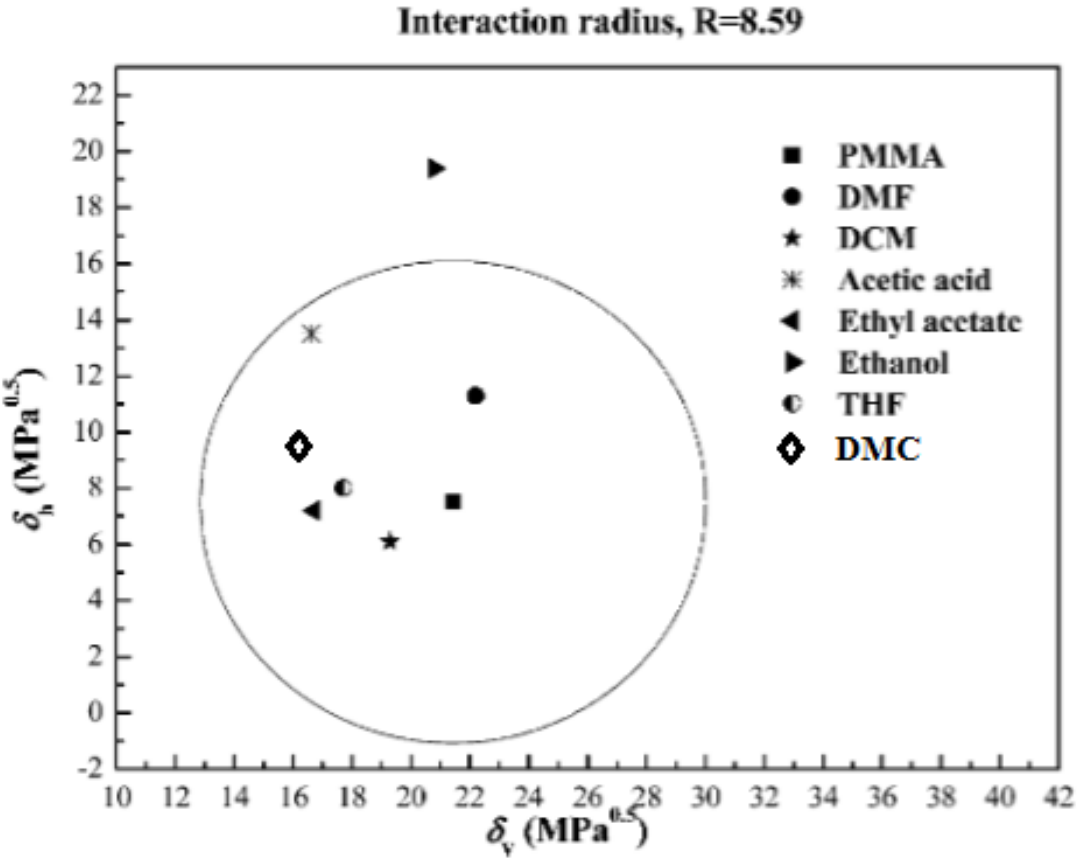


Figure 1: Bagley graph of Hansen solubility sphere for PMMA[32]

Chapter 3: Nanoparticle Synthesis and Comparison

3.1 Introduction

This chapter will describe the preparation processes of the three aforementioned nanoparticle synthesis methods, along with the materials used. The resulting particle characteristics are compared for each method. We also investigate how varying the size of the aqueous phase, the amount of polymer in the organic phase, and blend/pump rate from the various formulations affects the particle sizes, yields, and entrapment efficiencies.

Furthermore, we attempt to optimize the formulations in order to produce particles that are smaller, have a higher yield, and have a higher entrapment efficiency. The optimization is determined by giving each formulation a score for each of the three key characteristics mentioned above. This score will have equal weight for each of the characteristics, and further changes to the formulation will be based on the previous formulation that scored the highest.

3.2 Materials

Water. Deionized (DI) water was used in all experiments.

Polymer. Poly(methyl methacrylate) (PMMA) (MW 120,000) was obtained from Aldrich. PMMA is a versatile polymer that has been used in numerous nanoparticle formulations.[33,34,35,36] It was chosen for its characteristics of dissolving in various solvents and being insoluble with water, which make it suitable for nanoparticle synthesis. Some polymers can have potentially toxic effects from residual substances present after polymerization reactions, so nanoparticle formulation techniques utilizing pre-formed, well-defined polymers are preferred.[37] PMMA has also found uses in contact lenses, eye

prostheses, dentures, bone cement, and many other applications demonstrating its safety and biocompatibility.

Solvents. Acetone and ethyl acetate were obtained from Fisher Scientific. Dimethyl carbonate was obtained from Acros Organics (NJ, USA). Acetone is one of the least toxic of known solvents that possess many valuable characteristics for particle formation including being volatile, water-miscible, and able to dissolve PMMA. Ethyl acetate and dimethyl carbonate were chosen for their characteristics of being regarded as less toxic than traditional solvents used for particle formation such as toluene and dichloromethane. They are also partially soluble with water and fairly volatile.

Ethyl acetate and dimethyl carbonate were chosen for their characteristics of being relatively volatile and hydrophobic, but not extremely hydrophobic. Ethyl acetate is often used in nanoparticle formation,[38] but dimethyl carbonate has not, to our knowledge, been previously studied for these techniques or similar techniques of nanoparticle synthesis.

Encapsulated Ingredient. Beta-carotene was obtained from Sigma-Aldrich (St. Louis, MO, USA). Beta-carotene has been chosen as the model ingredient to be encapsulated for its various properties. It is highly hydrophobic which makes it suitable for encapsulation in hydrophobic polymeric nanoparticles. Beta-carotene degrades with exposure to UV light which make it suitable to examine the protective effects of encapsulation and UV absorbers.[39] Beta-carotene is also suspected to be susceptible to isomerization, thermal degradation, and chemical oxidations.[40]

Beta-carotene is also excellent for data analysis. Beta-carotene is bright orange in color, which corresponds to a definitive absorbance peak at about 455 nm when observed

using an ultraviolet-visible spectrophotometer (UV/Vis). Absorbance is directly proportional to concentration, so that a calibration curve can easily be drawn to correlate absorbance values to concentration of beta-carotene. This allows the entrapment efficiencies of the various methods to be determined by dissolving the particles and examining the concentration of beta-carotene in a known quantity of solution.

Antioxidant. α-Tocopherol is a water-insoluble compound that is a form of vitamin-E and is widely used in the food and cosmetic industries as an antioxidant and preservative. It removes free radical intermediates, thus disallowing oxidation reactions to continue. α-Tocopherol has also been shown to be a UV absorber and can act as a topical sunscreen.[41]

Surfactant. Poly(vinyl alcohol) (PVA) was used as a surfactant. PVA is a nonionic polymer that is often used to stabilize emulsions for the production of nanoparticles. PVA is quite non-toxic and even biocompatible as it is often used as a moisturizing agent or artificial tear to relieve eye dryness or soreness[42]

3.3 Preparation

First, a stock aqueous solution 4% by mass of PVA was prepared by dissolving 20 g of PVA in 480 g of DI water by heating to 80^{o}C for at least 4 hours and stirring at 250 rpm overnight.

Each oil phase formulation was prepared with 3mg of beta-carotene and 30 mg of α-tocopherol to act as a stabilizer for the beta-carotene, which is further discussed in Chapter 4.

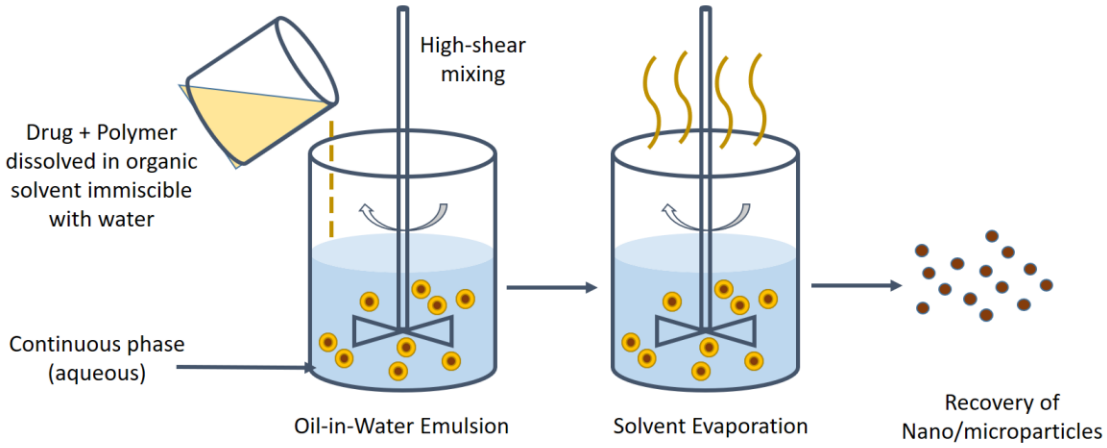
3.4 Synthesis Methods

3.4.1 Solvent Evaporation

Vials that can hold up to 20 mL of water were labeled and weighed. Then the following components were weighed and added to the vial to make up the organic phase: 3 mg of beta-carotene, 30 mg of α-tocopherol, either 100 mg, 200 mg, or 400 mg of PMMA, a 1 cm magnetic stir bar, and 10 mL of solvent. The vials were then weighed again and covered in foil and stirred on a stir plate at 250 rpm to dissolve the PMMA for at least two days.

In a 1 L beaker, the aqueous phase was made by diluting the stock 4% PVA solution with more DI water to create 0.5% by mass PVA solutions of either 150 g, 200 g, or 300 g. We used 0.5% PVA because in preliminary studies, this concentration produced relatively uniform and small particles. Using higher values of PVA would lead to more foaming, and at 4% PVA the solution would be too viscous to blend with homogenization. Once the PMMA in the organic phase was dissolved, the vial was re-weighed to ensure loss of solvent was minimal, otherwise more was added. The organic phase was then added to the aqueous phase and immediately blended using an IKA Ultra Turrax, T-25 homogenizer with S25N dispersing element for 3 minutes at 6,500 rpm or 13,500 rpm. A 2-inch magnet was placed in the beaker and the resulting emulsion was stirred to allow the solvent to evaporate for two days to yield solid polymeric nanoparticles. The particles were centrifuged at 20,000 rpm for 20 minutes, washed two more times, and deposited in a pre-weighed 20 mL vial with an additional 15 mL of DI water added to the wet particles. The vial with particles in water was then weighed again and stored covered in foil until analysis.

Figure 2: Schematic of Solvent Evaporation Method



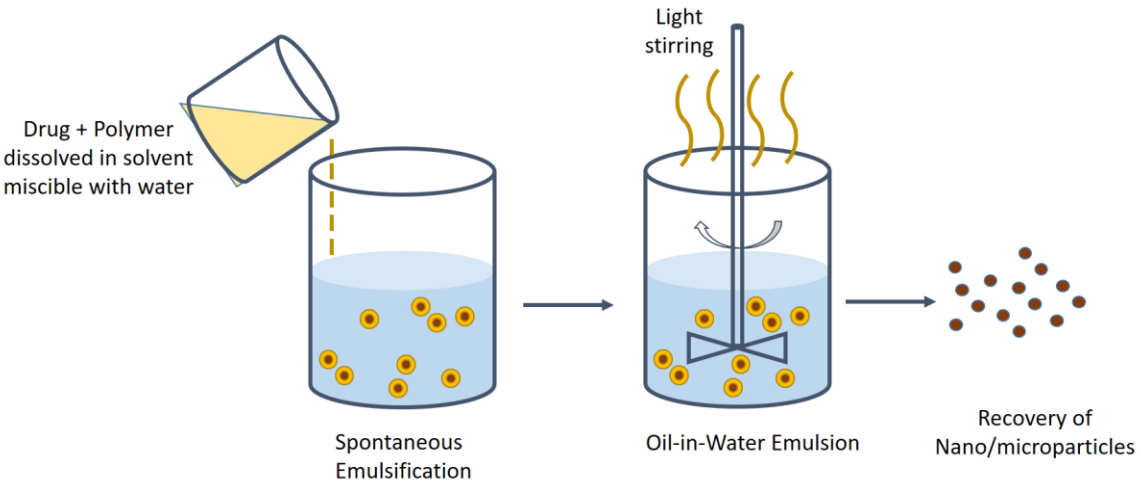
3.4.2 Spontaneous Emulsification

Vials that can hold up to 20 mL of water were labeled and weighed. Then the following components were weighed and added to the vial to make up the organic phase: 3 mg of beta-carotene, 30 mg of α-tocopherol, either 100 mg, 200 mg, or 400 mg of PMMA, a 1 cm magnetic stir bar, and 10 mL of solvent. The vials were then weighed again and covered in foil and stirred on a stir plate at 250 rpm to dissolve the PMMA for at least two days.

In a 1 L beaker, the aqueous phase was made by diluting the stock 4% PVA solution with more DI water to create 0.5% by mass PVA solutions of either 25 g, 50 g, or 100 g. Once the PMMA in the organic phase was dissolved, the vial was re-weighed to ensure loss of solvent was minimal, otherwise more was added. The organic phase was then added to the aqueous phase while stirring at 200 rpm. The emulsion formed spontaneously and the solvent was allowed to evaporate. The particles were centrifuged at 20,000 rpm for 20 minutes, washed 2 more times, and deposited in a pre-weighed 20

mL vial with an additional 15 mL of DI water added to the wet particles. The vial with particles in water was then weighed again and stored covered in foil until analysis.

Figure 3: Schematic of Spontaneous Emulsification Method



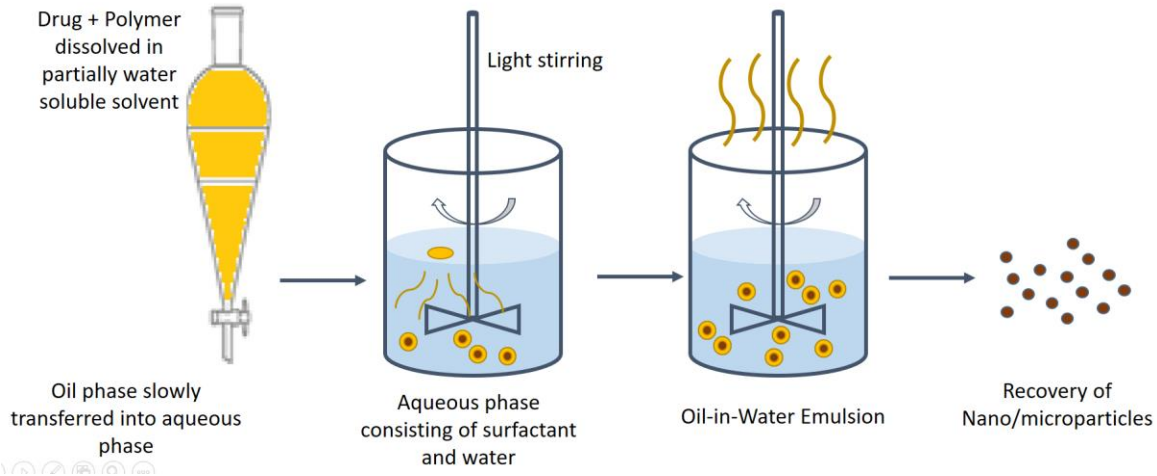
3.4.3 Spontaneous Emulsification Solvent Diffusion

Vials that can hold up to 20 mL of water were labeled and weighed. Then the following components were weighed and added to the vial to make up the organic phase: 3 mg of beta-carotene, 30 mg of α-tocopherol, either 100 mg, 200 mg, or 400 mg of PMMA, a 1 cm magnetic stir bar, and 10 mL of solvent. The vials were then weighed again and covered in foil and stirred on a stir plate at 250 rpm to dissolve the PMMA for at least two days.

The aqueous phase was prepared by pouring 50 mL, 100 mL, or 200 mL of previously prepared 4% PVA solution into a beaker or flask along with a magnetic stir rod. Once the PMMA in the organic phase was dissolved, the vial was re-weighed to ensure loss of solvent was minimal, otherwise more was added. The organic phase was drawn into a 10 mL syringe. A syringe pump was used to pump the organic phase into the aqueous phase under constant stirring at 400 rpm. The syringe pump rates used were 0.5

mL/min, 1 mL/min or 2 mL/min. The resulting emulsion was stirred to allow the solvent to evaporate for 2 days to yield solid polymeric nanoparticles. The particles were centrifuged at 20,000 rpm for 20 minutes, washed 2 more times, and deposited in a pre-weighed 20 mL vial with an additional 15 mL of DI water added to the wet particles. The vial with particles in water was then weighed again and stored covered in foil until analysis.

Figure 4: Schematic of Spontaneous Emulsion Solvent Diffusion Method



3.5 Characterization

3.5.1 Mean Particle Size

The z-average size and size distribution of the particles was determined by dynamic light scattering using a Zeta Nanosizer (Nano S90, Malvern Instruments, U.K.). A scattering angle of 90º was used and sizing experiments were performed at 25 ºC.

3.5.2 Yield

To expand on method for the collection of the particles, we will expand on the synthesis methods previously mentioned. The dye-loaded polymer dispersions were centrifuged at 20,000 rpm and 4 ºC for 20 minutes. The particles were washed two more

times to remove excess PVA. Then, 1.875 mL of DI water was added to each of the already wet particles in the eight centrifuge tubes and vortexed to separate the particles from the tubes. The particles in water were then stored in vials, noting the exact masses of the empty vials and vials with particles in order to obtain the exact mass of water and particles. A sample was removed from the vial for testing by first re-suspending the vial contents, and then pipetting 2.5 mL of particles into a petri dish whose empty mass was already noted. The mass of the vial was noted after pipetting for a more precise value of the mass of the sample used. The petri dish was then placed into an oven at 80 ºC and allowed to evaporate overnight. The mass of the particles was then recorded by subtracting the empty petri dish's mass from that of the dry petri dish with particles. The yield percent was then calculated based on the theoretical maximum mass the sample should have yielded in the 2.5 mL of particles taken from the vial and is as follows:

$$Yield\ Percent = \frac{Experimental\ Dry\ Mass\ of\ Sample}{Theoretical\ Maximum\ Dry\ Mass\ of\ Sample} * 100\%$$

3.5.3 Entrapment Efficiency of Beta-Carotene

A sample was removed from the vial for testing by first re-suspending the vial contents, and then pipetting 1.25 mL of particles into a 10 mL test tube. Then, 8 mL of DI water was added to the test tube. The test tube was then centrifuged at 20,000 rpm and 4 ºC for 20 minutes. The water was decanted off and 3 mL of acetone was added along with a stir bar and stirred at 250 rpm to allow the particles to dissolve. Once dissolved, the UV-visible spectra were recorded using a UV-visible spectrophotometer (Perkin Elmer, Lambda XLS+). The concentration of beta-carotene in the acetone solution was determined by comparing the absorption intensity at 455 nm, where there is a distinct absorbance peak from the beta-carotene, to a calibration curve based on the absorption

intensity at 455 nm for a beta-carotene in acetone. PMMA has negligible absorbance in the vicinity of this beta-carotene peak, thus its presence was acceptable for determining beta-carotene concentration. The experimental concentration of beta-carotene was then compared to the theoretical maximum concentration of beta-carotene from the mass of the sample used and the total mass of particles and water in the vial to determine entrapment efficiency. The equation to determine entrapment efficiency is:

$$Entrapment\ Efficiency = \frac{Experimental\ Concentration\ of\ BC}{Calculated\ Maximum\ Concentration\ of\ BC} * 100\%$$

3.6 Results and Discussion

Each organic phase in the three synthesis methods consisted of 10 mL of a solvent. One of the solvents used for these experiments is dimethyl carbonate (DMC). DMC has some desirable characteristics for particle formation, as does ethyl acetate. In some preliminary studies, we compared the quality of particles prepared with DMC and with ethyl acetate. It was found, based on a smallest size, highest yield, and highest entrapment, that DMC was slightly better than ethyl acetate. Therefore, it was used primarily for subsequent experiments.

Figures 2 and 3 show the micrographs of particles produced via SE and SESD. The SE particles from the micrograph were made with 200 g aqueous phase, 200 mg PMMA, and 6,500 rpm blend rate. The particles made by SESD had been made with, 200 g aqueous phase, 200 mg PMMA, and 2 mL/min blend rate.

Figure 5: Particles made by SE, 200mg PMMA, 6500rpm, 200g of 0.5% PVA

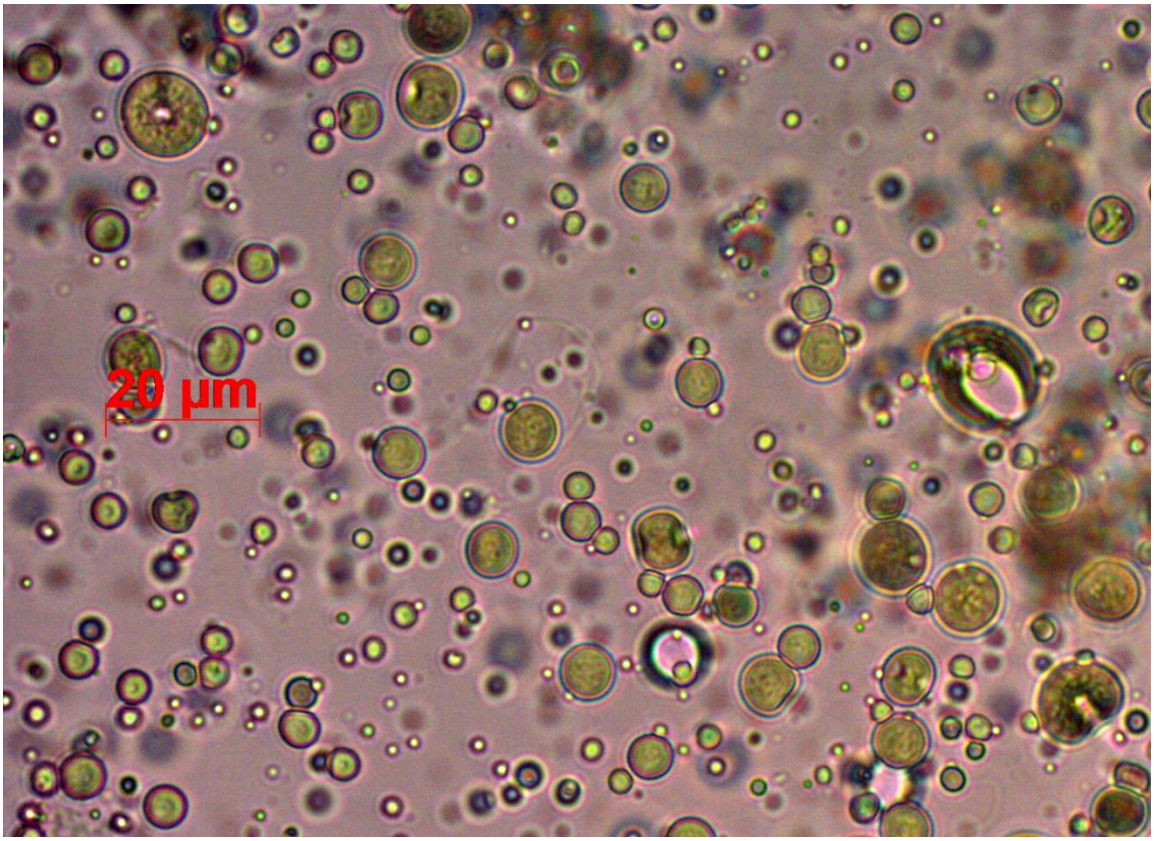


Figure 6: Particles made by SESD, 200mg PMMA, 2mL/min, 50g of 4% PVA

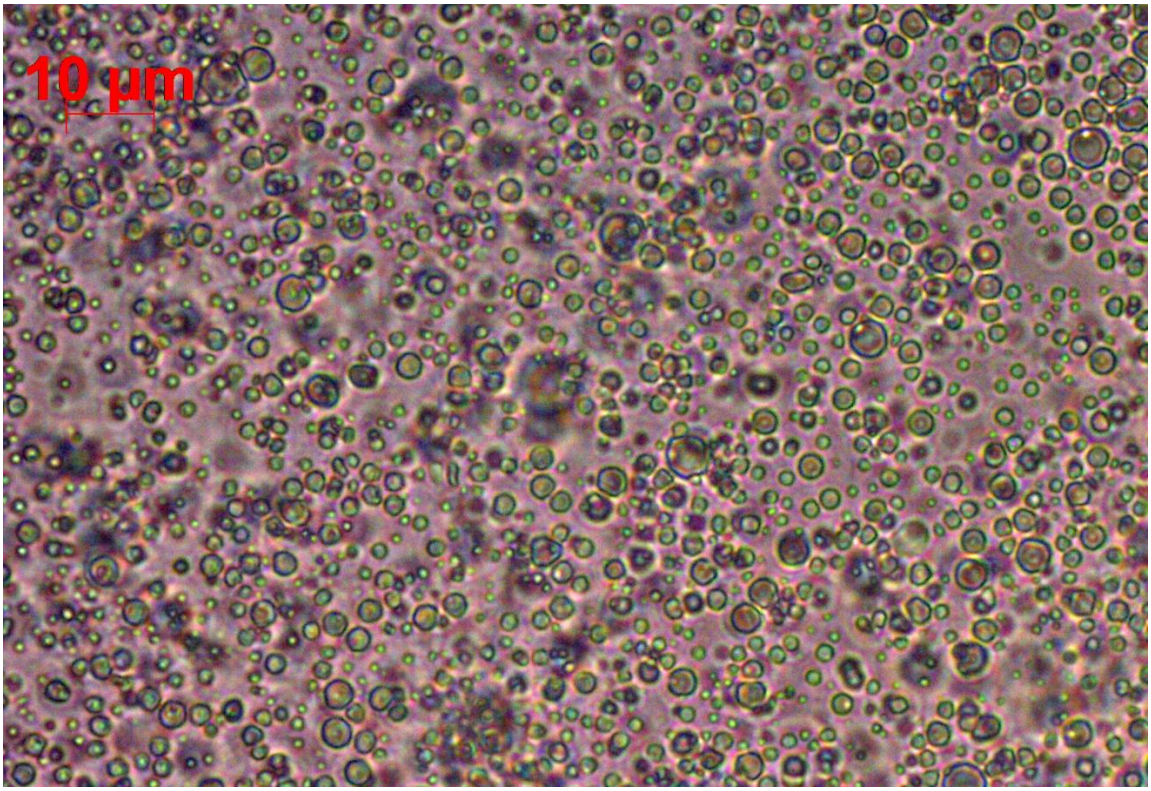


Table 4 shows how varying the size of the water phase affected the particles characteristics. The smallest sizes for solvent evaporation (SE) and SESD both were obtained from the middle water phase sizes, 200 g and 100 g respectively. The particles obtained from the larger water phase in SESD were much bigger, about 5 microns in diameter, even larger than the ones obtained from SE. The smallest particles. The entrapment efficiencies of the particles were generally higher for the larger particles within a mode.

Table 4: Varying Mass of the Water Phases

Mode	Solvent	Water Phase Comp.	Mass Water Phase [g]	Mass PMMA [g]	Pump/Blend Rate	Mean Z-Avg Size [nm]	Mean PDI	Yield [%]	Entrap. [%]
SE	DMC	0.5%	150.2	0.221	6,500rpm	3,418	0.09	60.81	31.02
SE	DMC	0.5%	201.1	0.201	6,500rpm	2,734	0.04	62.02	27.31
SE	DMC	0.5%	300.1	0.205	6,500rpm	2,998	0.10	61.69	31.18
SESD	DMC	4%	49.8	0.207	2.0mL/min	1,479	0.96	64.06	22.74
SESD	DMC	4%	100.0	0.205	2.0mL/min	1,218	0.79	40.63	14.18
SESD	DMC	4%	200.7	0.225	2.0mL/min	5,008	0.75	62.16	25.27
Ouzo	acetone	0.5%	25.0	0.204	-	655	0.47	52.67	26.49
Ouzo	acetone	0.5%	50.5	0.216	-	962	0.49	78.58	64.39
Ouzo	acetone	0.5%	100.0	0.199	-	856	0.60	87.92	53.39

Each variation was given a score based on the size, yield, and entrapment. The sizes of the smallest particles from each mode were divided by each of the sizes of particles that were made with the same method to yield a score between zero and one for each mass of water phase. The highest yields from each mode were divided by each yield from their respective modes to yield a score between zero and one. The same was done for entrapment. The three scores were then added up for each mode to compare overall particle quality. An example of this scoring chart is shown in Table 7 for optimizing of the mass of the water phase for SESD. Using this method of ranking formulations, we determined that the best water phase sizes for each mode were: 200 g for SE, 50 g for SESD, and 100 g for spontaneous emulsification (Ouzo).

The effect of varying the amount of PMMA used in each formation was also examined. As expected, the mass of PMMA used had a large effect on the size of the particles. When more PMMA was used, larger particles were produced. From Table 5, the PMMA masses that produced the smallest size, largest yield, and most entrapment overall were: 100 mg for SE, 100 mg for SESD, and 200 mg for Ouzo. The entrapment was relatively high for the Ouzo particles in this case. We think this was due to the

solution becoming slightly white and cloudy after acetone was added for these cases. Normally, the particles completely dissolve in the acetone, but the Ouzo particles were not dissolving readily.

Table 5: Varying Mass of PMMA in organic phase

Mode	Solvent	Water Phase Comp.	Mass Water Phase [g]	Mass PMMA [g]	Pump/Blend Rate	Mean Z-Avg Size [nm]	Mean PDI	Yield [%]	Entrap. [%]
SE	DMC	0.5% PVA	202.3	0.1091	6,500rpm	1,671	0.44	48.51	23.64
SE	DMC	0.5% PVA	201.1	0.2010	6,500rpm	2,734	0.04	62.02	27.31
SE	DMC	0.5% PVA	202.4	0.4053	6,500rpm	4,355	0.08	48.44	24.34
SESD	DMC	4% PVA	50.1	0.1077	2.0mL/min	1,039	0.15	61.66	25.25
SESD	DMC	4% PVA	49.8	0.2073	2.0mL/min	1,479	0.96	64.06	22.74
SESD	DMC	4% PVA	50.3	0.4073	2.0mL/min	2,008	0.23	58.89	25.12
Ouzo	acetone	0.5% PVA	100.3	0.1024	-	495	0.21	73.49	29.28
Ouzo	acetone	0.5% PVA	100.2	0.1011	-	478	0.13	66.81	27.66
Ouzo	acetone	0.5% PVA	100.0	0.1990	-	856	0.60	87.92	53.39

Varying the blending rate for SE had some peculiarities. The size should normally decrease, with faster blending, but for this case the particles became slightly larger. With the faster blending, more foam was produced, so we think that this could be due to some polymer solidifying in the foam instead of the bulk liquid. This could also explain why the polydispersity was much higher. Entrapment was improved by the faster pump rates of the SESD mode and remained relatively constant when varying blending speed in SE. Yields for all pump/blend variations, except 6,500 rpm in SE, were close to 62%.

Table 6: Varying Blend or Pump Rate

Mode	Solvent	Water Phase Comp.	Mass Water Phase [g]	Mass PMMA [g]	Pump/Blend Rate	Mean Z-Avg Size [nm]	Mean PDI	Yield [%]	Entrap. [%]
SE	DMC	0.5% PVA	202.3	0.1091	6,500rpm	1,671	0.44	48.51	23.64
SE	DMC	0.5% PVA	201.3	0.1039	13,500rpm	1,713	0.87	61.33	23.85
SESD	DMC	4% PVA	50.1	0.1026	0.5mL/min	1,008	0.17	62.52	21.42
SESD	DMC	4% PVA	50.0	0.1077	1.0mL/min	988	0.10	61.80	23.87
SESD	DMC	4% PVA	50.1	0.1077	2.0mL/min	1,039	0.15	61.66	25.25
SESD	DMC	4% PVA	50.1	0.1026	2.0mL/min	2,082	0.48	61.53	26.40

Throughout the methods, the entrapment efficiencies typically hovered around 25% for most cases. We found there was no direct comparison between our PMMA particles encapsulating beta-carotene to other literature values. But, others have reported some much higher entrapment efficiencies for different systems where they do not report any washing steps in their procedure.[43] In some preliminary studies, we tested the entrapment efficiencies of our particles without washing, and we found higher entrapment efficiencies generally around 50 to 70% as well. So, this indicates that much of the beta-carotene was not encapsulated, but merely on the outside of the particles and was removed during washing steps. This could also be why some others have attained such high reported values of entrapment.

Table 7: Score chart various masses of water phase in SESD

SESD - Water Phase Mass [g]	50	100	200
Size Score	0.82	1.00	0.19
Yield Score	1.00	0.63	0.97
Entrapment Score	0.95	0.59	1.00
Total out of 3	2.77	2.23	2.16

We also investigated particle production using a range of values of water phase masses and PMMA masses based on the preparation method of Aubry et al.[33] Their

system was much more dilute in the organic phase with about an order of magnitude less PMMA. The size values that were obtained, shown in Table 8, were in fairly good agreement with Aubry. The other values, yield and entrapment, were not able to be determined because they were too small to compared to the limits of measurement for the equipment used. The beta-carotene was also diluted to coincide with the level of dilution of PMMA, and so it was not possible to reasonably determine a distinct absorbance peak at this dilution. Nevertheless, it provides us with knowledge of how the average size behaves at such a dilution.

Table 8: Spontaneous Emulsification for very low PMMA mass – Varying mass of the water phase

Mode	Solvent	Water Phase Comp.	Mass Water Phase [g]	Mass PMMA [g]	Mean Z-Avg Size [nm]	Mean PDI
Ouzo	acetone	0.5% PVA	12.0	14.5	716	0.43
Ouzo	acetone	0.5% PVA	31.6	14.5	260	0.10
Ouzo	acetone	0.5% PVA	71.0	14.5	282	0.17
Ouzo	acetone	0.05% NaOH	31.7	14.5	127	0.06
Ouzo	acetone	0.05% NaOH	71.1	14.5	202	0.34

In general, we found that particles made using non-toxic chemical were able to be formed rather well. The solvent used for much of the experiments, DMC, has not been used for making these particles before to the authors' knowledge. This partially water-soluble solvent combined with the water-soluble friendly methods can allow for a much greener and safer nanoparticle formation techniques. The particles of sizes ranging from about 100 nm to 5,000 nm were able to be formed. The particles created from acetone through spontaneous emulsification were much smaller than the other methods.

Chapter 4: Beta Carotene Stability in Various Solvents

4.1 Introduction

Beta-carotene has been chosen as the model ingredient to be encapsulated for its various properties as mentioned in Chapter 3. It is highly hydrophobic which make it suitable for encapsulation in hydrophobic polymeric nanoparticles. Beta-carotene degrades with exposure to UV light, and is also suspected to be susceptible to isomerization, thermal degradation, and chemical oxidations.[40] This chapter examines the degradation behavior of beta-carotene in various solvents and stabilization effects of anti-oxidant α-tocopherol.

4.2 Materials

Acetone and ethyl acetate were obtained from Fisher Scientific (NJ, USA). Dimethyl carbonate, dichloromethane, and toluene were obtained from Acros Organics (NJ, USA). Poly(methyl methacrylate) (MW 120,000) was obtained from Aldrich (NJ, USA). Deionized water was used in all experiments. Beta-carotene and α-tocopherol were obtained from Sigma-Aldrich (St. Louis, MO, USA).

4.3 Method

Fifteen vials were prepared each containing roughly 1mg of beta-carotene and 20mL of a solvent. This concentration was chosen because it was determined to be suitable for the UV-visible spectrophotometer's absorbance range. Solvents ethyl acetate, dimethyl carbonate, dichloromethane, toluene, and acetone were each added to 3 of the vials. This preparation was repeated with fifteen more vials that also contained 10 mg of α-tocopherol.

The UV-visible spectra were obtained using a UV-visible spectrophotometer (Perkin Elmer, Lambda XLS+) and the main peak, ~455nm, was recorded. The vials were then stored in a cardboard box at 25 ± 1 ºC to prevent exposure to light until the next measurement was taken. This was repeated at various time points over a period of 49 days to observe the degradation behavior of beta-carotene in the various solvents and the stabilizing effect of α-tocopherol. The absorbance peaks were normalized by dividing their absorbance values by their initial absorbance to show comparative trends in degradation.

4.4 Results

The graphs of the beta-carotene degradation in various solvents is presented below. Presence of α-tocopherol is denoted by the points labeled with VitE.

Figure 7: Stability of Beta Carotene in Dichloromethane

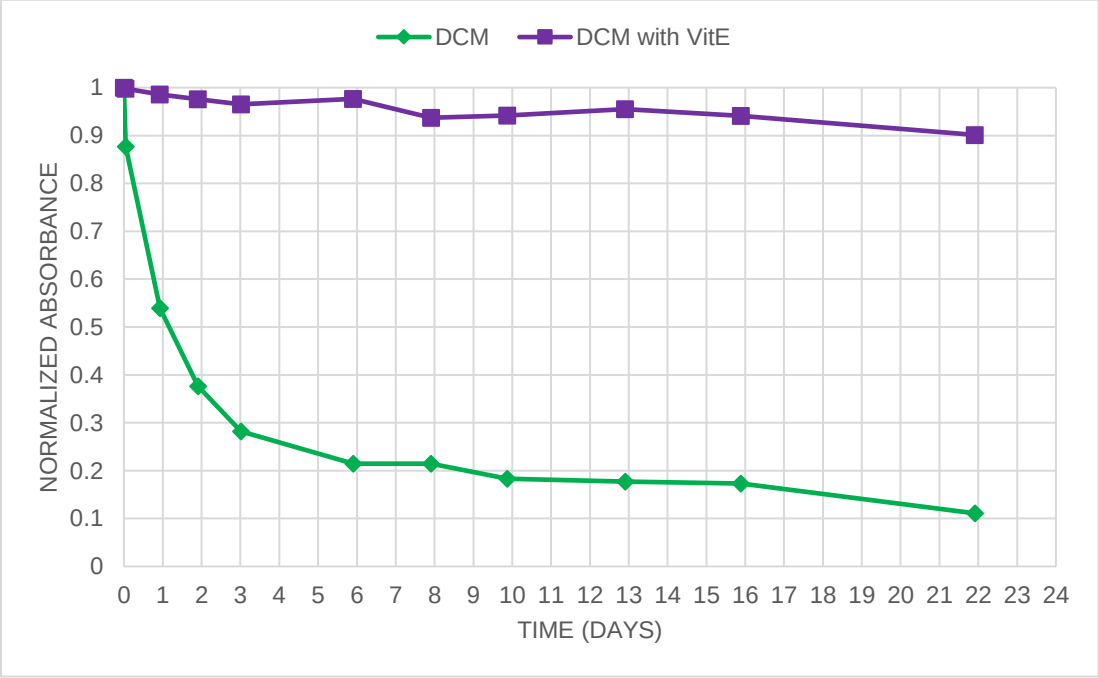


Figure 8: Stability of Beta Carotene in Toluene

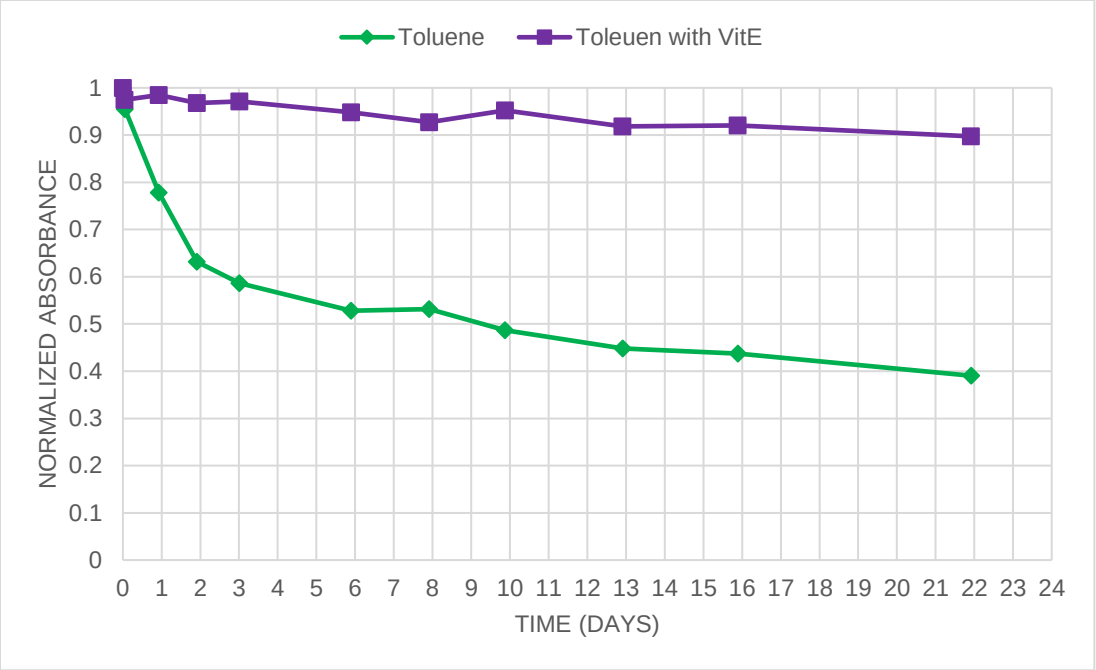


Figure 9: Stability of Beta Carotene in Acetone

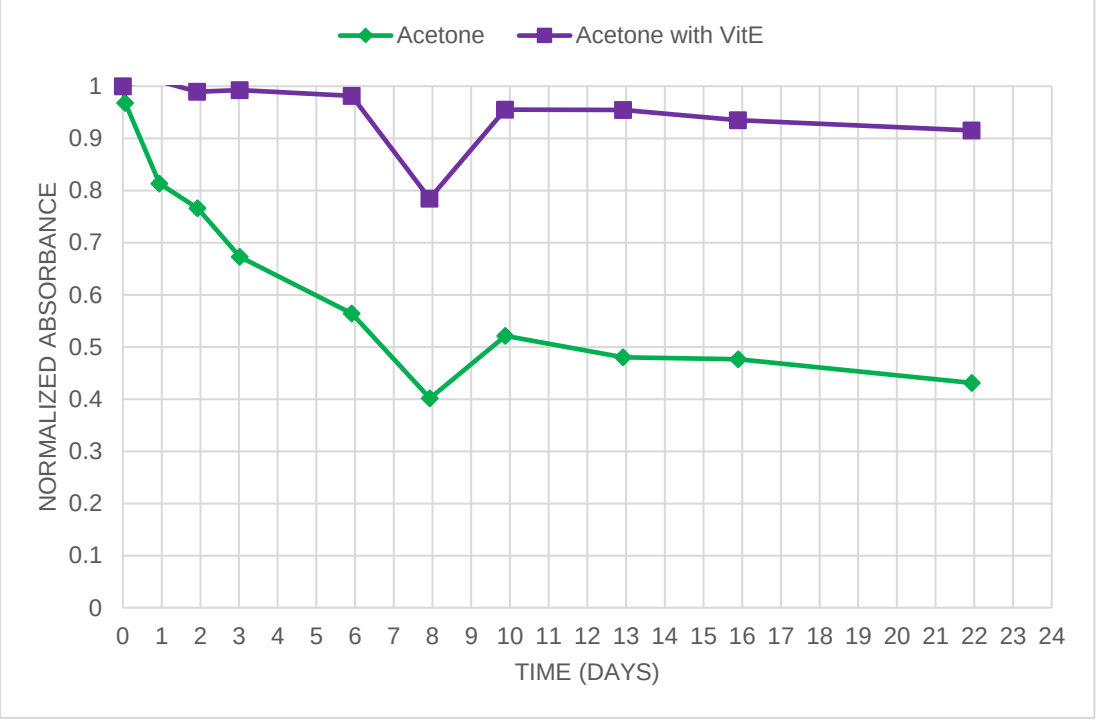


Figure 10: Stability of Beta Carotene in Dimethyl Carbonate

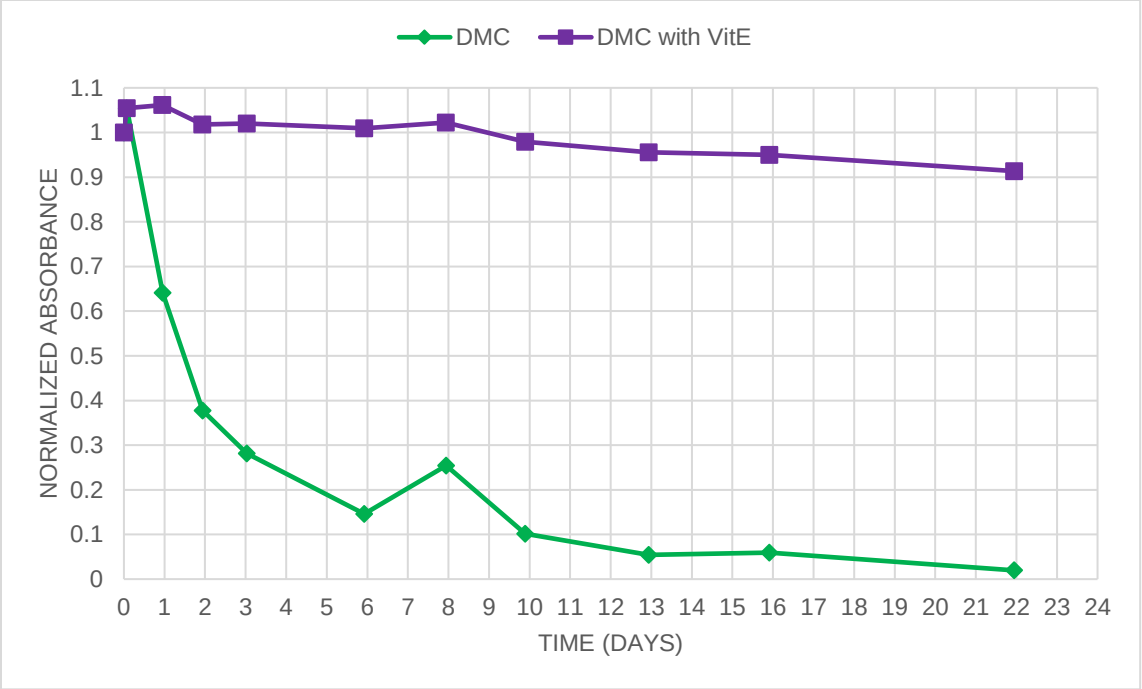
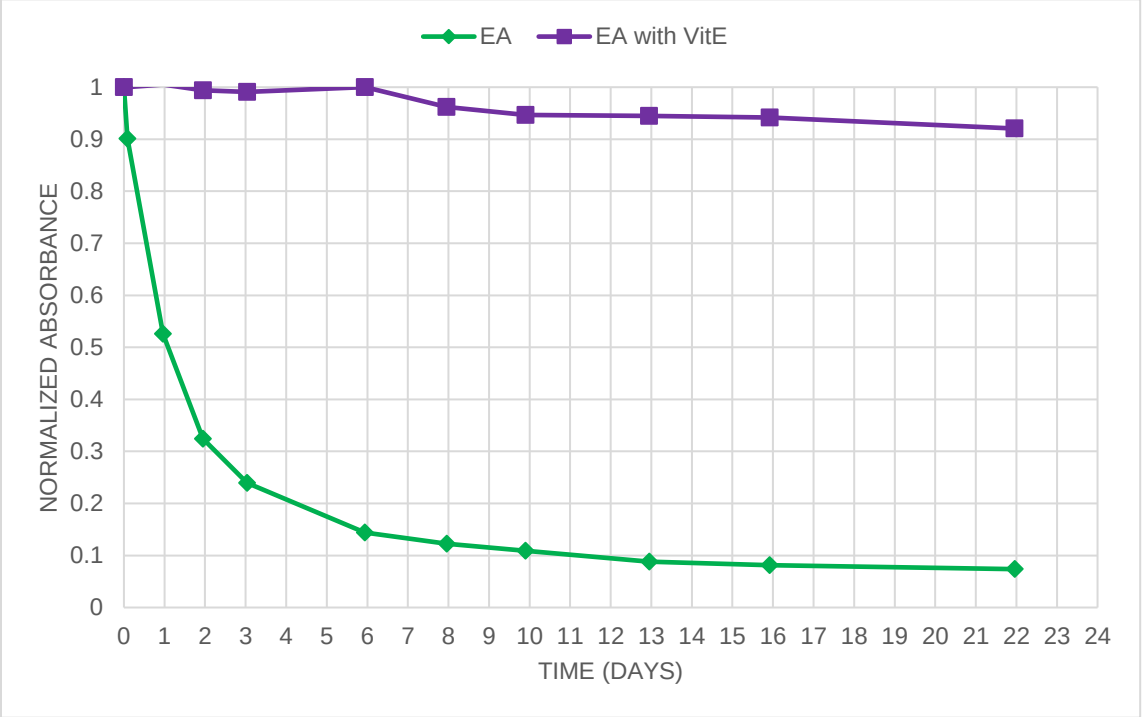


Figure 11: Stability of Beta Carotene in Ethyl Acetate



The results showed that beta-carotene degraded most rapidly in the first few time points when α-tocopherol was not present and slowed in degradation over time. After 10

days, about 50% of the beta-carotene was degraded in acetone and toluene. In the other solvents, around 80 - 90% degraded. When α-tocopherol, denoted as VitE in the graphs, was present, beta-carotene degradation was much slower in all cases and appeared to degrade linearly. After 10 days, only about 10% of the beta-carotene would be degraded when α-tocopherol was present.

The data shows that beta-carotene stability is greatly enhanced when α-tocopherol is incorporated into a formulation. Therefore, the organic phases that were used for the nanoparticle formulations each had α-tocopherol to prevent the degradation.

Chapter 5: Final Remarks

In this thesis, we explored and investigated three particle preparation methods. The quality of the particles was investigated in terms of their average sizes, yield percent, and entrapment percent. The effects of varying the mass of the aqueous phase, the mass of PMMA used, and the blending or pump rates were investigated. Particles made smaller than 1000 nm were easily achieved by spontaneous emulsification using acetone. There was also a formulation that created sub-micron particles with the SESD method. Solvent evaporation, was able to make particles as small as about 1,600 nm. For most cases, the entrapments efficiencies hovered around 25% with some peculiarities. It was found that in general, the larger particles had the larger entrapment efficiencies, therefore minimizing particles size while maximizing entrapment were contradictory goals. Comparing the methods, primarily to the size differences, particles made using the spontaneous emulsification ranked the best overall.

This work allows for a new approach to creating polymeric nanoparticles. By researching non-toxic solvents, we have found a green solvent, namely dimethyl carbonate, that has not been used in these techniques and performs well at creating particles between 1000 nm and 3000 nm with yields of around 65% and entrapment efficiencies of around 25%. This can lead to improvements in the future safety and the environment in the nanoparticle industry while still producing quality nanoparticles.

In the future, there are more avenues to explore that could have an effect on particle characteristics such as alternative polymerizations and extent hydrolysis of PVA, exploring mixtures of solvents used for the oil phases, and/or adjusting the pH of the aqueous phase. Others have indicated that less hydrolyzed and lower polymerization

PVA leads less aggregation in the SESD method.[21] Adjusting the pH to be lower than the pKa of the encapsulated material has been shown to increase encapsulation for a similar system.[15] Since there were only three variations of each of the input variables, there are likely values that were not tested that may produce even better particles than the ones obtained in this research. More formulations could be tested to further improve upon these methods.

We found through our beta-carotene stability experiments that the degradation of beta-carotene can be drastically slowed down with the addition of α-tocopherol. This stabilized beta-carotene to degrade less than 10% after 10 days. Where without α-tocopherol, between about 50% to 90% of beta-carotene degrades after 10 days.

Furthermore, the effect of UV radiation on the beta-carotene along with the stabilization effect of α-tocopherol and other UV-absorbers could be investigated. Also, encapsulation of other materials, including less hydrophobic materials, could be further investigated to determine particle characteristics such as encapsulation efficiency. Drug release could also be investigated for controlled release nanoparticles of various formulations.

References

[1] Perugini, P., et. al. (2002). Effect of nanoparticle encapsulation on the photostability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate. *International Journal of Pharmaceutics, 246*(1-2), 37-45.

[2] Steeley, K. G., et. al. (2014). Uniform polymer particles formulated with ultraviolet protective materials for the protection of UV sensative molecules. *Dyes and Pigments, 105*, 12-22.

[3] Naito, T., et. al. (1991). Photochemistry in polymer solids. 11. The effects of the size of reaction groups and the mode of photoisomerization on photochromic reactions in polycarbonate film. *Macromolecules, 24*(10), 2907-2911.

[4] Morabito, K., et. al. (2012). Proximal effects of ultraviolet light absorbers and polymer matrix in the photostability of β-carotene. *Dyes and Pigments, 92*(1), 509-516.

[5] Weiss-Angeli, V., et. al. (2008). Nanocapsules of octyl methoxycinnamate containing quercetin delayed the photodegradation of both components under ultraviolet a radiation. *Journal of Biomedical Nanotechnology, 4*(1), 80-89.

[6] Birnbaum, D. T., et. al. (2000). Controlled release of b-estradiol from PLAGA microparticles: The effect of organic phase solvent on encapsulation and release. *Journal of Controlled Release, 65*, 375-387.

[7] Wang, J., & Schwendeman, S. P. (1999). Mechanisms of solvent evaporation encapsulation processes: prediction of solvent evaporation rate. *Journal of Pharmaceutical Sciences, 88*(10), 1090-1099.

[8] Mao, S., et. al. (2008). Effects of process and formulation parameters on characteristics and internal morphology of poly(d,l-lactide-co-glycolide) microspheres formed by the solvent evaporation method. *European Journal of Pharmaceutics and Biopharmaceutics, 68*(2), 214-223.

[9] Bodmeier, R., & McGinity, J. W. (1987). The preparation and evaluation of drug-containing poly(dl-lactide) microspheres formed by the solvent evaporation method. *Pharmaceutical Research, 4*(6), 465-471.

[10] Anselmi, C., et. al. (2002). New microencapsulated sunscreens: technology and comparative evaluation. *International Journal of Pharmaceutics, 242*(1-2),207-211.

[11] Mu, L., & Feng S. S. (2003). A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS. *Journal of Controlled Release, 86*(1),33-48.

[12] Herrman, J., & Bodmeier, R. (1995). Somatostatin containing biodegradable microspheres prepared by a modified solvent evaporation method based on W/O/W-multiple emulsions. *International Journal of Pharmaceutics, 126*(1-2), 129-138.

[13] O'Donnell, P. B., & McGinity. (1997). Preparation of microspheres by the solvent evaporation technique. *Advanced Drug Delivery Reviews, 28*(1), 25-42

[14] Sternling, C. V., & Scriven, L. E. (1959). Interfacial turbulence: Hydrodynamic instability and the marangoni effect. *AiChE Journal, 5*(4), 514-523.

[15] Niwa, T., et. al. (1993). Preparation of biodegradable nanospheres of water-soluble and insoluble drugs with D,L-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *Journal of Controlled Release, 25*, 89-98.

[16] Davies, J. T., & Rideal, E. K. (1961) Disperse systems and adhesion. *Interfacial Phenomena.* Academic Press, Inc.: New York, 343-450.

[17] Ruschak, K. J., & Miller, C. A. (1972). Spontaneous emulsification in ternary systems with mass transfer. *Industrial & Engineering Chemistry Fundamentals, 11*(4), 534-540.

[18] Lopez-Montilla, J. C., et. al. (2002). Spontaneous emulsification: Mechanisms, physicochemical aspects, modeling, and applications. *Dispersion Science and Technology, 23*(1-3), 219–268.

[19] Bouchemal, K., Briancon, S., Perrier, E., & Fessi, H. (2004). Nano-emulsion formation using spontaneous emulsification: solvent, oil and surfactanct optimization. *International Journal of Pharmaceutics, 280*, 241-251.

[20] Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R., & Rudzinski, W. E. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release*, 20, 1-20.

[21] Murakami, H., et. al. (1999). Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. *International Journal of Pharmaceutics, 187*, 143-152.

[22] Berman, J., Zorrilla-Lopez, U., Farre, G., Zhu, C. F., Sandmann, G., Twyman, R. M., Capell, T.; Christou, P. (2015). Nutritionally important carotenoids as consumer products. *Phytochemistry Reviews, 14*(5), 727-743.

[23] Hodge, H. C., & Sterner, J. H. (1943). Determination of substances acute toxicity by LD50. *American Industrial Hygiene Association, 10*, 93.

[24] Smith, M. P. (2012). Divergent Regulatory Trajectories. In *Environmental and Health Regulation in the United States and the European Union: Protecting Public and Planet* (pp. 1-21). Palgrave Macmillan US.

[25] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labeling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No. 1907/2006. *Official Journal of the European Communities: Legislation*, (2008). L353/1.

[26] Green, D. W., & Perry. (2008). *Perry's Chemical Engineers' Handbook* (8th ed.). New York: McGraw-Hill.

[27] Benazzouz, A., et. al. (2013). Selection of a Greener Set of Solvents Evenly Spread in the Hansen Space by Space-Filling Design. *Industrial & Engineering Chemistry Research, 52*, 16585-16597.

[28] Hansen, C. M. (2007). *Hansen Solubility Parameters: A User's Handbook* (2nd ed.). Boca Raton, Florida: CRC Press.

[29] Hildebrand, J., & Scott, R. L. (1950). *The Solubility of Nonelectrolytes* (3rd ed.). Reinhold, New York.

[30] Blanks, R. F., & Prausnitz, J. M. (1964). Thermodynamics of Polymer Solubility in Polar and Nonpolar Systems. *Industrial & Engineering Chemistry Fundamentals, 3*(1), 1-8.

[31] Stefanis, E., & Panayiotou, C. (2008). Prediction of Hansen Solubility Parameters with a New Group-Contribution Method. *International Journal of Thermophysics, 29*(2), 568-585.

[32] Li, L., et. al. (2014). Theoretical selection of solvent for production of electrospun PMMA fibers with wrinkled surfaces. *RSC Advances, 4*, 27914-27921.

[33] Aubry, J., et. al. (2009). Nanoprecipitation of Polymethylmethacrylate by Solvent Shifting: 1. Boundaries. *Langmuir, 25*(4), 1970-1979.

[34] Mallepally, R. R., et. al. (2014). Hydrogen peroxide filled poly(methyl methacrylate) microcapsules: Potential oxygen delivery materials. *International Journal of Pharmaceutics, 475*, 130-137

[35] Schoener, C. A., & Peppas, N. A. (2013). pH-Responsive hydrogels containing PMMA nanoparticles: an analysis of controlled release of a chemotherapeutic conjugate and transport properties. *Journal of Biomaterials Science, Polymer Edition, 24*(9), 1027-1040.

[36] Sivakumar, M., & Rao, K. P. (2000). Synthesis and characterization of poly(methyl methacrylate) functional microspheres. *Reactive and Functional Polymers, 46*(1), 29-37.

[37] Quintanar-Guerrero, D. et. al. (1997). A mechanistic study of the formation of polymer nanoparticles by the emulsification-diffusion technique. *Colloid and Polymer Science, 275*, 640-647.

[38] Lee, S. J., & McClements, D. J. (2010). Fabrication of protein-stabilized nanoemulsions using a combined homogenization and amphiphilic solvent dissolution/evaporation approach. *Food Hydrocolloids, 24*(6-7), 560-569.

[39] Gloria, M. B. A., et. al. (1993). Effect of type of oxidation on b-carotene loss and volatile products formation in model systems. *Food Chemistry, 46*(4), 401-406.

[40] Qiu, D., et. al. (2012). Stability of b-Carotene in Thermal Oils. *Journal of Food Biochemistry, 36*, 198-206.

[41] McVean, M., & Liebler, D. C. (1997). Inhibition of UVB induced DNA photodamage in mouse epidermis by topically applied alpha-tocopherol. *Carcinogenesis, 18*(8), 1617-1622.

[42] Winterton, L. C., et. al. (2007). The elution of poly (vinyl alcohol) from a contact lens: The realization of a time release moisturizing agent/artificial tear. *Journal of Biomedical Materials Research Part B: Applied Biomaterials, 80B*(2), 424-432.

[43] Teeka, P., Chaiyasat, A., & Chaiyasat, P. (2014). Preparation of Poly (methyl methacrylate) microcapsule with encapsulated Jasmine oil. *Energy Procedia, 56*, 181-186.