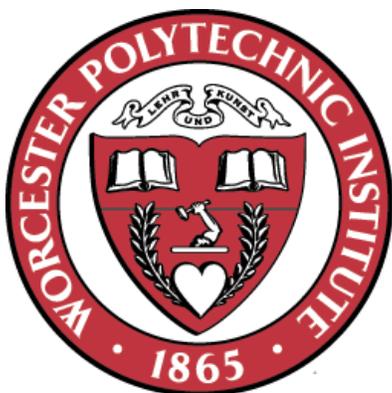


Near-PIT Nanoparticle Formulation

A Major Qualifying Project Report:
Submitted to the faculty of
Worcester Polytechnic Institute
In partial fulfillment of the requirements for the
Degree of Bachelor of Science

By: David Saxner

April 28, 2011



This report represents the work of a Worcester Polytechnic Institute undergraduate student submitted to the faculty as evidence of completion of a degree requirement. Worcester Polytechnic Institute routinely publishes these reports on its website without editorial or peer review.

Abstract

Interest in nanoparticles has skyrocketed in the past decade. With uses in medicine, cosmetics, and specialty chemicals there is a competition to synthesize new formulations of nanoparticles. Using the low energy, Near-PIT emulsification method followed by polymerization, nanoparticles containing a mixture of styrene and glycidyl methacrylate were synthesized. It was found that the average synthesized nanoparticle size was 91.5 nm, making the Near-PIT nanoparticles smaller than those synthesized by ultrasound, where the average particle size was 123.4 nm. The percent of epoxy groups on the surface of the synthesized nanoparticles was found to be 12.1%, slightly higher than that on the surface of nanoparticles synthesized by ultrasound, which was 11.6%.

Acknowledgements

I would like to acknowledge Professor Terri Camesano, of Worcester Polytechnic Institute, who was my main advisor for this paper. Without her assistance the quality of this paper would have been significantly diminished.

I would like to thank Dr. Johanna Galindo-Alvarez, my laboratory advisor at Ecole Nationale Supérieure des Industries Chimiques, for her constant assistance while I was conducting this research. Without her this paper would not exist.

I would also like to express my gratitude to Professors Alain Durand and Véronique Sadtler of Ecole Nationale Supérieure des Industries Chimiques' Laboratoire de Chimie Physique Macromoléculaire and Laboratoire Réactions et Génie des Procédés respectively. Their assistance proved extremely valuable when difficulties arose.

I would like to extend my thanks to the research professors, post doctorate researchers, and doctorate students at the Laboratoire de Chimie Physique Macromoléculaire who were always willing to help me when I required assistance.

I would like to also like to thank Marie Blanchard who assisted me with the transition to Nancy, France. She assisted with the paperwork and significantly reduced the amount of energy required to transition.

Finally, I would like to thank all of the people who assisted in the editing process for this paper. The comments they proved critical in improving the quality of this paper.

Executive Summary

Interest in nanoparticles has grown dramatically in the last decade. New technologies have overcome many of the limitations on the synthesis of nanoparticles that previously hindered nanoparticle research and development. The new technology has resulted in intense competition among a growing number of researchers to create and adapt new and better methods of synthesizing nanoparticles. Currently, the main uses of nanoparticles are medicine, cosmetics, and specialty chemicals (Scientific Committee on Emerging and Newly Identified Health Risks, 2006). This paper focuses on the exploration of a recently adapted method for synthesizing miniemulsions, called the near phase inversion temperature (Near-PIT) method, and its viability in synthesizing nanoparticles that contain a mixture of styrene and glycidyl methacrylate. This research was conducted in an attempt to enhance Near-PIT nanoparticle synthesis applications.

Background

Nanoparticles have gained popularity as a result of their small size. This small size provides the particles with a high surface area to volume ratio, the ability to get into places that larger particles cannot, less light obstruction, and more. In addition to uses in medicine and cosmetics, nanoparticle uses include but are not limited to: specialty chemicals, food products, and environmental technology (Scientific Committee on Emerging and Newly Identified Health Risks, 2006). Nanoparticles are also opening possibilities for new products that have not been possible until now (Scientific Committee on Emerging and Newly Identified Health Risks, 2006).

Emulsions are systems in which two immiscible liquids are mixed together to form a single phase. One form of emulsion is a miniemulsion, which is defined by the droplet size of the dispersed liquid, with a diameter ranging from 20 to 200 nm (Solans, Izquierdo, Nolla, Azemar, & Garcia-Celma, 2005). Miniemulsions are not thermodynamically stable and thus require energy input in order to form (Fernandez, André, Rieger, & Kuhnle, 2004). As a result, the produced emulsion is dependent upon the method of preparation (Márquez, Mirra, Peña, Tyrode, & Salager, 2003).

A low energy method that was recently adapted from a previous method is the near phase inversion temperature method (Near-PIT), which makes use of transitional phase inversion,

where the emulsion switches from an oil in water emulsion to a water in oil emulsion (Tadros, Izquierdo, Esquena, & Solans, 2004). The Near-PIT emulsification method utilizes the chemical potential of the components in the emulsion as well as temperature (Anton & Vandamme, 2009). In the Near-PIT method, the system is heated close to but kept below the phase inversion temperature (PIT), and then quenched by rapid cooling, which fixes the droplet size. The emulsion is then used to synthesize nanoparticles via polymerization.

The Near-PIT method has the potential for changing the way in which industrial nanoparticles are synthesized (Vauthier & Bouchemal, 2009). Unlike some other forms of emulsification, the Near-PIT method is easily scalable from the laboratory to manufacturing. In addition, certain chemical compounds are unable to be synthesized through high energy emulsification methods, but can be synthesized via low energy methods (Anton, Benoit, & Saulnier, 2008).

Methodology

The synthesis of nanoparticles varies upon the method being used. In this case, the Near-PIT emulsification method was tested and the ultrasound emulsification method served as the control. To synthesize nanoparticles using the Near-PIT method the solution was heated to 5 degrees Celsius below the PIT where it held for 15 minutes, quenched in an ice bath, and then allowed to polymerize at 45 degrees Celsius for 24 hours. To synthesize nanoparticles by ultrasound, the solution was subjected to two 120 second ultrasound pulses and then polymerized at 45 degrees Celsius for 24 hours.

The evaluation of the synthesized nanoparticles contained three elements: evaluation of nanoparticle size, characterization of nanoparticle size dispersion, and determination of the percent of epoxy groups on the surface of the nanoparticle. To evaluate the size of the synthesized nanoparticles a High Performance Particle Sizer (HPPS) was utilized. The nanoparticle size dispersion was characterized through the use of a Mastersizer. The percent of epoxy groups on the surface of the particle was determined by titration.

Results and Discussion

Through the conducted research, it was found that nanoparticles containing glycidyl methacrylate and styrene could be synthesized through the Near-PIT method followed by polymerization. This process produces nanoparticles with a smaller size than nanoparticles

synthesized via ultrasound emulsification followed by polymerization. The average size of nanoparticles containing a three to one ratio of styrene to glycidyl methacrylate synthesized by the Near-PIT method was 91.5 nm, while the average size of nanoparticles of the same initial composition synthesized via ultrasound was 123.4 nm. This result is in agreement with published literature, in which Galindo-Alvarez et al. found that nanoparticles synthesized by the Near-PIT method followed by polymerization were smaller than those synthesized by ultrasound followed by polymerization (Galindo-Alvarez et al., 2011). It was found that there was a bimodal size distribution for nanoparticles synthesized by the Near-PIT method, while the nanoparticles synthesized via ultrasound yielded only a single, more stable, distribution of particle size.

The nanoparticles synthesized via the Near-PIT method that contained a ratio of three to one styrene to glycidyl methacrylate had just over 12% of the epoxy groups on the surface of the particle, while nanoparticles of the same ratio synthesized by ultrasound contained 11.6% epoxy groups on the surface of the particle. In light of potential variance in synthesizing nanoparticles, the epoxy groups on the surface of the nanoparticles for both solutions can be viewed as potentially equivalent.

Recommendations

Based upon the collected data I recommend that future researchers focus their efforts on three areas. First, I recommend a comprehensive examination of the effects of higher agitation to reduce the discontinuity in synthesized nanoparticle size. I recommend that research be conducted into modification of the nanoparticles, utilizing the epoxy groups on the surface of the particle. Finally, I recommend that future research be conducted to evaluate the ability of the Near-PIT emulsification method followed by polymerization to synthesize nanoparticles using other formulations of emulsions containing different mixtures of oils.

Conclusion

The research conducted at Ecole Nationale Supérieure des Industries Chimiques, Laboratoire de Chimie Physique Macromoléculaire, demonstrates the viability of synthesizing nanoparticles that contain a mixture styrene and glycidyl methacrylate, by the Near-PIT emulsification method followed by polymerization. The Near-PIT synthesized nanoparticles were smaller than those synthesized by ultrasound; however, the nanoparticles synthesized by ultrasound had a uniform size distribution, while the Near-PIT nanoparticles did not. Nanoparticles synthesized by the

Near-PIT method were found have just over 12% of the total epoxy groups in the particle on the surface.

Table of Contents

Abstract	i
Acknowledgements	ii
Executive Summary	iii
Table of Contents	vii
List of Figures	viii
List of Tables	ix
Introduction.....	1
Background	3
Emulsions	3
Miniemulsion Formulation.....	7
Applications	10
Methodology.....	12
Solutions.....	12
Equipment	13
Procedure.....	14
Results and Discussion	17
PIT Determination.....	17
Nanoparticle Size	20
Epoxy Content of the Nanoparticles	24
Recommendations.....	26
Conclusion	27
References.....	28
Appendix A: Titration Data	31
Appendix B: HPPS Nanoparticle Size Data	32
Appendix C: Mastersizer Results.....	33

List of Figures

Figure 1: The Different Kind of Emulsions (Salager, 1999)	3
Figure 2: The Different Kinds of Surfactant.....	5
Figure 3: A Micelle (Wikimedia, 2010)	6
Figure 4: Effect of Temperature on an Emulsion System (Solans et al., 2005)	7
Figure 5: Phase Inversion (Galindo-Alvarez et al., 2011)	9
Figure 6: Emulsion Properties as Related to Temperature (Nielloud & Marti-Mestres, 2000)....	10
Figure 7: Mastersizer Results for 1:1 Styrene to Glycidyl Methacrylate Nanoparticles Synthesized by Near-PIT	22
Figure 8: Mastersizer Results for 3:1 Styrene to Glycidyl Methacrylate Nanoparticles Synthesized by Near-PIT	23
Figure 9: Mastersizer Results for 3:1 Styrene to Glycidyl Methacrylate Nanoparticles Synthesized by Ultrasound	23
Figure 10: Percent of Epoxy Groups on the Surface of the Nanoparticles.....	24

List of Tables

Table 1: Visual PIT Determination.....	18
Table 2: Visual PIT Determination Average	19
Table 3: Comparative PIT Determination.....	20
Table 4: Nanoparticle Size and PDI.....	21

Introduction

Interest in nanoparticles has sky-rocketed in the last decade, with more research into nanoparticle synthesis and modification than ever before. Once extremely limited by the available equipment, new technologies have partially removed the barriers to research and development, and allow for new methods of synthesis. The main uses of nanoparticles are in medicine for drug delivery and fluorescence, cosmetics, and the synthesis of specialty chemicals (Scientific Committee on Emerging and Newly Identified Health Risks, 2006). This paper focuses on the exploration of a method, recently adapted from an older method, used to synthesize miniemulsions, called the near phase inversion temperature (Near-PIT) method, and its viability in synthesizing nanoparticles.

The Near-PIT method was created through the adaptation of the phase inversion temperature (PIT) method. The PIT method and Near-PIT methods are low energy ways of forming miniemulsions and synthesizing nanoparticles via polymerization. Unlike the PIT and Near-PIT methods, the majority of the other methods of synthesis involve high energy processes which limit the potential use of certain chemicals in the process. In addition to being viable for more chemicals, the PIT and Near-PIT methods are easily adaptable from lab scale to plant scale as they rely upon temperature instead of the mechanical devices used in the high energy methods (Anton et al., 2008).

This research was based upon the research conducted by Galindo-Alvarez et al. in which styrene nanoparticles were synthesized using the Near-PIT emulsification method followed by polymerization (Galindo-Alvarez et al., 2011). For this research, the nanoparticles synthesized contained a mixture of styrene and glycidyl methacrylate. Glycidyl methacrylate was used so that the synthesized nanoparticles would have exposed epoxy groups on their surface. After the synthesis of the nanoparticles, the exposed epoxy rings provide a way of modifying the nanoparticles, and allow for the addition of chemical compounds to the surface of the particle.

The research included in this paper was conducted between January and March, 2011, at Ecole National Supérieure des Industries Chimiques, Laboratoire de Chimie Physique Macromoléculaire. The research was a joint research project between the Laboratoire de Chimie

Physique Macromoléculaire and Laboratoire Réactions et Génie des Procédés, with an overseas advisor from Worcester Polytechnic Institute.

Background

Emulsions

Introduction to Emulsions

An emulsion is a system in which two insoluble or nearly insoluble phases are thoroughly mixed together to form a single phase. In an emulsion, one phase envelops the other, leaving the dispersed (interior) phase to form spherical droplets inside of the continuous (exterior) phase.

The most common types of emulsions are oil in water (O/W) and water in oil (W/O). There are also water in oil in water ($W_1/O_1/W_2$) and oil in water in oil ($O_1/W_1/O_2$) emulsions, where W_1 and W_2 or O_1 and O_2 can be the same liquid, but these emulsions are less common. The last type of emulsion is biemulsions in which two oil phases are contained in a water phase (O_1+O_2/W) (Salager, 1999).

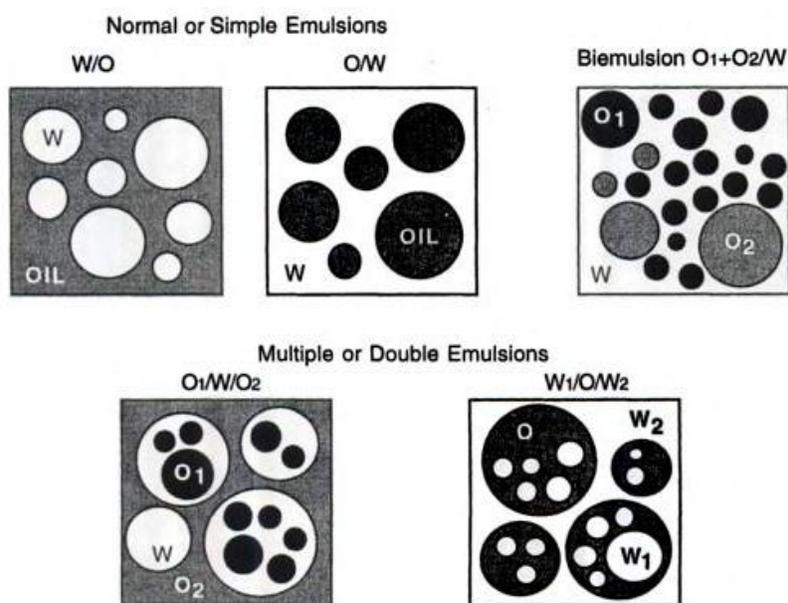


Figure 1: The Different Kind of Emulsions (Salager, 1999)

There are two size categories of emulsions, microemulsions and miniemulsions. Each of which has its own benefits and drawbacks.

Microemulsions

Microemulsions are defined by their thermodynamic stability. This stability stems from their ability to become a single phase system in which there is neither an internal nor external phase. As a result of this single phase, microemulsions are unable to be diluted; because a change in

composition would alter the system as a whole. Microemulsion's droplet size can range from 1 μm to 500 μm (Nielloud & Marti-Mestres, 2000).

Miniemulsions

Miniemulsions are defined by their small droplet size ranging from 20-200 nm (Solans et al., 2005). This extremely small drop size allows the emulsion to be either transparent or translucent, unlike microemulsions. Also, unlike microemulsions, miniemulsions are dependent upon the method of preparation (Anton, Gayet, Benoit, & Saulnier, 2007). This includes, but is not limited to the emulsification method, composition of the solution, surfactants present, temperature, and pressure.

The small drop size allows for greater stability in the solution, as a result of Brownian motion, which allows the drops to counteract the effects of creaming and sedimentation (Solans et al., 2005). As a result, the main method of degradation of miniemulsions is Oswald rippling. One way of reducing the effects of Oswald rippling is through the addition of surfactant (Tadros et al., 2004).

Surfactants

Surfactants are organic amphiphilic compounds. This means that they contain both a hydrophilic head and a hydrophobic tail. This combination allows them to adsorb on the oil-water interface of an emulsion. The adsorbed surfactant reduces the interfacial tension between the two phases, which aids the breakup of the droplets and helps resist recoalescence (Salager, 1999).

Surfactants are classified by the charge of their polar head. Figure 2, below, shows the four different categories of surfactants: anionic (negatively charged), cationic (positively charged), non-ionic (no charge), zwitterionic (both positive and negatively charged).

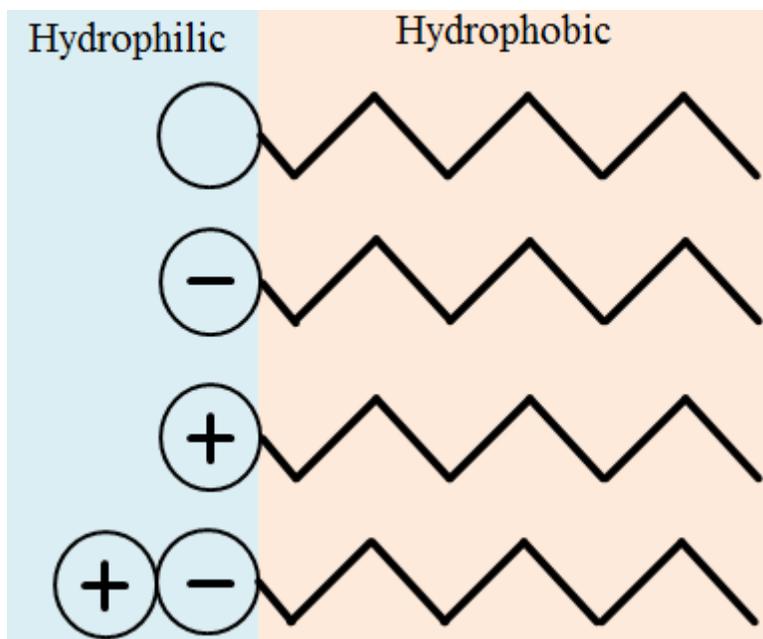


Figure 2: The Different Kinds of Surfactant

The surfactant plays the deciding role in determining the external phase, as described by the Bancroft rule, “a hydrophile colloid will tend to make water the dispersing phase while a hydrophobe colloid will tend to make water the dispersed phase” (Ruckenstein, 1996). As summarized by the Bancroft rule, the surfactant plays a large role in the type of emulsion that is formed.

Micelles

If the surfactant concentration is above the Critical Micelle Concentration (CMC), micelles will form (Jones & Leroux, 1999). Micelles are drops of surfactant that are that contain no internal phase. As seen in Figure 3, below, in an attempt to meet the desired conditions, the hydrophilic surfactant heads form a barrier around the hydrophobic tails when immersed in an oil in water emulsion.

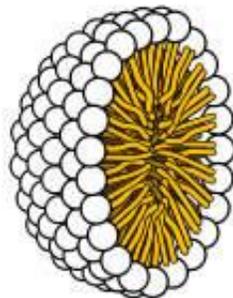


Figure 3: A Micelle (Wikimedia, 2010)

Hydrophile-Lipophile Balance

The hydrophilic or hydrophobic nature of surfactants is described by a surfactant's Hydrophile-Lipophile Balance (HLB) number. The HLB number ranges from 1 to 20, where 7 represents a neutral HLB. HLB numbers less than 8 are not dispersible in oil, while HLB numbers greater than 12 are fully soluble in water (Solans et al., 2005).

Winsor Ternary Diagram

Winsor ternary diagrams are used to determine where the surfactant is located in the solution and the type of micelles present (Salager, 1999). Figure 4, below, provides information on Winsor types I, II, and III.

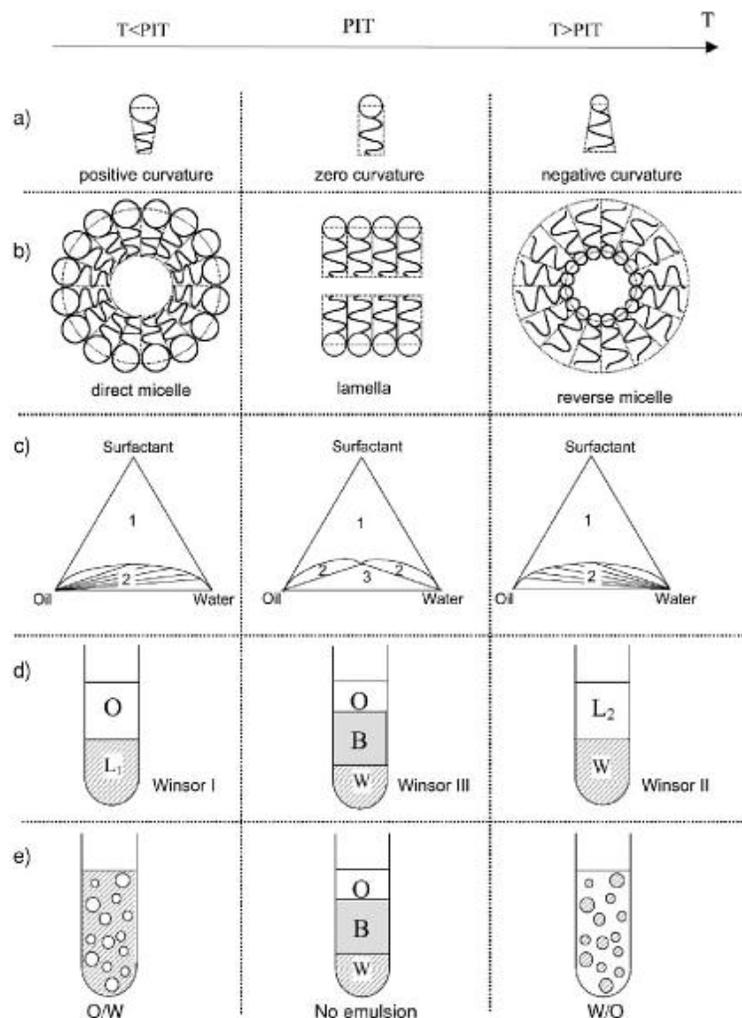


Figure 4: Effect of Temperature on an Emulsion System (Solans et al., 2005)

The Winsor type I diagram shows a solution in which normal micelles (O/W) are able to occur. The Winsor type II diagram is the opposite of the type I diagram, in which inverse micelles (W/O) are able to occur. Winsor type III is the intermediary phase between types I and II, in which a three phase system can occur. In a Winsor type III, the surfactant is located between the oil and aqueous phases, favoring neither normal nor inverse micelles (Solans et al., 2005).

Miniemulsion Formulation

Miniemulsions are non-equilibrium systems that cannot form spontaneously. As a result, the solution needs to have energy added in order to reach the particle size that defines a

miniemulsion. This added energy can come from two sources, either mechanical devices or the system's chemical potential (Anton et al., 2008).

High Energy Emulsification Methods

Until recently the main method of synthesizing miniemulsions was through the use of high energy methods. The three main methods of forming miniemulsions through high energy methods are ultrasound emulsification, high shear homogenization, and high pressure homogenization.

Ultrasound Emulsification

In ultrasound emulsification, an ultrasound probe is used to break apart larger oil droplets into nano sized oil droplets. An ultrasound probe uses sound pressure to break up the droplets. Li and Fogler proposed two mechanisms of how an ultrasound probe synthesizes miniemulsions (Li & Fogler, 1978a). The first mechanism is that the energy produced by the ultrasound causes oil droplets to become unstable, causing their surface to rupture. This in turn causes the oil phase to spill into the aqueous phase, creating smaller oil droplets. The second mechanism relies upon the cavitation caused by the ultrasonic emissions, which causes the creation and collapse of nano scale bubbles. Upon collapsing these bubbles create a high level of local turbulence, and it is this turbulence that breaks apart the oil droplets (Li & Fogler, 1978b).

High Pressure Homogenization

For high pressure homogenization, a pump, filter, and interaction chamber are required. The pump propels the fluids through the filter into micro channels in the interaction chamber. In the micro chambers dynamic interactions occur between the oil and the aqueous phase. The dynamic interactions result in the production of a miniemulsion (Maa & Hsu, 1999).

High Shear Homogenization

For high shear homogenization a machine such as an Ultra Turrax is used. In this process, the head of the probe is inserted into the solution. The head contains two pieces, a still part and a rotating part. The moving section of the Ultra Turrax rotates, causing the solution to be continuously sucked in to and ejected from between the two sections, which are spaced at varying lengths apart, normally less than 0.1 centimeters. This creates a large amount of turbulence which results in the synthesis of a miniemulsion (Maa & Hsu, 1999).

Low Energy Emulsification Methods

The low energy emulsification methods make use of oil and water physical properties, relying upon either temperature or concentration to form miniemulsions (Anton et al., 2008). The two main methods of low energy emulsification are catastrophic phase inversion and phase inversion temperature (PIT)/ near phase inversion temperature (Near-PIT) (Tadros et al., 2004). To understand these methods it is necessary to understand how oil in water and water in oil emulsions change with respect to concentration and temperature.

Phase Inversion

Phase inversion can occur in two ways, through catastrophic phase inversion or through transitional phase inversion. Catastrophic phase inversion is caused when the concentration of the emulsion is changed so that it crosses either of the vertical lines as seen below in Figure 5. Transitional phase inversion is caused by a change in temperature so that the emulsion crosses the horizontal line as seen below in Figure 5 (Fernandez et al., 2004).

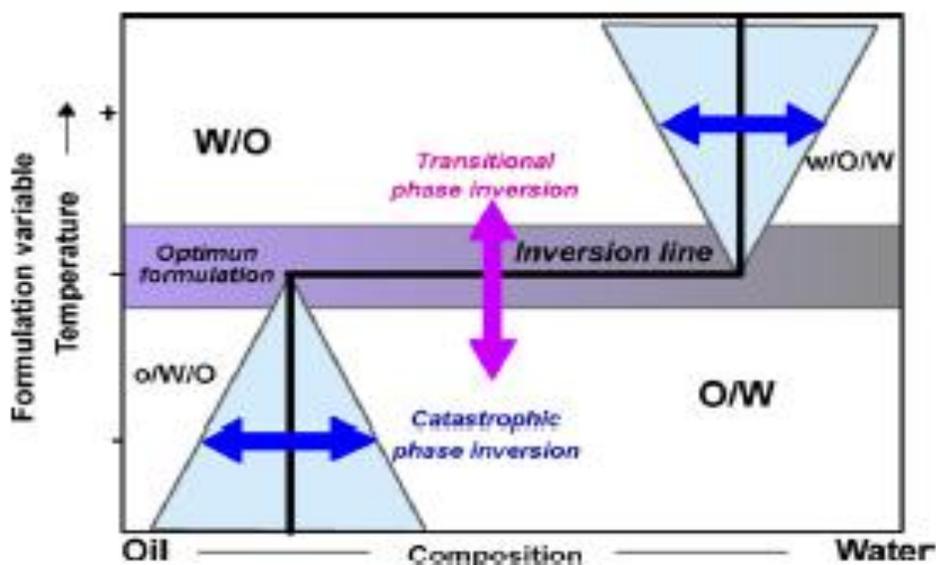


Figure 5: Phase Inversion (Galindo-Alvarez et al., 2011)

Catastrophic Phase Inversion

The exact mechanism that drives catastrophic phase inversion is currently unknown, but Pacek et al. surmises that the sudden change in the emulsion occurs when an excess of the dispersed phase exceeds the critical volume of liquid allowed by the system for the droplets to be in their closest packed formation (Bouchama, Van Aken, Autin, & Koper, 2003). The shift that occurs as a

result of catastrophic phase inversion is non-reversible. One reason behind this is that the catastrophic phase inversion line changes based upon the direction from which it is approached, as seen above in Figure 5.

Transitional Phase Inversion

As with catastrophic phase inversion, the exact mechanism behind transitional phase inversion is unknown. It is known that as the temperature nears the phase inversion temperature (PIT), the curvature of the droplets decreases and at the PIT the curvature of the droplets is zero (Fernandez et al., 2004). Unlike catastrophic phase inversion, transition phase inversion is reversible; by either cooling or heating across the PIT (Fernandez et al., 2004). Utilizing transitional phase inversion, two low energy methods of emulsification have been created. The first is PIT, where the emulsion is heated above the PIT and then cooled to slightly below the PIT before being quenched by a sudden large drop in temperature. The second is Near-PIT where the emulsion is heated almost to the PIT and then quenched (Tadros et al., 2004). Figure 6, below, shows the surface tension, emulsion stability, and drop size as the temperature varies. T^* is the PIT temperature.

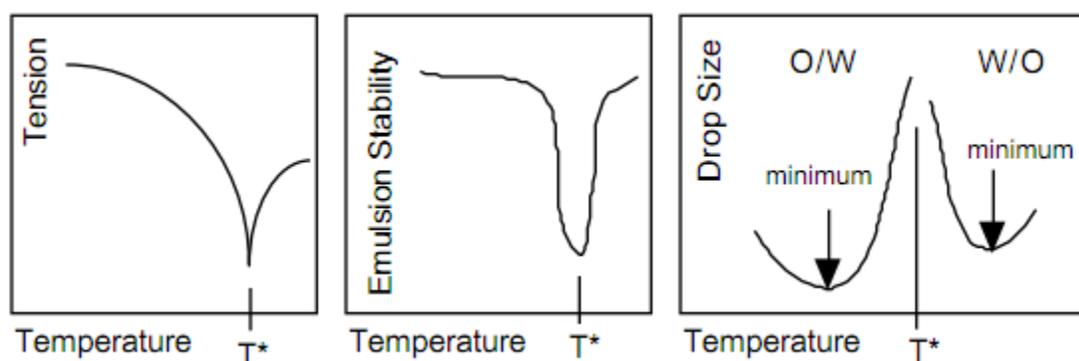


Figure 6: Emulsion Properties as Related to Temperature (Nielloud & Marti-Mestres, 2000)

Applications

The use of nanoparticles in products began in medicine for drug delivery and imaging purposes, and has since spread to cosmetics, specialty chemicals, food, environmental technology, and much more (Scientific Committee on Emerging and Newly Identified Health Risks, 2006). With the recent influx of attention to nanoparticles, this list is sure to continue to grow, with products once viewed as not viable turning into a reality.

The medical industry has benefited greatly from the initial formulation of nanoparticles. In particular, drug delivery has been extremely enhanced as a result of the initial synthesis of nanoparticles. Some current uses of nanoparticles for drug delivery include, but are not limited to: porous silica for chemotherapy, nanoparticles with attached proteins for targeted delivery to fight cardio vascular disease, and nanoparticles that release nitric oxide in order to fight drug resistant staph bacteria (Scientific Committee on Emerging and Newly Identified Health Risks, 2006).

As a result of their translucent nature nanoparticles have also been adopted by the cosmetic industry. Nanoparticles have become commonly used in creams to fight aging, enhance skin strength with vitamins, reduce the effects of nickel allergies, and even to fight sun damage (Scientific Committee on Emerging and Newly Identified Health Risks, 2006).

Methodology

Solutions

Aqueous (Phase) Solution

The aqueous solution consisted of 1% (weight per volume) sodium chloride dissolved in Milli-Q water. The sodium chloride was added to increase the conductivity of the solution, which is important for PIT determination.

Surfactant Solution

The surfactants were dissolved in the aqueous solution, to the amount of 5.00 g of Brij 78 and 2.69 g of Brij 700, and then completed to 100 ml with the aqueous solution to form the surfactant solution. The amount of surfactant was calculated so that there would be 5% (weight per volume) surfactant in the nanoparticle solution, with a 65:35 ratio of Brij 78 to Brij 700.

Brij 78 (S20)

Brij 78 is a non-ionic surfactant consisting of polyoxyethelene stearyl ether with a polyoxyethelene chain length of approximately 20. The chemical formula is $C_{18}H_{37}(OCH_2CH_2)_nOH$, where n is approximately 20. The HLB number for Brij 78 is 15.

Brij 700

Brij 78 is a non-ionic surfactant consisting of polyoxyethelene stearyl ether with a polyoxyethelene chain length of approximately 100. The chemical formula is $C_{18}H_{37}(OCH_2CH_2)_nOH$, where n is approximately 100. The HLB number for Brij 78 is 18.

Oil (Phase) Solution

The oil solution consisted of 5% hexadecane, to reduce Oswald ripping, and a mixture of styrene and glycidyl methacrylate with compositions ranging from 25% styrene to 80% styrene. The styrene, 99% purity, was distilled under reduced pressure in order to remove the inhibitor added during manufacturing. The glycidyl methacrylate used was 97% pure.

Nano emulsion Solution

The nanoemulsion solution consisted of 35% oil solution and 65% surfactant solution.

Initiator Solution

The initiator solution consisted of 0.07 g of potassium persulfate, 99.99% purity, dissolved in 2 ml of Milli-Q water.

Cresol Color Indicator Solution

The cresol color indicator solution consisted of 1% (weight per volume) cresol, 95% purity, dissolved in a 50% ethanol 50% water mixture. Cresol color indicator solution is pink at pH's lower than 7.2, yellow between 7.2 and 8.8, and purple at pH's higher than 8.8.

Phenolphthalein Color Indicator Solution

The phenolphthalein color indicator solution consisted of 1% (weight per volume) phenolphthalein, pure, dissolved in a 50% ethanol and 50% water mixture. Phenolphthalein color indicator solution is transparent at pH's lower than 8.2 and pink at pH's greater than 8.2.

Hydrogen Chloride Solution

A 0.2 M hydrogen chloride solution was prepared by mixing hydrogen chloride into water.

Sodium Hydroxide Solution

A 0.1 M sodium hydroxide solution was prepared by mixing sodium hydroxide into water.

Equipment

Jacketed Heating Vessel and Associated Equipment

The conductivity in relation to temperature of the pre-emulsified solution was tested in a 200 ml glass heating vessel, which was attached to a thermostated bath. The vessel was placed upon a stir bar apparatus and agitated at 800 rpm. Inside of the heating vessel were conductivity and temperature probes, which relayed the collected data to a nearby laptop.

Temperature Controlled Reactor

The temperature controlled reactor vessel that utilized vaseline oil for heat transfer was heated from room temperature to five degrees Celsius below the PIT at approximately two degrees per minute. The apparatus supported the use of a stir bar, which was set fixed 800 rpm.

Temperature Controlled Polymerization Bath

The temperature controlled polymerization bath that utilized vaseline oil for heat transfer was set at 45 degrees Celsius. The apparatus supported the use of a stir bar, which was set fixed 800 rpm.

Ultrasound

The ultrasound probe was situated in the middle of a containment vessel lined with aluminum foil. The ultrasound probe was placed in the solution and allowed to run for two 120 second

periods at 300 W. The solution was placed in a water bath in order to prevent any possible overheating.

Titration Pipette

The titration pipette was a 50 ml pipette.

High Performance Particle Sizer

The High Performance Particle Sizer (HPPS) was made by Malvern Industries. The HPPS was connected to a computer which calculated the data using Dispersion Technology Software. To calculate average particle size and polydispersity index (PDI) the HPPS took numerous readings of particle sizes. The HPPS data was used as the main size estimate for the nanoparticles, as it was better suited for determining nanoparticle size in the expected size range.

Mastersizer

The Mastersizer used was a Mastersizer HYDRO 2000. A Mastersizer measures particle size and the scattering pattern by using detectors which takes snapshots, called sweeps, of the dispersal pattern of the particles. In a typical Mastersizer analysis over 2,000 sweeps are performed (Malvern Instruments Ltd., 1997). The collected data is then used to determine the size and scattering pattern of the nanoparticles. It was connected to a computer that measured the sizes of the different particles. The Mastersizer was primarily used for determining the nanoparticles' size range(s) and size dispersal pattern.

Procedure

PIT determination

The PIT of the emulsion solution was determined by subjecting the sample to a vortex for three minutes to ensure a well-mixed solution. The solution was then poured into a 200 ml jacked heating vessel that was connected to a thermostated bath. Temperature and conductivity probes were positioned near the top of the emulsion. These probes relayed all collected data to a computer for analysis. The temperature of the system was slowly raised one degree per minute, under constant low agitation from a magnetic agitator. The solution was closely monitored for a separation of phase and drops in conductivity.

Near-PIT Nanoparticle Synthesis

To synthesize nanoparticles by the Near-PIT emulsification method, the solution was subjected to three minutes in the vortex to ensure a well-mixed emulsion. The solution was then placed in the temperature controlled reactor, and the temperature was slowly raised from room temperature to 5 degrees below PIT, 60 degrees Celsius, at approximately 2 degrees a minute. Upon reaching 60 degrees Celsius the solution was allowed to sit for 15 minutes.

The emulsion was then removed from the reactor and quenched in an ice bath until the temperature had dropped to 20 degrees Celsius. After the quench was complete, the initiator solution was added to the emulsion. The quenched emulsion was submerged in a temperature controlled polymerization bath, set at 45 degrees Celsius, and allowed to polymerize for 24 hours. During the entire process, except in the vortex, the emulsion was under constant agitation from a magnetic stir bar rotating at 800 rpm. The vaseline oil inside of the reactor and polymerization bath was also subjected to agitation by stir bar, rotating at 800 rpm.

Ultrasound Nanoparticle Synthesis

To synthesize nanoparticles by ultrasound, the solution was subjected to three minutes in the vortex to ensure a well-mixed emulsion. The emulsion was then put through two 120 second ultrasound pulses, at 300 W and a 50% cycle. For the first 120 seconds, the tip of the ultrasound probe was located in the top half of the emulsion, and for the second period the probe was located in the bottom half. During both pulses, the solution was immersed in water in order to reduce the amount of heat added to the system by the probe. The initiator was then added to the emulsion. The emulsion was then submerged in the temperature controlled polymerization bath set at 45 degrees Celsius and allowed to polymerize for 24 hours. During that time both the emulsion and the polymerization bath were subjected to constant agitation from magnetic stir bars rotating at 800 rpm.

Titration to Determine Epoxy Content on the Surface of the Nanoparticles

In order to determine the epoxy content on the surface of the nanoparticles, titration was used. 2.86 g of nanoparticles were added to a 250 ml Erlenmeyer flask containing 25 ml of 0.2 M hydrochloric acid solution. The solution was then agitated for 2 hours by a magnetic stir bar, spinning at 100 rpm. After the nanoparticles were thoroughly dispersed throughout the solution, 25 ml of ethanol and 5 ml of color indicator solution were added. This solution, under constant

agitation from a magnetic stir bar at 1,000 rpm, was titrated with the 0.1M sodium hydroxide solution. This process was also used for the titration of the surfactant solution (1.86 g of surfactant) and for a solution containing neither nanoparticles nor surfactant.

Particle Size Determination

The average size of the particles was determined through the use of the High Performance Particle Sizer (HPPS). A single drop of nanoparticle solution was placed in a disposable cuvet and diluted with 3 ml of Milli-Q water. The cuvet containing the sample was inserted into the HPPS for testing.

The size distribution of the synthesized nanoparticles was found through the use of a Mastersizer. The Mastersizer liquid well was filled with Milli-Q water and a few drops of nanoparticle solution were added, so that the opacity of the sample was eight percent.

Results and Discussion

The data collected is divided into three sections: PIT determination, nanoparticle size, and percent of epoxy groups on the surface of the synthesized nanoparticles. The PIT determination data was used to determine the temperature at which the nanoparticles were synthesized, while the nanoparticles' size and percent of epoxy groups on the surface were used to evaluate the synthesized nanoparticles.

PIT Determination

Previous research determined the PIT by measuring the conductivity of the solution and when the PIT was crossed and the emulsion inverted, the conductivity dropped to zero (Galindo-Alvarez et al., 2011). In this research, the conductivity drop did not occur as the glycidyl methacrylate in combination with the styrene prevented the formation of a uniform oil phase and thus prevented a water in oil emulsion. This in turn prevented a full and instantaneous inversion from an oil in water emulsion to a water in oil emulsion. Without the sudden inversion, the aqueous solution was not fully encapsulated in drops and able to act as a conductor between the probes. There was a partial drop in conductivity after crossing the PIT, as recorded by the conductivity probes, but the conductivity drop was minor and could easily be seen as part of the background noise on the computer generated graphs.

As a result of the conductivity readings yielding no definitive result, visual signs were used to determine the PIT. The indication that the PIT had been reached was the appearance of small oil bubbles (less than 1 mm) in the emulsion. This was followed by the formation of a flow pattern in the shape of a vortex and the formation of midsized bubbles (1-3 mm). After additional heating, the bubbles gave way to a patch of oil, located on the surface, which would form around the probe and would be followed by a color change in the system and then the formation of a complete oil layer. The collected data has been included below in Table 1.

Table 1: Visual PIT Determination

Ratio of styrene to glycidyl methacrylate	3:7	1:1	1:1	1:1	4:1	4:1	4:1
Small bubbles on surface (°C)	64	62	66	63	64	67	66
Flow pattern (°C)	64	71	-	68	69	70	72
Midsized bubbles (°C)	66	-	74	71	74	73	71
Oil patch (°C)	69	72	73	72	77	74	75
Color change (°C)	76	78	77	78	77	80	81
Oil layer (°C)	81	-	81	81	-	82	77

Before the PIT determination test, the emulsion was subjected to three minutes in the vortex at 2,500 cycles per minute. While the vortex successfully yielded smaller droplets, a small number of droplets that were able to be classified as small bubbles remained. This posed a problem and holds the potential to have influenced the results. The averages of each composition are shown below in Table 2.

Table 2: Visual PIT Determination Average

Ratio of styrene to glycidyl methacrylate	3:7 (n=1)	1:1 average (n=3)	4:1 average (n=3)
Small bubbles on surface (°C)	64	64	65
Flow pattern (°C)	64	69	70
Midsized bubbles (°C)	66	72	73
Oil patch (°C)	69	73	75
Color change (°C)	76	78	79
Oil layer (°C)	81	81	79

This data shows that the emulsion begins breaking down at approximately 65 degrees Celsius, which was determined to be the PIT temperature. Due to the nature of the measurements, this is only an estimate. From this data, the relative stability of each emulsion system was determined by comparing the averages against each other. Table 3, below, shows the difference in the average temperatures between the varying compositions of styrene for each surface pattern.

Table 3: Comparative PIT Determination

Ratio of styrene to glycidyl methacrylate	4:1 compared to 3:7	4:1 compared to 1:1	1:1 compared to 3:7
Small bubbles on surface (°C)	1	2	-1
Flow pattern (°C)	6	1	5
Midsized bubbles (°C)	6	0	6
Oil patch (°C)	6	3	3
Color change (°C)	4	2	2
Oil layer (°C)	-2	-2	0

Table 3, above, shows how much higher or lower the higher fraction of styrene's average temperature was than the lower fraction of styrene. From this data, it was concluded that the higher the fraction of styrene in the system, the greater the system stability with respect to temperature.

Nanoparticle Size

The nanoparticle sizes were determined via the use of a High Performance Particle Sizer (HHPS). Table 4, below, shows the average size and the polydispersity index (PDI) of nanoparticles synthesized by either ultrasound or Near-PIT.

Table 4: Nanoparticle Size and PDI

Ratio of styrene to glycidyl methacrylate and method of synthesis	1:1 Ultrasound	1:1 Ultrasound	1:1 Near-PIT	3:1 Near-PIT
Average particle size (nm)	119.4	123.4	108.8	91.5
Average particle size standard deviation (nm)	0.76	0.86	1.20	0.49
Average polydispersity index	0.055	0.053	0.324	0.102

A Student T-Test was used to determine if two sets of data were able to be considered equal. A Student T-Test evaluates if data falls within a bell shaped curve, with larger tails, and is used for situations where there is a small sample size. The Student T-Tests of the samples revealed that none of the samples can support the possibility that they are equivalent, as all returned values supported a null hypothesis. Of the solutions, the most similar were the two synthesized via ultrasound, which yielded a T-Test result of 0.003, where the other T-tests yielded numbers of approximately 10^{-10} . For a T-Test, the value must be greater than 0.05 for the sets of data to be considered equivalent.

The data provided in Table 4 yields two major trends. The first is that the particle size of nanoparticles synthesized via ultrasound was larger than that of nanoparticles synthesized by the Near-PIT method; however, the PDI was lower for those particles synthesized by ultrasound. This result was expected, as ultrasound is an extremely consistent way of synthesizing nanoparticles, but is unable to synthesize particles as small as those synthesized by the PIT or Near-PIT methods, as mentioned in published literature. Galindo et al., found that nanoparticles synthesized by the Near-PIT emulsification method were between a third to two thirds smaller than those synthesized via ultrasound (Galindo-Alvarez et al., 2011).

The other major trend is that as the nanoparticles synthesized by the Near-PIT method decrease in styrene content, they become less stable and consistent. The nanoparticles containing a ratio of three to one styrene to glycidyl methacrylate had a PDI that is within the accepted range of 0.2 or lower, but the one to one ratio nanoparticles' PDI was large enough to raise worries with the measurement. Attempts at synthesizing nanoparticles that contain a one to three ratio of styrene to glycidyl methacrylate, by the Near-PIT method proved unsuccessful. This instability is believed to occur as a result of the difference in densities between glycidyl methacrylate and styrene. Under ideal conditions, the solution would be stirred at a high enough rate to prevent separation. Using the available equipment, this option was deemed impossible and is the reason that the vortex was used before the formation of the emulsions. While the vortex provided enough agitation to initially provide the desired mixture quality, the Near-PIT process takes approximately 30 minutes, which gave the glycidyl methacrylate enough time to separate from the styrene.

Further investigation through the use of a Mastersizer revealed that the nanoparticles in both the one to one and the three to one styrene to glycidyl solutions synthesized by the Near-PIT method contained a bimodal size distribution. Figure 7, Figure 8, and Figure 9, below, show Mastersizer graphs of a one to one styrene to glycidyl methacrylate nanoparticle solution synthesized via the Near-PIT method, a three to one styrene to glycidyl methacrylate nanoparticle solution synthesized via the Near-PIT method, and a three to one styrene to glycidyl methacrylate nanoparticle solution synthesized via ultrasound respectively.

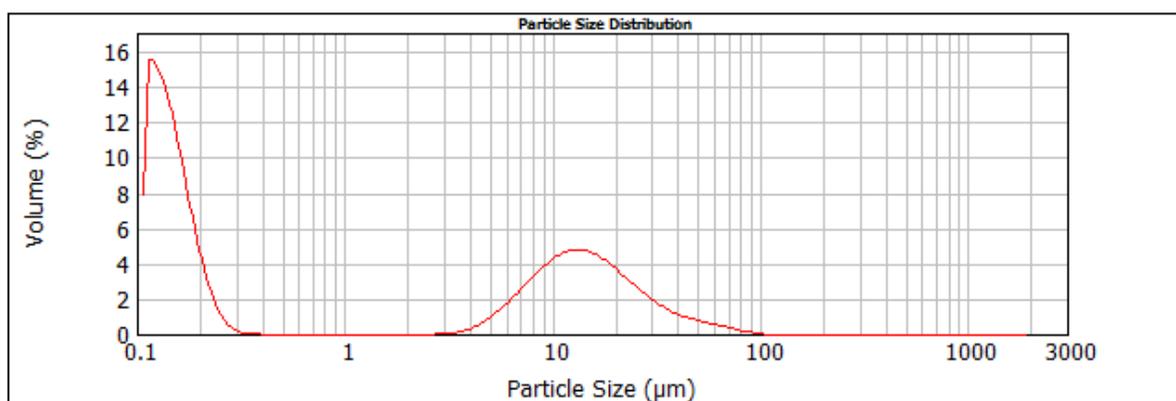


Figure 7: Mastersizer Results for 1:1 Styrene to Glycidyl Methacrylate Nanoparticles Synthesized by Near-PIT

As Figure 7 shows, the nanoparticle solution containing a ratio of one to one synthesized via the Near-PIT method contains a significant bimodal distribution. The two peaks show that there were two groupings of nanoparticles present in the solution, one with a size range of 0.1 μm (smallest recordable size) to 0.275 μm and the other with a range of 3.311 μm to 91.201 μm .

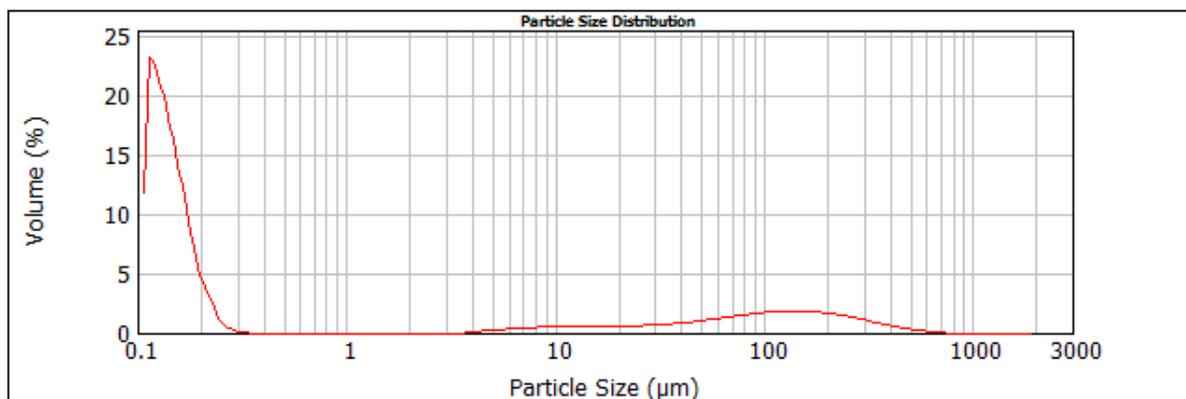


Figure 8: Mastersizer Results for 3:1 Styrene to Glycidyl Methacrylate Nanoparticles Synthesized by Near-PIT

As Figure 8 shows, the nanoparticle solution containing a three to one ratio synthesized by the Near-PIT method had a significant bimodal distribution. The two peaks show that there were two groupings of nanoparticles present in the solution, one with a size range of 0.1 μm to 0.275 μm and the other with a range of 3.802 to 630.957 μm .

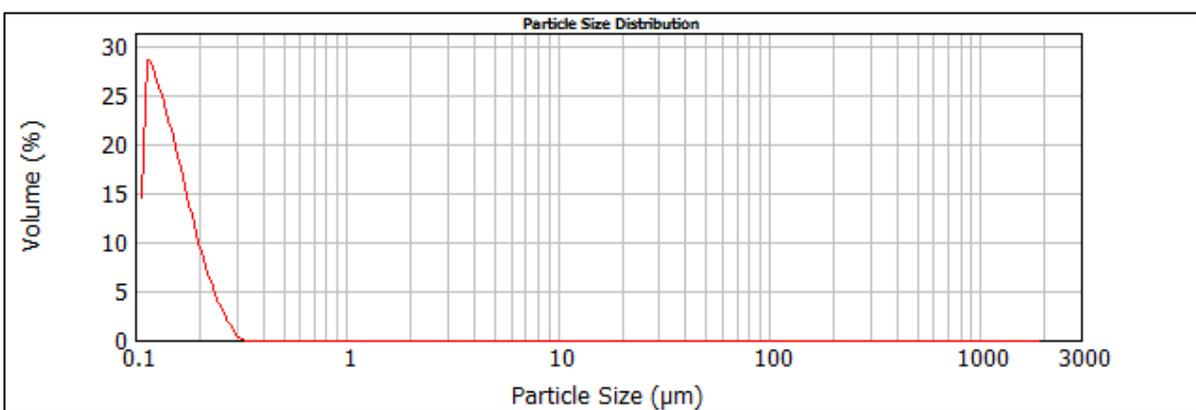


Figure 9: Mastersizer Results for 3:1 Styrene to Glycidyl Methacrylate Nanoparticles Synthesized by Ultrasound

As Figure 9 shows, the nanoparticle solution containing a three to one ratio of styrene to glycidyl methacrylate synthesized by ultrasound has a single distribution. The single peak holds true for

all nanoparticle solutions synthesized through the use of an ultrasound. This means that all of the particles synthesized by ultrasound were within the range of 0.1 μm to 0.275 μm .

This disparity in the number of peaks between the Near-PIT method and ultrasound is the difference in consistency between the two methods. As a result of this disparity in the quality of particle size, all data regarding the collected nanoparticles must be viewed with a shade of caution.

Epoxy Content of the Nanoparticles

The titration of the nanoparticles in order to determine the epoxy content on the surface of the particles in relation to the initial number of epoxy groups is shown below in **Error! Reference source not found.**

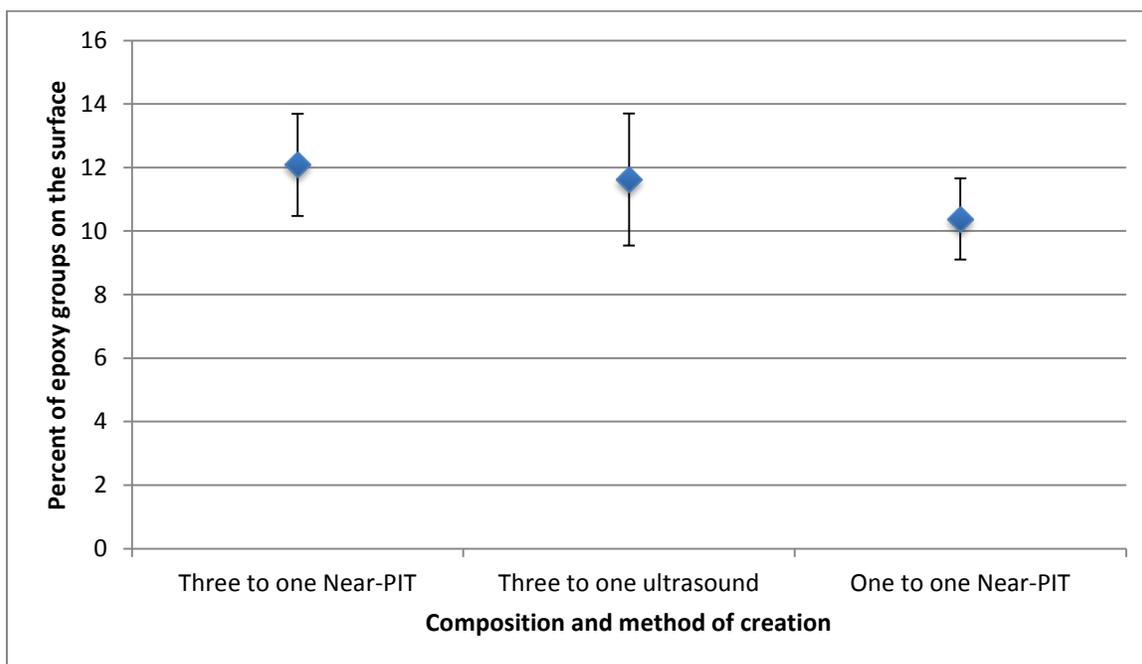


Figure 10: Percent of Epoxy Groups on the Surface of the Nanoparticles

As seen in Table 4, the percent of initial epoxy groups on the surface of the nanoparticles varies from 9.9%, as found in one of the one to one ratio Near-PIT nanoparticle solution, to 13.1%, as seen in one of the three to one ratio Near-PIT nanoparticle solution. In analyzing these numbers there are a number of hidden factors that must be accounted for, including but not limited to the average nanoparticle size, the multiple peaks in the nanoparticle solutions synthesized via the

Near-PIT method, and the difference in the concentration of glycidyl methacrylate in the solutions.

As a result of the size distributions of the nanoparticles, as well as the use of the many solutions to synthesize and determine the epoxy groups on the surface of the nanoparticles, which increased potential variance, it is difficult to draw any solid conclusions about the percent of epoxy groups on the surface of nanoparticles synthesized by the Near-PIT method versus those synthesized through the use of an ultrasound probe.

Recommendations

Based upon the research conducted on the synthesis of nanoparticles containing a mixture of styrene and glycidyl methacrylate and results acquired through the analysis of the synthesized particles, I have created a list of recommendations for future researchers.

I recommend that further research be conducted to determine the effects of stirring on the synthesis of the nanoparticles. While nanoparticles containing styrene and glycidyl methacrylate were successfully synthesized in the laboratory using the Near-PIT method, there were difficulties in synthesizing a solution containing only uniform nanoparticles. The most promising way to address this issue is through evaluation of the effects of stirring on the synthesis of nanoparticles.

I recommend that the synthesized nanoparticles be modified through the use of the epoxy rings on the surface so that they can be deemed viable for purposes beyond that of research. This research focused solely on synthesizing nanoparticles with epoxy rings that would then be able to be modified. While the synthesis was successful, the full potential of the nanoparticles will only be realized when they can be adapted to serve non-academic purposes.

I recommend that further research be conducted into synthesizing nanoparticles through the use of the Near-PIT method followed by polymerization, using different oil combinations. This research covered only one combination of oils in the synthesis of nanoparticles. Using different combinations of oils will lead to a better understanding of the viability of the Near-PIT method. In addition, further research using oils with more similar densities may help eliminate the bimodal dispersion. As a result, I recommend that future research use two different oils and that the density of the oils be similar.

Conclusion

Through my research at Ecole Nationale Supérieure des Industries Chimiques, Laboratoire de Chimie Physique Macromoléculaire, it was shown that the synthesis of nanoparticles containing styrene and glycidyl methacrylate by the Near-PIT emulsification method followed by polymerization is viable. While some issues have not yet been resolved related to the process, such as synthesizing particles greater than the desired size, further research, as recommended above, may show the way to synthesize uniform nanoparticles of appropriate size. The conducted research shows that the Near-PIT method followed by polymerization is able to synthesize nanoparticles containing a mixture of two oils. The conducted research also shows that nanoparticles synthesized by the Near-PIT method have epoxy groups on the surface that are available for modification.

References

- Anton, N., Benoit, J. P., & Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates--A review. *Journal of Controlled Release*, 128(3), 185-199.
- Anton, N., Gayet, P., Benoit, J. P., & Saulnier, P. (2007). Nano-emulsions and nanocapsules by the PIT method: An investigation on the role of the temperature cycling on the emulsion phase inversion. *International Journal of Pharmaceutics*, 344(1-2), 44-52.
- Anton, N., & Vandamme, T. F. (2009). The universality of low-energy nano-emulsification. *International Journal of Pharmaceutics*, 377(1-2), 142-147.
- Binks, B. P., Murakami, R., Armes, S. P., & Fujii, S. (2005). Temperature-induced inversion of nanoparticle-stabilized emulsions. *Angewandte Chemie*, 117(30), 4873-4876. doi:10.1002/ange.200501073
- Bouchama, F., Van Aken, G., Autin, A., & Koper, G. (2003). On the mechanism of catastrophic phase inversion in emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 231(1-3), 11-17.
- Calderó, G., García-Celma, M. J., & Solans, C. (2010). Formation of polymeric nano-emulsions by a low-energy method and their use for nanoparticle preparation. *Journal of Colloid and Interface Science*.
- Corkery, R. W., Blute, I. A., Friberg, S. E., & Guo, R. (2010). Emulsion inversion in the PIT range: Quantitative phase variations in a two-phase emulsion. *Journal of Chemical & Engineering Data*.
- Falk, B., & Crivello, J. V. (2004). Synthesis of epoxy-functional microspheres by cationic suspension photopolymerization. *Chemistry of Materials*, 16(24), 5033-5041.
- Falk, B., & Crivello, J. V. (2005). Synthesis and modification of epoxy-and hydroxy-functional microspheres. *Journal of Applied Polymer Science*, 97(4), 1574-1585.
- Fernandez, P., André, V., Rieger, J., & Kuhnle, A. (2004). Nano-emulsion formation by emulsion phase inversion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 251(1-3), 53-58.
- Galindo-Alvarez, J., Boyd, D., Marchal, P., Tribet, C., Perrin, P., Marie-Begue, E., et al. (2011). Miniemulsion polymerization templates: A systematic comparison between low energy emulsification (near-PIT) and ultrasound emulsification methods. *Colloids and Surfaces A: Physicochemical and*, (374), 134-134-141.

- Gutiérrez, J., González, C., Maestro, A., Solè, I., Pey, C., & Nolla, J. (2008). Nano-emulsions: New applications and optimization of their preparation. *Current Opinion in Colloid & Interface Science*, 13(4), 245-251.
- Jahanzad, F., Chauhan, G., Mustafa, S., Saha, B., Sajjadi, S., & Brooks, B. W. (2007). Composite polymer nanoparticles via transitional phase inversion emulsification and polymerisation. Paper presented at the *Macromolecular Symposia*, , 259(1) 145-150.
- Jones, M. C., & Leroux, J. C. (1999). Polymeric micelles-a new generation of colloidal drug carriers. *European Journal of Pharmaceutics and Biopharmaceutics*, 48(2), 101-111.
- Kentish, S., Wooster, T., Ashokkumar, M., Balachandran, S., Mawson, R., & Simons, L. (2008). The use of ultrasonics for nanoemulsion preparation. *Innovative Food Science & Emerging Technologies*,9(2), 170-175.
- Leal-Calderon, F., Schmitt, V., & Bibette, J. (2007). *Emulsion science: Basic principles* Springer Verlag.
- Li, M., & Fogler, H. (1978). Acoustic emulsification. part 1. The instability of the oil-water interface to form the initial droplets. *Journal of Fluid Mechanics*, 88(03), 499-511.
- Li, M., & Fogler, H. (1978). Acoustic emulsification. part 2. Breakup of the large primary oil droplets in a water medium. *Journal of Fluid Mechanics*, 88(03), 513-528.
- Maa, Y. F., & Hsu, C. C. (1999). Performance of sonication and microfluidization for liquid-liquid emulsification. *Pharmaceutical Development and Technology*, 4(2), 233-240.
- Malvern Instruments Ltd. (1997). *Getting started*
- Márquez, L., Mirra, I., Peña, A., Tyrode, E., & Salager, J. (2003). Principles of emulsion formulation engineering. *Adsorption and Aggregation of Surfactants in Solution*,
- Moddarese, M., Brown, M. B., Tamburic, S., & Jones, S. A. (2010). The role of vehicle-nanoparticle interactions in topical drug delivery. *International Journal of Pharmaceutics*,
- Nielloud, F., & Marti-Mestres, G. (2000). *Pharmaceutical emulsions and suspensions* Marcel Dekker.
- Rao, J., & McClements, D. J. (2010). Stabilization of phase inversion temperature nanoemulsions by surfactant displacement. *Journal of Agricultural and Food Chemistry*, 58(11), 7059-7066.
- Roger, K., Cabane, B., & Olsson, U. (2009). Formation of 10– 100 nm size-controlled emulsions through a sub-PIT cycle. *Langmuir*, 26(6), 3860-3867.

- Ruckenstein, E. (1996). Microemulsions, macroemulsions, and the Bancroft rule. *Langmuir*, 12(26), 6351-6353.
- Salager, J. (1999). Pharmaceutical emulsions and suspensions. *Marti-Mestres, FN, Ed*, , 73–121.
- Sarker, D. (2005). Engineering of nanoemulsions for drug delivery. *Current Drug Delivery*, 2(4), 297-310.
- Scientific Committee on Emerging and Newly Identified Health Risks. (2006). The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies. *IO* 1-1-79.
- Solans, C., Izquierdo, P., Nolla, J., Azemar, N., & Garcia-Celma, M. (2005). Nano-emulsions. *Current Opinion in Colloid & Interface Science*, 10(3-4), 102-110.
- Tadros, T., Izquierdo, P., Esquena, J., & Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, 108, 303-318.
- Trotta, M., Pattarino, F., & Ignoni, T. (2002). Stability of drug-carrier emulsions containing phosphatidylcholine mixtures. *European Journal of Pharmaceutics and Biopharmaceutics*, 53(2), 203-208.
- Vauthier, C., & Bouchemal, K. (2009). Methods for the preparation and manufacture of polymeric nanoparticles. *Pharmaceutical Research*, 26(5), 1025-1058.
- Velev, O., Furusawa, K., & Nagayama, K. (1996). Assembly of latex particles by using emulsion droplets as templates. 1. Microstructured hollow spheres. *Langmuir*, 12(10), 2374-2384.
- Velev, O., & Nagayama, K. (1997). Assembly of latex particles by using emulsion droplets. 3. Reverse (water in oil) system. *Langmuir*, 13(6), 1856-1859.
- Wikimedia. (2010). *Micellecolor*.
<http://upload.wikimedia.org/wikipedia/commons/d/d1/MicelleColor.png>

Appendix A: Titration Data

All values are in ml of NaOH solution required to titrate the solution.

	HCl solution	Surfactant and HCl solution	3:1 Near-PIT and HCl solution	3:1 Ultrasound and HCl solution	1:1 Near-PIT and HCl solution
Run 1	49.3	49.2	47.6	47.4	46.1
Run 2	49.6	49.1	47.8	47.7	46
Run 3	49.4	49.5	47.5	47.5	46.5
Run 4			47.1	47.4	46.4
Run 5			47.4		46
Run 6			47.8		45.9
Run 7			47.3		46.1
Run 8			47.5		46
Run 9			47.2		
Run 10			47.1		

Appendix B: HPPS Nanoparticle Size Data

Nanoparticle size: all values are in nm.

	3:1 Near-PIT	3:1 Ultrasound	1:1 Near-PIT	1:1 Ultrasound
Run 1	91.5	124.5	107.7	119
Run 2	91.8	122.6	108.9	120.3
Run 3	91.1	123.1	106.5	119
Run 4	91.7		111.8	
Run 5	91.2		107.7	
Run 6	91.6		109.9	

PDI

	3:1 Near-PIT	3:1 Ultrasound	1:1 Near-PIT	1:1 Ultrasound
PDI 1	0.105	0.058	0.343	0.049
PDI 2	0.084	0.075	0.334	0.031
PDI 3	0.118	0.026	0.296	0.084
PDI 4	0.111		0.347	
PDI 5	0.067		0.355	
PDI 6	0.066		0.358	

Appendix C: Mastersizer Results

1:1 Near-PIT

Size (µm)	Volume in %										
0.010	0.00	0.105	12.31	1.096	0.00	11.482	4.29	120.226	0.00	1258.925	0.00
0.011	0.00	0.120	13.44	1.259	0.00	13.183	4.30	138.038	0.00	1445.440	0.00
0.013	0.00	0.138	11.25	1.445	0.00	15.136	4.06	158.489	0.00	1659.587	0.00
0.015	0.00	0.158	8.06	1.660	0.00	17.378	3.63	181.970	0.00	1905.461	0.00
0.017	0.00	0.182	4.88	1.905	0.00	19.953	3.10	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	2.42	2.188	0.00	22.909	2.52	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.91	2.512	0.00	26.303	1.99	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.23	2.884	0.01	30.200	1.53	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.02	3.311	0.10	34.674	1.18	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.30	39.811	0.92	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.63	45.709	0.73	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	1.09	52.481	0.58	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	1.67	60.256	0.43	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	2.32	69.183	0.30	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	2.98	79.433	0.14	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	3.58	91.201	0.03	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	4.05	104.713	0.00	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	4.05	120.226	0.00	1258.925	0.00		

3:1 Near-PIT

Size (µm)	Volume in %										
0.010	0.00	0.105	18.43	1.096	0.00	11.482	0.46	120.226	1.47	1258.925	0.00
0.011	0.00	0.120	18.96	1.259	0.00	13.183	0.46	138.038	1.47	1445.440	0.00
0.013	0.00	0.138	14.64	1.445	0.00	15.136	0.46	158.489	1.46	1659.587	0.00
0.015	0.00	0.158	9.87	1.660	0.00	17.378	0.47	181.970	1.42	1905.461	0.00
0.017	0.00	0.182	5.02	1.905	0.00	19.953	0.49	208.930	1.37	2187.762	0.00
0.020	0.00	0.209	2.59	2.188	0.00	22.909	0.52	239.883	1.29	2511.886	0.00
0.023	0.00	0.240	0.70	2.512	0.00	26.303	0.56	275.423	1.19	2884.032	0.00
0.026	0.00	0.275	0.17	2.884	0.00	30.200	0.61	316.228	1.07	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.68	363.078	0.92	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.06	39.811	0.77	416.869	0.75	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.17	45.709	0.86	478.630	0.56	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.24	52.481	0.97	549.541	0.38	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.31	60.256	1.09	630.957	0.11	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.37	69.183	1.20	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.41	79.433	1.30	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.44	91.201	1.38	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.45	104.713	1.44	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.45	120.226	0.00	1258.925	0.00		

3:1 Ultrasound

Size (µm)	Volume in %										
0.010	0.00	0.105	22.79	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	0.00
0.011	0.00	0.120	23.67	1.259	0.00	13.183	0.00	138.038	0.00	1445.440	0.00
0.013	0.00	0.138	19.50	1.445	0.00	15.136	0.00	158.489	0.00	1659.587	0.00
0.015	0.00	0.158	14.65	1.660	0.00	17.378	0.00	181.970	0.00	1905.461	0.00
0.017	0.00	0.182	9.86	1.905	0.00	19.953	0.00	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	5.86	2.188	0.00	22.909	0.00	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	2.92	2.512	0.00	26.303	0.00	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.76	2.884	0.00	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.00	3.311	0.00	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.00	3.802	0.00	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.00	4.365	0.00	45.709	0.00	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.00	5.012	0.00	52.481	0.00	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.00	5.754	0.00	60.256	0.00	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.00	6.607	0.00	69.183	0.00	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.00	7.586	0.00	79.433	0.00	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.00	8.710	0.00	91.201	0.00	954.993	0.00	10000.000	0.00
0.091	0.00	0.955	0.00	10.000	0.00	104.713	0.00	1096.478	0.00		
0.105	0.00	1.096	0.00	11.482	0.00	120.226	0.00	1258.925	0.00		