Pseudo-ternary Phase Diagrams of a Drug Delivery System

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Ziheng Wang

Abstract

The purpose of this research was to develop the pseudo-ternary phase diagrams for a model drug delivery system consisting of vitamin E (model drug) + soybean oil + surfactant + co-surfactant (anhydrous glycerol) + water. The model drug (vitamin E) was loaded in the oil phase. The effects of different surfactants (pure and mixed) on the phase diagram, especially the microemulsion region, were investigated. The influence of drug loading level on the phase diagram was also determined. The surfactants studied were Tween 20, Tween 80, Cremopher EL, and their mixtures. The size (area) of the microemulsion region of the phase diagram was found to be dependent on the type of surfactant used and the loading level of drug (vitamin E).

The phenomenon of phase inversion from W/O microemulsion to O/W microemulsion was also investigated for the drug delivery system consisting of soybean oil (0% w/w Vitamin E loading or 30% w/w Vitamin E loading) + Tween 80 + anhydrous glycerol + water. The inversion of phases was detected by observing changes in the viscosity of the system.

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Chapter 1 Introduction and objectives

1.1 Introduction

Oral-based drug delivery system is the most common way to deliver drugs into the bloodstream. The water-soluble drugs can diffuse freely and easily in gastrointestinal tract and they have a high bioavailability. However, more and more drugs being discovered nowadays with the advances in biotechnology and pharmaceutical technology are oil-soluble [1]. The oil-soluble drugs pose serious problems in that they cannot diffuse freely and easily in gastrointestinal tract because of their poor solubility. One way to deliver oil-soluble drugs is to incorporate the drug into an inert lipid vehicle, such as microemulsions, oils [2], surfactant dispersions [3], and liposome [4]. At present, at least four drug products are available in the pharmaceutical market that are delivered in emulsion form; they are: Sandimmune and Sandimmun Neoral (cyclosporin A), Norvir (ritonavir), and Fortovase (saquinavir). A significant improvement in the oral bioavailability of these drug compounds has been demonstrated [5]. Therefore, much attention is focused on using emulsions as a vehicle to deliver oil-based drugs. Recently, microemulsions have been used to deliver oil-based drugs. Microemulsions offer several advantages over the usual (coarse) emulsions. Microemulsions are thermodynamically stable and the droplets of microemulsions are of very small size. The microemulsion delivery system

is also referred to as the “self-microemulsifying drug delivery system (SMEDDS)”.

Self-microemulsifying drug delivery system (SMEDDS) is a pre-mixture of drug, oil, surfactant and co-surfactant that can be used to deliver oil-based drugs. Upon gentle shaking and gastric juice dilution in stomach, it can form microemulisons spontaneously [6]. It is a highly suitable drug delivery system for hydrophobic drugs because it can be self-emulsified to microemulsion easily and steadily under mild condition in stomach. The pre-mixture can be stored for a very long period in capsules because of the high thermodynamic stability. However, a major disadvantage is the large amount of surfactant needed to form a SMEDDS. Normally the amount of surfactant required to form a microemulsion is around 4 to 5 times of oil [7]. Another disadvantage related to large surfactant requirement is the potential toxic effects associated with the surfactant [8]. Therefore, it is highly desirable to reduce the usage of surfactants and at the same time, maintain the droplet size at a microemulsion level.

1.2 Objectives

The broad objective of this research is to develop the pseudo-ternary phase diagrams for a drug delivery system. The phase diagrams are needed to identify the microemulsion region and to find out the optimal composition for the self-microemulsifying drug within the microemulsion region.

The specific objectives are:

(1) To develop the phase diagrams for the system: Soybean oil (Vitamin E: dissolved in soybean oil) + Surfactant + Anhydrous Glycerol + Water.

(2) To study the influence of different surfactants on the phase diagram and to select the optimal surfactant system.

(3) To study the influence of drug loading on the microemulsion region of the selected system.

(4) To investigate the phase inversion phenomenon occurring in these systems.

Vitamin E, or alpha-tocopherol, is a typical hydrophobic antioxidant that can be used to treat cardiovascular diseases and cancer [9, 10]. Vitamin E can reach a higher absorption rate and bioavailability after self-microemulsifying preparation [11] and was chosen as the model drug in this work. Tween 20, Tween 80, and Cremopher EL are chosen as surfactants in this research. All of the surfactants are nonionic and are considered relatively safe. Anhydrous glycerol, which is one of the most common

chemicals used in pharmaceutical industry, is the co-surfactant used in this study. Water was used as the continuous phase.

The particle size is a crucial property to evaluate microemulsion products [12]. Smaller microemulsion particles can ease the drug absorption. Therefore, efforts were made to minimize the particle size of microemulsions as much as possible.

In the development of the phase diagrams, three types of microemulsion (water-in-oil microemulsion, oil-in-water microemulsion and bicontinuous microemulsion) were encountered. A phase inversion from W/O (water-in-oil) microemulsion to O/W (oil-in-water) microemulsion happened during the titration process involved in the development of the phase diagrams. The inversion phenomenon was detected by measuring the viscosity of microemulsions.

Chapter 2 Literature Review

2.1 Emulsion

2.1.1 Introduction

Emulsion consists of two immiscible liquids (e.g. oil and water) that are brought together into one pseudo phase by using surfactants. They are prepared using shearing force or shaking [13]. The O/W (oil-in-water) emulsion consists of oil droplets dispersed in the water phase. Oil can be called dispersed phase and water can be called continuous phase. Similarly, W/O (water-in-oil) emulsion consists of water droplets dispersed in the oil phase.

The word ‘emulsion’ can be found in ‘macroemulsion’ as well as in ‘microemulsion’ [14]. One major difference between macroemulsion and microemulsion is the particle size. For macroemulsion, the drop size typically ranges from 0.5 μm to 500 μm. These droplets can easily settle down under the influence of gravity. Also, macroemulsion is thermodynamically unstable system because the interfacial free-energy is always positive [15-17]. Phase inversion, flocculation, phase separation, coalescence, and creaming can happen during the storage of macroemulsion. Microemulsion, on the other hand, can be readily prepared with the diameter of the droplets in the range of 100nm to 600nm [18]. Compared with macroemulsion, microemulsion is optically clear (transparent) and thermodynamically stable.

Microemulsion is known to have tiny interfacial tension (close to 0), large interfacial area and zero interfacial free energy [19]. Due to its unique properties, microemulsion has many applications in industry.

2.1.2 Types of Emulsions

Emulsions can be commonly classified as water-in-oil (W/O) emulsion or oil-in-water (O/W) emulsion. Generally speaking, hydrophilic surfactant [20] forms O/W emulsion easily and hydrophobic surfactant [20] is likely to form W/O emulsion. There are several methods that can be used to distinguish the two types of emulsions:

The first method of testing is called the dying method. A powdered, oil-soluble dye such as Sudan II is sprinkled in the emulsion. Then the emulsion is inspected under the microscope. The red background can be seen if the emulsion is W/O type and the red discrete dots can be detected if the emulsion is O/W type. The second method is the dilution method. A sample of emulsion is added to both oil and water. W/O emulsion disperses readily in oil whereas O/W emulsion quickly disperses in water. The type of emulsion can also be determined by testing electrical conductivity. O/W emulsion has a much higher conductivity than W/O emulsion. Also note that a sudden change in viscosity occurs during the phase inversion [20]. With the addition of aqueous phase liquid to emulsion, the viscosity of O/W emulsion will decrease, and the viscosity of W/O emulsion will increase.

Microemulsions can be classified into three types: W/O microemulsion, O/W microemulsion and bicontinuous microemulsion [21]. Bicontinuous microemulsions consist of a net structure which is twined by oil, water, and surfactants. Bicontinuous microemulsion is less toxic and more electro catalytic active than the polar and nonpolar substrates [22].

Double emulsions are “emulsions of emulsions”. The droplets of double emulsions contain a number of inner droplets and are much greater in size as compared with the droplets of single emulsions [23]. Two main types of double emulsions are: O/W/O (oil-in-water-in-oil) emulsions and W/O/W (water-in-oil-in-water) emulsions.

2.2 Microemulsions

Microemulsions are isotropic, thermodynamically stable systems containing a very high concentration of surfactant [24]. Microemulsion is an excellent carrier of oil-based drugs. It has a small particle size, high stability, larger interfacial area and low interfacial tension and forms spontaneously [25]. A key distinctive property of microemulsion is its nano-scale particle size.

2.2.1 Microemulsions are not Nanoemulsions

The main difference between microemulsions and nanoemulsions is that microemulsions are self-assembling nano-scale emulsions whereas nanoemulsions are nano-scale emulsions formed under intense mechanical shear [26].

Microemulsions are isotropic solutions of oil and water and are prepared using a high surfactant concentration of around 40 percent under gentle stirring or shaking. Microemulsions form spontaneously without mechanical shear [27]. An extremely high concentration of surfactants ensures self-assembling with particle size at the nano-scale level. Bowcott and Schulman have proved that the self-microemulsification can happen when the oil-water interfacial tension is zero [28].

The interfacial tension is given as:

$$\gamma_i = \gamma_{ow} - \pi \quad (1)$$

where γ_{ow} is the interfacial tension without the presence of surfactant. π is the spreading pressure of surfactants at the interface. A large amount of surfactant can result in a high value of π. Therefore, the interfacial tension will reach a negative value when $\pi > \gamma_{ow}$. A negative interfacial tension results in negative free energy and as a consequence microemulsion possesses high stability. Coarse emulsions are formed when $\pi < \gamma_{ow}$. The droplets of coarse emulsion tend to coalesce as the interfacial tension is positive [29].

The preparation of nanoemulsions requires extreme shear in order to rupture large droplets into nano-scale droplets. The mechanical shear should be intensive enough to overcome the large interfacial tension [30]. Unlike microemulsions, nanoemulsions are thermodynamically unstable systems as the interfacial tension between oil and water phase is high.

2.2.2 Mechanism of Forming Microemulsions

All types of emulsions should be prepared with a certain amount of surfactant. Surfactants can promote the formation of emulsion as they reduce the interfacial tension between oil and water by attaching on to the liquid-liquid interface [31]. Surfactants can be thought of as “pollywogs” with hydrophilic head and hydrophobic tail. There are three types of surfactants: anionic, cationic and nonionic surfactants. Anionic surfactants have a negative charge on the hydrophilic part and cationic

surfactants have a positive charge on the hydrophilic part. Nonionic surfactants have no charge on the molecules. In the pharmaceutical field, nonionic surfactants are widely used as they are less irritative than ionic surfactants. Ionic surfactants are used rarely in special cases [14]. When the surfactant concentration exceeds a certain value, aggregates of surfactant called "micelle" are formed. The critical concentration of surfactant where micelles are formed is called critical micelle concentration (CMC). The surfactant distributes in an energetically favorable way. In water, the hydrophilic heads of the surfactant molecules are surrounded by water molecules and the hydrophobic tails of the surfactant molecules are gathered up in the inner portion of the micelles. In oil, the hydrophilic heads of the surfactant molecules are inside the micelles (reverse micelles) and the hydrophobic tails of the surfactant molecules extend away form the core of the micelles to the oil phase [32]. The main difference between surfactant micelles and emulsion is the liquid phase. Typically, micelles are formed by adding surfactant to a single liquid phase, either oil (reversed micelles) or water whereas emulsions are prepared by adding surfactant to a double liquid phase, such as soybean oil and water.

Micelles have a unique inside structure in that the hydrophobic tails of the surfactant molecules aggregate in the core center only. When the concentration of the surfactant reaches CMC, a small amount of the oil droplets can penetrate the hydrophilic "shield" of the micelles and stabilize into the core center of the micelles [33]. Penetration process results in a spreading of the interface area and consequently

increases the spreading pressure of surfactants at the interface. According to equation (1), when the spreading pressure of surfactant at the interface (π) is greater than the interfacial tension without the presence of surfactant (γ_{ow}), the oil-water interfacial tension (γ_i) becomes very small (close to zero). Hereby the thermodynamically stable microemulsions are formed [34-37]. Figure 2-1 shows the structures of typical micelles and microemulsions.

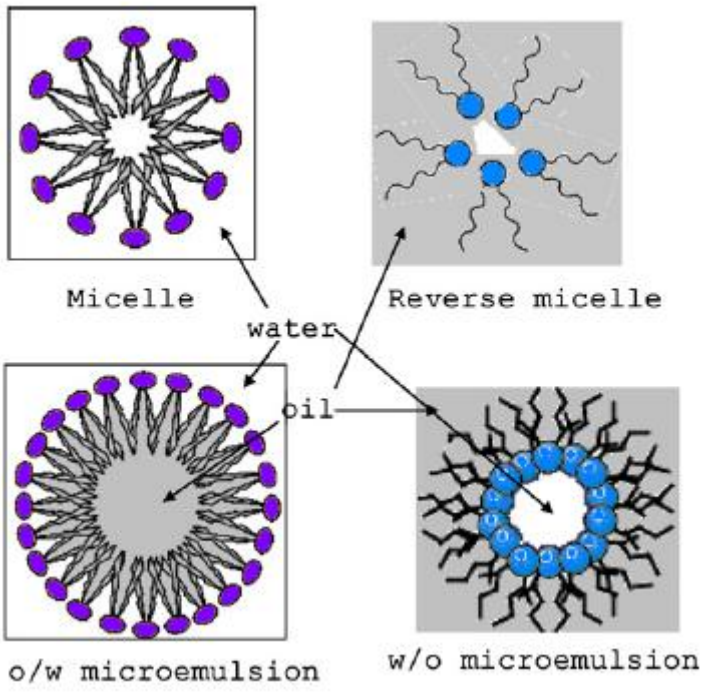


Figure 2-1: Schematic representation of the dispersed phase structure of micelles, reverse micelles, o/w microemulsion and w/o microemulsion [55]

2.2.3 The Applications of Microemulsions

Microemulsions have received a lot of attention in both research and industry due to their unique properties. The characteristic properties of microemulsions are:

extremely low interfacial tension, large interfacial area, and capability to solubilize two immiscible liquids. The distinctive advantages of microemulsions are: small particle size and high thermodynamic stability [38].

One of the main applications of microemulsions is in the pharmaceutical industry. Oil-based drugs are easily dissolved in oil but have a very low solubility in water. Due to this disadvantage, oil-based drugs have a poor bioavailability after oral administration because of the low solubility and absorption rate in gastrointestinal lumen. Microemulsions are suitable carriers for oil-based drugs because oil-based drugs can be dispersed easily in gastrointestinal juice in microemulsion form. Microemulsions can enhance the oil-based drugs absorption due to their small particle size. Also the drugs can be stored longer because of the stability of microemulsions [39]. The only disadvantage of microemulsions as drug carriers is that the toxicity of the drugs tends to increase due to a large amount of the surfactant utilized in microemulsion formulation.

Microemulsions have many other applications. For example, hair care product which contains an amino-functional polyorganosiloxane (a nonionic surfactant) is prepared in microemulsion form. In microemulsion form, the fragrance and the flavored oils can be stabilized very well [38]. As microemulsions can easily solubilize organic components, they can be used as detergents to remove grease, oil and protein during the cleaning and washing processes [40]. In the oil industry, microemulsions are used

to enhance the oil recovery from the reservoir. A lot of oil remains trapped in the reservoir because of the high interfacial tension between oil and brine. One way to reduce the interfacial tension and extract the residual oil from the porous media is to inject surfactant to form microemulsions. This mode of enhanced oil recovery is called surfactant/microemulsion flooding method [41-43]. Other applications of microemulsions include: fuels to purge soot, and paint to resist scrub. These applications show a promising and significant contribution of microemulsion to the chemical industry.

2.2.4 Phase inversion in microemulsions

Phase inversion is a phenomenon whereby the discrete phase changes into the continuous phase while the original continuous phase becomes the discrete phase [44]. Figure 2-2 shows the process of the phase inversion schematically [45]. The phase inversion point, as shown in the Figure 2-2, is the point where the dispersed phase changes into the continuous phase suddenly. Phase inversion is a severe form of instability. The inversion can happen when changes are made in the composition of the system [46]. The physical properties, such as viscosity and conductivity, undergo a sharp change during the inversion process.

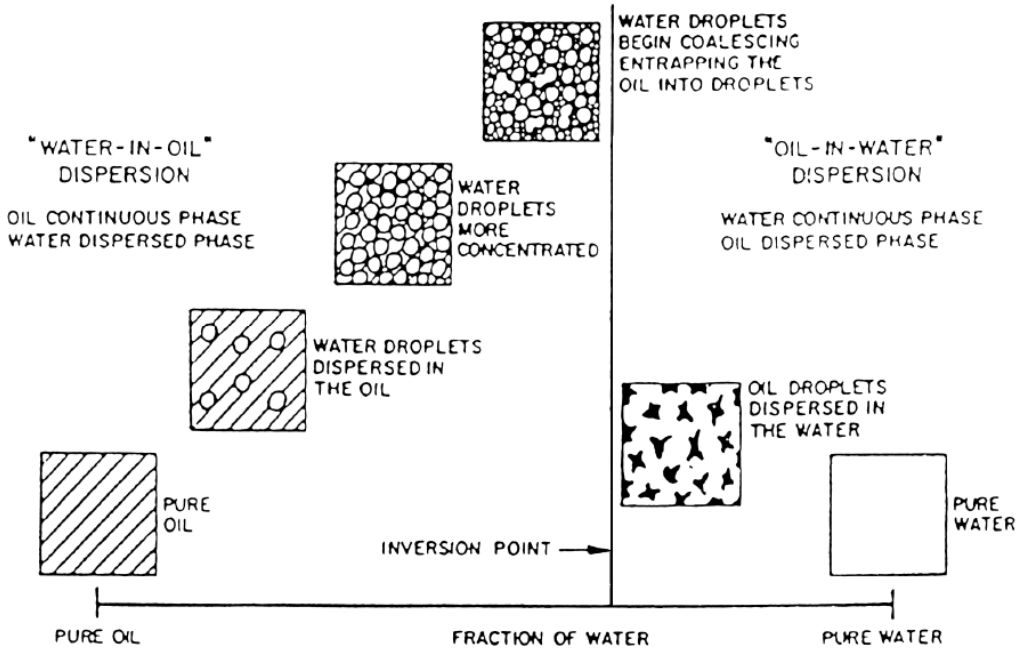


Figure 2-2: Phase inversion Process form W/O emulsion to O/W emulsion [45]

Phase inversion can happen during the preparation of microemulsions. O/W microemulsions and W/O microemulsions can invert from one form to another (O/W to W/O or W/O to O/W) by going through a third type of microemulison phase called bicontinuous microemulsions. Bicontinuous microemulsions are a network structure of oil and water twined together with surfactant. Figure 2-3 [21] captured by Watanabe et al. shows the structure of bicontinuous microemulsions. The micrograph was obtained by transmission electron microscopy with freeze-fracture replication method (FF-TEM) [21]. Bicontinuous microemulsions do not have the classical emulsion structure as there are no aggregates dispersed in the continuous phase. They are sponge-like structure with intertwined lines of oil and water in the mixture [47].

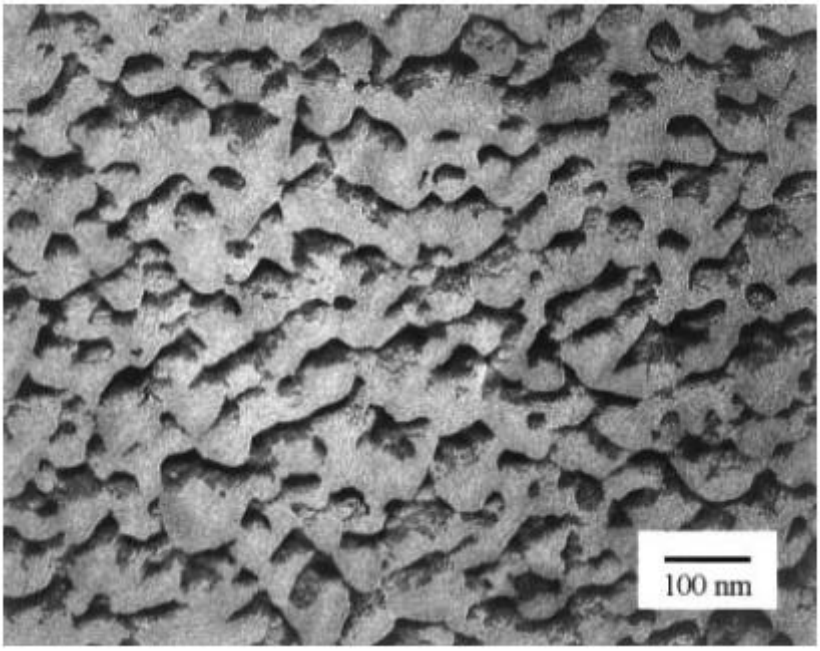


Figure 2-3: Electron Micrograph of a typical texture of bicontinuous microemulsions [21]

Research work carried out by Garti et al. [48] revealed the principle of phase inversion for microemulsions. These researchers chose Celecosib as the drug, R (+)-limonene/EtOH (1:2 w/w) as the oil phase, Tween 80 as the surfactant, and water/glycerol (3:1 w/w) as the aqueous phase. The results showed that W/O microemulsions invert to O/W microemulsions by going through an intermediate bicontinuous microemulsion phase. The process is shown in Figure 2-4. The W/O microemulsion droplets first convert to bicontinuous microemulsion structure when diluted with water. And then the surfactant molecules re-assemble to form O/W microemulisons with further dilution. The drug solubilization is lower in O/W microemulsions as they are more compact [48]. It is important to consider the phase inversion phenomena in formulating microemulsions as phase inversion can have a detrimental effect.

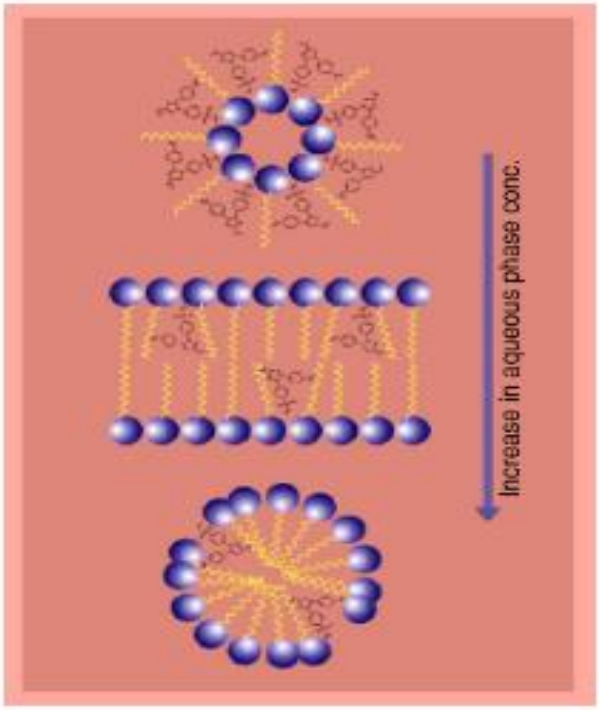


Figure 2-4: Schematic illustration of phase inversion (up: W/O microemulsion, middle: bicontinuous microemulsion, down: O/W microemulsion) [1]

2.3 Self-microemulsifying Drug Delivery System

Oral administration is a very general way to deliver drugs. Microemulsion-based drugs can also be delivered orally. Therefore, the general background of drug absorption and distribution after oral administration is discussed briefly.

2.3.1 Drug Absorption, Distribution, Metabolism and Excretion after Oral Administration [49]

Drugs can only function when they reach the right organs or tissues. Orally administrated drugs have a long journey before they can reach the targeted place. Absorption, distribution and excretion are the main processes that the drugs go through after oral administration.

After a drug is administrated by mouth, it firstly enters gastrointestinal (GI) tract. The drug is absorbed in GI tract by diffusing across the cell membrane or mucosal on the tract. The drug dissolves in gastric juice and stays in stomach for a while. During this period the drug may be decayed by the digestion process (for example, insulin is commonly delivered by injection to avoid the digestion process). The drug may also be destroyed in stomach because of pH environment or due to the attack from enzymes. The drug may lose activity. From the GI tract, the drug reaches the small intestine, the main organ for drug absorption, and penetrates across the mucosal on the small intestine. Water-soluble drugs are more easily absorbed than oil-based drugs

because they are easy to disperse in the aqueous gastric juice. The oil-soluble drugs are always present in the form of droplets in GI tract because of their low solubility. It is important that the drug is present in the form of small droplets so as to penetrate the mucosal barriers.

Figure 2-5 shows the process of orally administrated drug entering the blood circulation after absorption. Normally the drugs are bound by plasma, lipoproteins, antibody and proteins in the blood stream after absorption. The drugs present in the portal vein have to enter the hepatic portal system (the liver). The majority of the drugs will degrade and inactivate or undergo a change in chemical-structure in endothelial reticulum of the liver cell because of the inhibition from hepatic enzymes. This phenomenon is called “first pass effect”. The first pass effect can reduce the drug bioavailability significantly and can be avoided by using other drug delivery systems, such as transdermal drug delivery system. Drug then goes to systematic circulation. Inspection of drug loading in oxygen-carried blood at the start of systematic circulation can interpret the drug bioavailability, which is determined by the drug absorption rate in GI tract, first pass effect and the metabolism of drug before entering into systematic system [50]. Only a certain amount of well-designed drug is delivered to the targeted tissues or organs successfully after the first pass effect with the blood flow and then metabolize in the targeted place. Some organs, such as brain, can eliminate most kinds of drugs because of the physical protection of the special barrier. The delivery rate is highly dependent on the drug itself, the characteristic of the

organs or tissues and the way of delivering drugs.

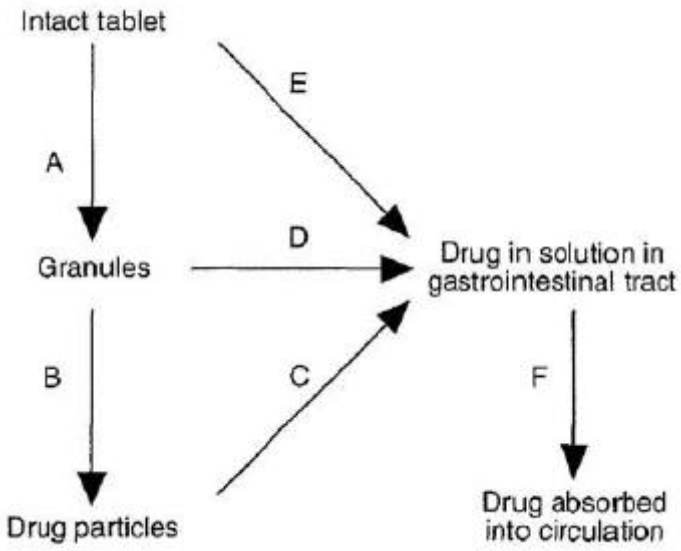


Figure 2-5: The various absorption processes of tablets. Steps A and B: disintegration to coarse and fine particles respectively. Steps C, D and E: drug dissolution rate C＞D＞E. Step F: drug absorption [50]

Most drugs that enter the systematic circulation for treatment are excreted by kidneys. Renal clearance is the most common way for drug excretion. The drug bioavailability can be inferred by inspecting the drug recovery in urine samples. Other routes to eliminate drug are: tears, sweat glands, bile channels, latex, feces or gas. The excretion rate is related to the ability of the organs (the kidney, bowel, and lungs function) and the physical property of the drug.

2.3.2 Self-microemulsifying Drug Delivery System

Self-microemulsifying drug delivery system (SMEDDS) is a very promising drug delivery system for oil-soluble drugs. It is a pre-mixture of drug, oil, surfactants and co-surfactants and is able to form microemulsion under gentle shaking or stirring

spontaneously [51]. Microemulsion is a very clear, isotropic, transparent and thermodynamically stable system with a very small particle size (below 100nm).

A pseudo-ternary phase diagram of drug, oil, surfactant, co-surfactant, and water can be very helpful in formulating a suitable composition of SMEDDS. Usually there are three types of phases encountered in a pseudo-ternary phase diagram: microemulsion (ME), liquid crystal (LC) and coarse emulsion (EM). Microemulsion (ME) region is the main region of interest in the formulation of SMEDDS. A large microemulsion region can offer more flexibility to find the optimal dosage composition. Microemulsions are identified with their clear and transparent appearance. Liquid crystal (LC) is a gel-like material that exhibits oil streaks under stirring condition. They also exhibit birefringence under crossed polarized microscope. Coarse emulsion (EM) is the traditional thermodynamically unstable emulsion; it appears as milky white during the preparation and storage. The droplet size of coarse emulsion can range from sub-microns to microns [52]. The boundary lines between the two emulsion regions (ME and EM) are drawn out according to the emulsion appearance and droplet size.

Figure 2-6 is a typical ternary phase diagram. It represents a three component system (oil, water and surfactant in the present case). Ternary phase diagram can be read following the solid lines in the figure. For example, point A corresponds to a composition of 30% water phase, 60% surfactant phase and 10% oil phase. The

region to which point A belongs depends on the particle size and the appearance of the sample. A titration technique is employed for the preparation of the ternary phase diagrams (In this work, phase diagrams are referred to as “pseudo-ternary” phase diagrams as the surfactant phase was a mixture of surfactant and co-surfactant). The titration procedure begins with zero loading of water. The dashed line (tie line) shown in the figure is followed with the addition of water. The titration procedure ends at a point of 100% water loading. An infinite number of tie lines can be drawn in any ternary phase diagram [53]. The titration begins by fixing two components and varying the third component. In the present work, the titration begins using different ratios of surfactant phase to the oil phase and following the tie lines with the addition of water. The mixture of surfactant and co-surfactant (referred to as surfactant phase) was fixed at 1/1 ratio.

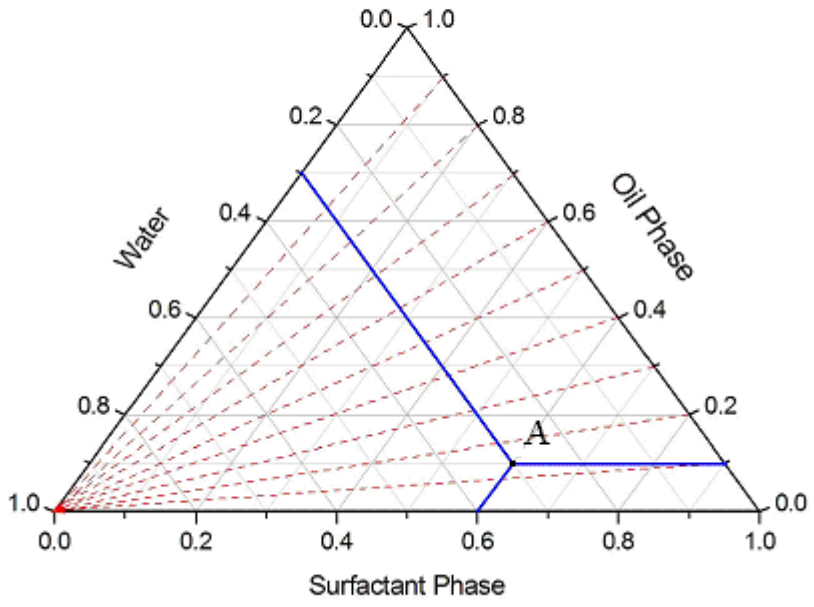


Figure 2-6: Tie lines of a pseudo-ternary phase diagram

2.3.3 Drug Capacity in Microemulsions

Self-microemulsifying drug delivery system can be described as oil (with drug) + surfactant + co-surfactant + water. Water comes from the aqueous phase present *in vivo.* No water is loaded in drug preparation. The system with zero water loading is stored in capsules as reverse micelles before drug administration. The solubilization or the amount of drug present in reverse micelles is very important to evaluate the system. The drug solubilization ability of the system is one of the most important properties in the selection of ingredients.

Drugs are always solubilized at the interface of microemulsion droplets or micelles. Research work carried out by Spernath et al. [56] showed that reverse micelles have a higher drug capacity than the individual components. They entrapped lycopene in the reverse micelle of R-(+)-limonene and polysorbate 60 (Tween 60). The drug capacity reached 2500ppm (700 ppm in (R)-(+)-limonene and 800 ppm in polysorbate 60) [54]. The reason for increasing capacity of drug solubilization is that the drug can distribute at the surface of the reverse micelles rather than occupy the core. It is known that the drug solubilization at reverse micelles surface is highly dependent on the physical properties of surfactant, co-surfactant, drug, interaction between drug and surfactant, and Hydrophile-Lipophile Balance Number (HLB) of surfactant. Different components can result in different solubilization capacity of drugs [55]. However, drug solubilization reduces after oral administration due to aqueous phase dilution and structural changes shown in Figure 2-4. Phase inversion also affects solubilization.

During the phase inversion, water miscible components, such as co-surfactant, will move away from the surface and lead to a decrease in drug solubilization [56]. Also drug solubilization in microemulsions can be influenced by the type of oil, the type of surfactant, and rate of aqueous phase dilution.

2.3.4 Mechanism of Enhancement of Drugs Absorption in SMEDDS

The droplet size and polarity of oil droplets can influence the bioavailability of self-microemulsifying drug delivery system. However, the polarity of oil droplets has a limited impact as the oil droplets are extremely small [57]. An increase in bioavailability of oil-based drugs delivered in the form of microemulsions is reported extensively. A decrease in the particle size can enhance the drug absorption to a large extent.

The factors that influence the bioavailability of self-microemulsifying drugs were discussed by Gyrsoy and Bentia [5]. The first factor is the surfactant. Surfactants can increase the drugs permeability. They disrupt the lipid bilayer on the epithelial cell membrane, a barrier to drug absorption and diffusion, to enhance the dissolution rate of the drugs [58]. The second factor is the lipids. Oil phase can work not only as a carrier but also a ‘shield’ to protect the attack and degradation from enzymes. Oil phase is necessary to deliver hydrophilic proteins to lymph systems. The hydrophilic proteins are incorporated in the water droplets of a W/O microemulsion. Hydrophilic proteins delivered in the form of W/O microemulsion are called lipoproteins.

Lipoproteins are highly lymph-philic and can be transported to lymph systems after absorption in small intestine. Drugs in lymph systems can reach systematic circulation directly without the first pass effect. It has been proven that lipoproteins have a higher bioavailability than non-lipids [59, 60]. The third factor is called P-glycoprotein (P-gp) inhibition. P-glycoprotein is a type of combined protein existing in normal cells. It expels the drugs out of the cells as a self-biological defense and can reduce the drugs absorption. A recent study shows that drugs incorporated in SMEDDS (self-microemulsifying drug delivery system) can inhibit the activity of P-glycoprotein which results in an enhancement of oral absorption [61].

2.4 Applications of SMEDDS and the Challenges

The O/W microemulsions are mainly used for the delivery of lipophilic peptide, such as cyclosporine. In recent years, with the commercialization of Sandimmune Neoral® (Cyclosporine, an oral hydrophobic drug to treat rejection in organ transplant, is available as a self-microemulsifying drug), a significant amount of research effort is being directed to this new pursuit of using microemulsion as a vehicle to deliver hydrophobic drugs. Sandimmune Neoral® is the second generation of Sandimmune®. It uses self-microemulsifying drug delivery system to enhance the drug absorption. According to *in vivo* study, Sandimmune Neoral® increases area under the plasma concentration time curve (AUC), which gives a measure of how much and how long a drug exposures occurs in a body [62]. Therefore, a higher bioavailability can be reached. For pharmaceutical purposes, microemulsion-based drugs are stored in capsules which contain a low volume of dosage, around 0.5ml [57]. Besides, the fluidity of the dosage can also influence the drugs absorption. A common recommendation is to use less viscous digestible oil, such as medium-chain triglycerides [63]. But it is still unclear how much can the fluidity influence the drugs bioavailability as compared with other factors. Also the *in vivo* behavior of drugs is difficult to predict because of the following reasons: (1) the accurate route of drugs after absorption is still not clear, (2) whether the drug will stay in microemulsion form or release out after absorption is not known, (3) when and where the drugs will be released is not clear, (4) it is also not clear if the drugs can reach the targeted organ,

and (5) it is not known if the particle size change occur after absorption [57]. Another challenge is the toxicity of surfactants used in SMEDDS.

2.4.1 Toxicity and Safety of SMEDDS

As mentioned in the section 2.3.4, surfactants can enhance the drug absorption by disrupting the lipid bilayer of the epithelial cell membrane. As a large amount of surfactant is required to form microemulsions, the toxicity of surfactants should be also taken into account [63]. A large amount of surfactant can cause irritation or tissue damage because surfactants can disrupt the lipid bilayer and interact with the mucosa. For the repeated administration, a large dosage of surfactant can also result in serious toxicological impact on humans and must be carefully evaluated [58].

Toxicity studies can be divided into two parts: acute oral toxicity and chronic oral toxicity. Swenson et al. [57] studied the effect of different surfactants on a single pass rat intestinal perfusion system. They uncovered the enhancement ability of drug absorption for different surfactants (Tween 80, bile salts and sodium dodecyl sulfate) and studied the damage on the intestinal wall resulting from surfactants [64]. Further studies have shown that the epithelial cells can repair damage upon termination of drug administration. However, long-term effects for repeated drug administration not can be ignored. A study of chronic oral toxicity is necessary for all the surfactant-containing microemulsion drugs. The study can be executed on a proper animal model by using gelatin capsules. The results will reveal relations between the

therapeutic effects and the toxicity of a specific surfactant [57]. Extensive research is needed to reduce the usage of surfactants and maintain the drugs absorption rate at the same time.

2.4.2 Scale-up and Manufacture

Compared to the challenge of reducing the drug toxicity, scale-up and manufacture of SMEDDS is easier. This is because of the intrinsic properties of SMEDDS. Two characteristics, spontaneous formation and thermodynamic stability, are helpful in the scale-up and manufacture processes. Burskirk et al. [65] discussed the general issues related to the SMEDDS manufacturing. Because of the advantages of SMEDDS, the manufacturing process only needs very basic mixing equipment to provide mild agitation to form micelles. And the preparation does not require careful in- process control needed in the manufacture of other drugs [65].

In batch-by-batch manufacturing, the degree of the purity and the chemical instabilities should be monitored carefully. The selection of capsules (soft or hard gelatin capsules), the selection of oil which can maximize drug solubility, the hygroscopicity of the contents that can either dehydrate or dissolve the gelatin shell, are also very important in the pharmaceutical manufacture [57]. Furthermore, the dynamic changes of the drug should be investigated thoroughly before the manufacture. The manufacturing conditions are highly dependent on the drug. Different drugs should be considered separately to obtain the optimum conditions.

Chapter 3 Development of Pseudo-ternary Phase Diagrams

3.1 Selection of Materials

Soybean oil was used as the oil phase and anhydrous glycerol was used as the co-surfactant. These materials are very commonly used in the pharmaceutical industry and are considered safe. A literature study has shown that no significant differences in the microemulsion are observed by using water, simulated gastric juice or simulated intestinal juice as dilution medium [52]. Therefore, deinoized water was used as aqueous phase in this research. Three different surfactants were employed: Tween 80 (polysorbate 80), Tween 20 (polysorbate 20) and Cremophor EL (polyethoxylated castor oil). All of the surfactants are non-ionic, less toxic and widely used in the pharmaceutical industry. For each of the surfactant, a corresponding pseudo-ternary phase diagram consisting of Soybean Oil + Surfactant (and co-surfactant: anhydrous glycerol) + Water was developed. Previous research [51] has shown that the emulsifying effect is good if the ratio of the surfactant to the co-surfactant is higher than 1/2.5 but stability properties are inferior at this ratio. Fixing the surfactant/co-surfactant ratio at 1/1 is a better choice from the stability point of view [51]. In accordance with this conclusion, the ratio of surfactant to co-surfactant (anhydrous glycerol) was fixed at 1/1 in the present work.

As a larger microemulsion region (ME region) given more flexibility to find the optimal dosage composition, it is important to select components which would lead to a large microemulsion region. Some studies have shown that mixtures of two surfactants (Cremophor EL + Tween 20) can enlarge microemulsion region (ME region) significantly in the pseudo-ternary phase diagram for the system Capmul PG8 + Surfactant (either single or combined) + Water [52]. Therefore, mixtures of surfactants were also used in this research to explore an enlargement of ME region. Vitamin E was employed as a model drug.

The materials used in the research can be summarized as follows: Vitamin E (α-Tocopherol, HPLC grade), anhydrous glycerol (GC grade) and Cremophor EL, all purchased from Fluka. Soybean oil, Tween 80, Tween 20, all purchased from Sigma. The chemicals were used as received. The solubility of Vitamin E in various components is given in the table 3-1 [66].

Table 3-1: Vitamin E solubility in different vehicles [66]

Vehicles	**Solubility of Vitamin E (kg/L)**
Soybean Oil	＞5.0
Tween 80	2.0-2.5
Tween 20	＜1.0
Cremopher EL	2.5-3.0
Anhydrous Glycerol	＜1.0

3.2 Experimental Methods

3.2.1 Construction of Pseudo-ternary Phase Diagrams

The ratio of surfactant to co-surfactant was fixed at 1:1 on the weight basis. The mixture of surfactant and co-surfactant is referred to as “surfactant phase” in the following discussion. Six types of surfactant phases were prepared: Tween 80 + Anhydrous Glycerol, Tween 20 + Anhydrous Glycerol, Cremophor EL + Anhydrous Glycerol, Tween 80 + Tween 20 + Anhydrous Glycerol, Tween 80 + Cremophor EL + Anhydrous Glycerol, Tween 20 + Cremophor EL + Anhydrous Glycerol. The soybean oil was mixed with each of the surfactant phases in the ratios (weight basis) of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 . A titration technique was employed for the preparation of the pseudo ternary phase diagrams. Deionized water was added in small increments (less than 5% w/w) to the mixture of soybean oil/surfactant phase at room temperature. After each water addition, the mixture was stirred in a beaker for 2-3 min using a stirring bar and a magnetic stirring plate. The titration process followed the tie lines (dash lines) shown in the pseudo-ternary phase diagram of Figure 2-6. The phases were identified using visual inspection, microscopic inspection, and measurement of droplet size. The pseudo-ternary phase diagram consisted of three regions: EM region representing coarse emulsion region, LC region representing liquid crystal region, and ME region representing microemulsion region. In each of the titration runs, several points were noted down as critical points between LC region and ME region or between LC region and EM region. The critical point was a specific

composition where a significant change in the appearance of the mixture occurred. The boundaries between LC and ME regions, and between LC and EM regions were drawn on the phase diagram by joining together the critical points.

3.2.2 Particle Size Measurement

The particle size distribution was determined using Dynamic Light Scattering (DLS) technique. DLS technique, also known as Photon Correlation Spectroscopy, is one of the most widely used methods to measure the size of nanoparticles. This technique assumes that all the particles are in Brownian motion in the solution and that all the particles are very small and spherical.

Scattering of light (normally a laser) takes place when particles are hit by light. The particle size can be determined based on the physical properties of the scattered light: the angular distribution, frequency shift, the polarization and the intensity of the light. [67]. Figure 3-1 shows the principle of DLS technique [68].

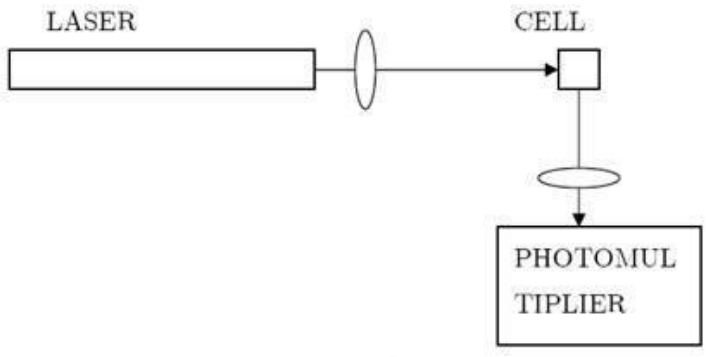


Figure 3-1: The illustration of DLS technique [68]

Several steps should be implemented to measure the particle size. The first step is the

calibration and the purpose of calibration is to confirm that the laser beam is at the same height in its entire path [68]. The cuvette (container for the sample) should be clean enough for the measurement. The filtered deionized water is used as the dispersant phase. Before the measurement, 1ml filtered deionized water was taken in a disposable sizing cuvette and the cuvette was put into the cell of the DLS instrument. The refractive index (RI) was set at 1.330, the viscosity was set at 0.8873cP, and absorption was set at 0.00 based on the physical properties of filtered deionized water. The cuvette was equilibrated for 3 minutes before the measurement. When the pure filtered deionized water was employed for the run, a message "Data not suitable for analysis. Do you wish to continue the measurement?" should be shown on the control panel, which means the cuvette is clean enough for the measurement.

Then the particle size measurement can be started. A 20 µ L sample is sucked into the clean cuvette. 1ml filtered deionized water is added to dilute the sample. It has been shown by Li et al. [52] that the dilution process does not influence the particle size. After the dilution, the sample is placed into the cuvette box to measure the particle size distribution.

3.3 Experimental Results and Discussion

As pointed earlier, the phase diagrams were constructed using titration along different tie lines while following a tie line different regions were encountered as described below.

(1) Transition from LC (liquid crystal) region to ME (microemulsion) region

This situation happened when the amount of surfactant phase (surfactant and co-surfactant) was very high, such as 95% surfactant phase and 5% oil phase at the beginning of titration. As an example, consider the composition ratio at the start of the titration to be as follows: surfactant phase/oil phase=90%/10%. In this case, the mixture can finally form microemulsion. The mixture was very clear and transparent before the titration (before water was added). At the beginning of the titration, a small amount of white precipitate was observed and the mixture became a little bit turbid. After approximately 7% to 10% (w/w) water was added, the mixture instantly turned to a very clear and transparent material. The liquid streaks or liquid lines were visible under mild stirring at this moment. With further addition of water in small amounts, the mixture was still very clear and transparent and liquid lines or streaks were present. After a certain amount of water was titrated, the liquid streaks disappeared completely. The solution is now transparent and clear. The final product is thermodynamically very stable for several months. No change can be observed under the optical microscope.

(2) Transition between ME (microemulsion) region and EM (coarse emulsion) region

This situation happened when the surfactant phase (surfactant and co-surfactant) amount was moderately high, such as 70% surfactant phase and 30% oil phase at the beginning of titration. In this case too, the mixture can form microemulsion in the end. The mixture is very clear and transparent before water is added. At the beginning of the titration, a small amount of white precipitate was observed and the mixture became turbid. After approximately 7% to 10% (w/w) water was added, the mixture suddenly turned to a very clear and transparent material. The liquid streaks or liquid lines were detected under gentle stirring. By continuing the addition of water, the liquid streaks disappeared. However, unlike the first situation (transition from LC region to ME region), the mixture was somewhat cloudy. The final product can be called microemulsion because most of the particles were under 100nm. Strictly speaking, these products are in transition region between microemulsion and coarse emulsion.

(3) Transition from LC (liquid crystal) region to EM (coarse emulsion) region

This type of transition happened when the ratio of surfactant phase to oil phase was 60%/40% or less. Similarly to previous situations, the titration led to the formation of liquid crystal firstly with 5% (w/w) water addition. However, coarse emulsions were formed by continuing addition of water. The mixture became very cloudy and opaque after the disappearance of liquid streaks. The coarse emulsions were thermodynamically unstable and were found to decay (creaming or phase separation

occurred) after storage for several days. However, compared with common coarse emulsions, the self-assembled coarse emulsions have a smaller particle diameter (around 600nm).

(4) Only EM (coarse emulsion) region with no transition

When the surfactant phase is relatively low in amount (30% w/w or less) before the titration is started, the titration leads to EM region directly. The coarse emulsions are milky in appearance and are thermodynamically unstable. The particles size (diameter) is around 1000nm.

Figure 3-2 to 3-7 show the pseudo-ternary phase diagrams obtained for the system (soybean oil + surfactant + anhydrous glycerol + water) using different surfactants. No drug (Vitamin E) was present in the system.

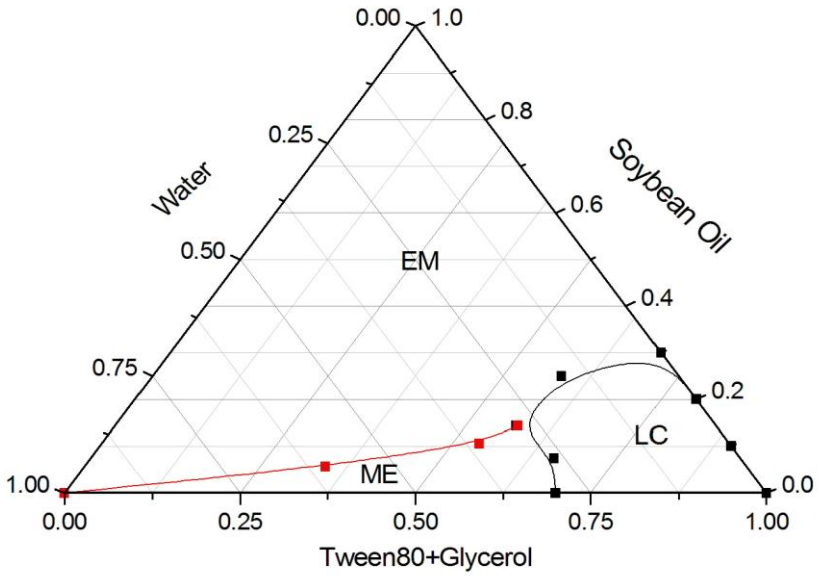


Figure 3-2: Pseudo-ternary phase diagram for the system: soybean oil (0% w/w Vitamin E loading in the oil phase) + Tween 80 + anhydrous glycerol + water

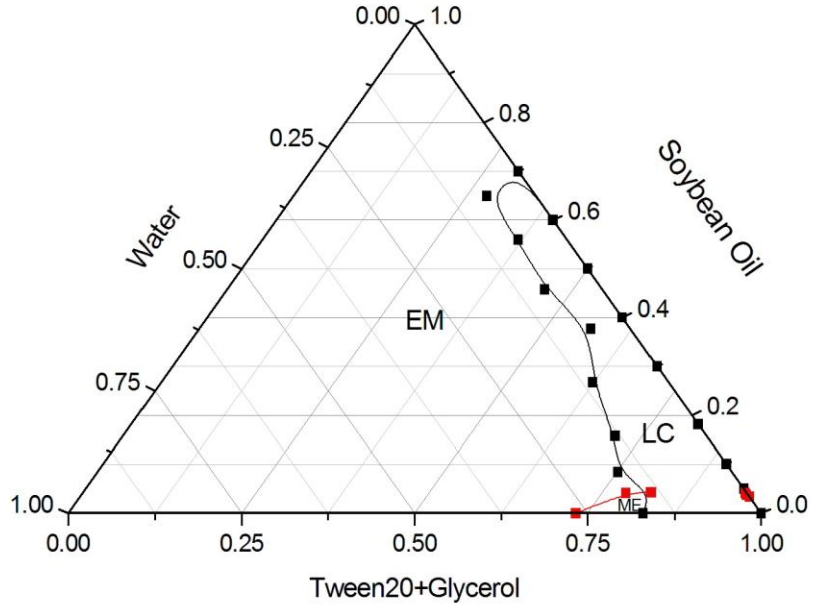


Figure 3-3: Pseudo-ternary phase diagram for the system: soybean oil + Tween 20 + anhydrous glycerol + water

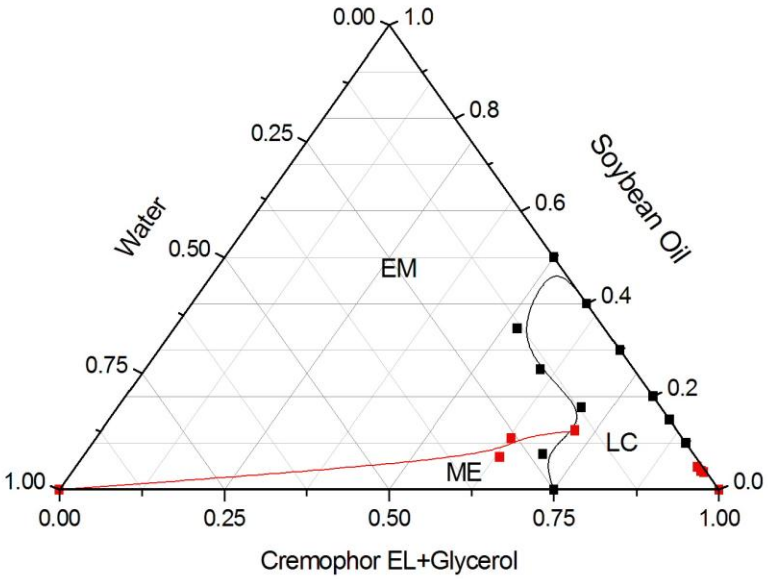


Figure 3-4: Pseudo-ternary phase diagram for the system: soybean oil + Cremophor EL + anhydrous glycerol + water

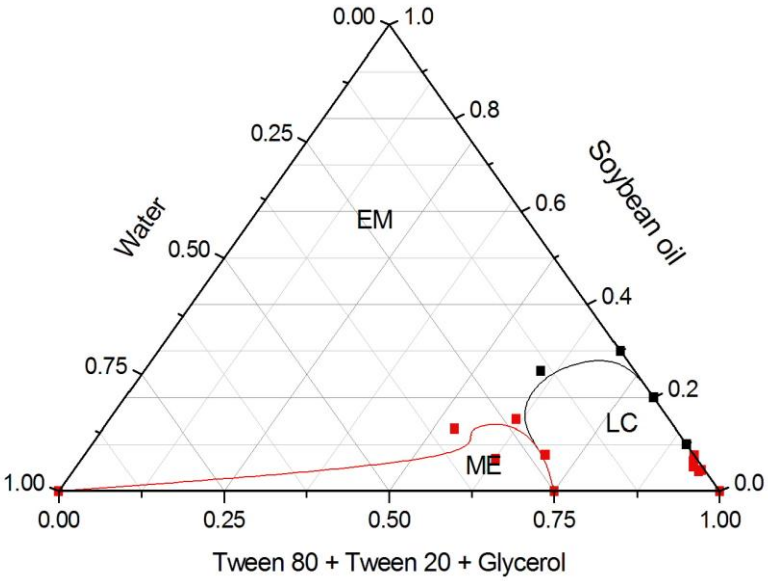


Figure 3-5: Pseudo-ternary phase diagrams for the system: soybean oil + Tween 80 + Tween 20 + anhydrous glycerol + water

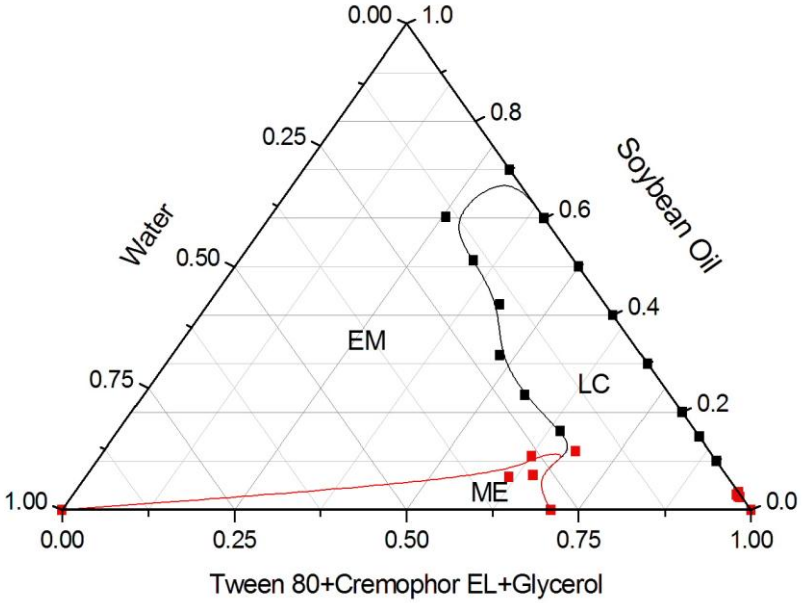


Figure 3-6: Pseudo-ternary phase diagrams for the system: soybean oil + Tween 80 + Cremophor EL + anhydrous glycerol + water

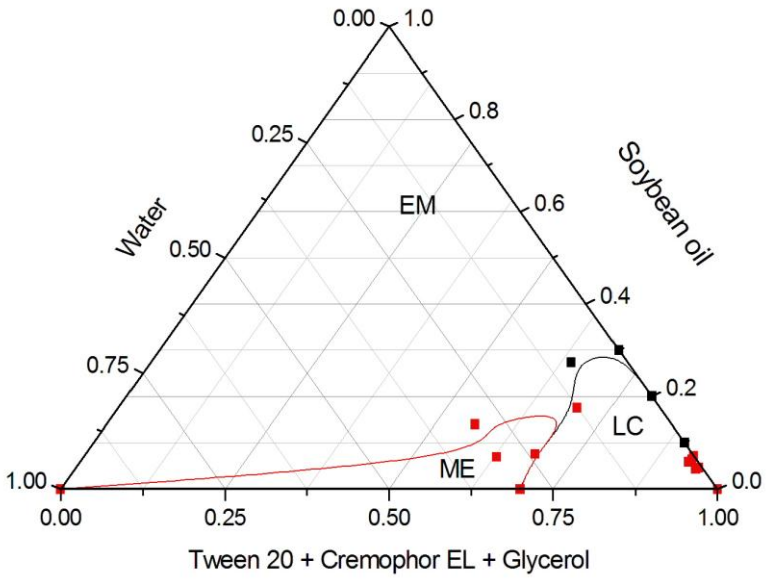


Figure 3-7: Pseudo-ternary phase diagrams for the system: soybean oil + Tween 20 + Cremophor EL + anhydrous glycerol + water

Figures 3-2 to 3-4 present the pseudo-ternary phase diagrams using three different surfactants (Tween 80, Tween 20, Cremophor EL). Apparently, Tween 20 is the worst surfactant in that it gives a negligibly small ME region. Isotropic microemulsions are very difficult to form even when Tween 20 + co-surfactant (anhydrous glycerol) is in high concentration (90% surfactant phase + 10% oil phase). Thus, choosing Tween 20 as the candidate surfactant for self-emulsifying drug delivery system, the potential toxic risk will be high as an extremely high amount of surfactant will be required. Furthermore, creaming occurred after few hours of storage. Therefore, Tween 20 is not a suitable surfactant for the drug delivery system under consideration.

Using Tween 20 in a mixture of surfactants is also not helpful. No enlargement in the ME region can be found in Figures 3-5 and 3-7. Furthermore, the appearance of microemulsions prepared from a mixture of Tween 20 and other surfactants is a little different. Using a single pure surfactant (Tween 80 or Cremophor EL), very clear, isotropic and transparent microemulsions are formed in ME region. However, the microemulsions look turbid and cloudy when prepared with mixture of Tween 20 and other surfactants. In conclusion, Tween 20 has a negative impact on the size of the ME region for the system: soybean oil + surfactant + anhydrous glycerol + water. Therefore, Tween 20 was not considered as a candidate surfactant for this specific system for further studies.

Comparing Figures 3-2, 3-4 and 3-6, one finds that no significant enlargement in ME

region occurs when mixture of surfactants Tween 80 and Cremophor EL is used. Therefore, usage of mixture of different surfactants is not a good choice for the system under consideration. The combination of two or more surfactants can actually increase the potential risk of drug administration due to unknown interactions between chemicals and human body.

Figures 3-2 and 3-4 show that Tween 80 and Cremophor EL have similar ME areas in the pseudo-ternary phase diagrams. However, Cremophor EL has a lower HLB value of 13.5 as compared with Tween 80 (HLB of 15). HLB value is a measure of the degree of hydrophilicity of surfactant [69]. The HLB value ranges from 0 to 20. The larger the HLB value, more hydrophilic is the surfactant and easier it is for the surfactant to form oil-in-water emulsions. From HLB point of view, Tween 80 is a little better than Cremophor EL. Also, Tween 80 is preferred from phase inversion point of view. As discussed earlier, phase inversion from W/O microemulsion to O/W microemulsion occurs at the beginning of titration. Tween 80 needed shorter stirring time to convert to O/W microemulsion than Cremophor EL [12]. This means that Tween 80 can emulsify the soybean oil faster than Cremophor EL.

In conclusion, the best surfactant (among the pure and mixed ones investigated in this work) for the system (soybean oil + surfactant + anhydrous glycerol + water) appears to be Tween 80. Therefore the system soybean oil + Tween 80 + anhydrous glycerol + water was investigated further for drug loading.

Chapter 4 Influence of Drug Loading on the Phase Diagrams

4.1 Introduction

The purpose of this chapter is to discuss the influence of drug loading on the system soybean oil + Tween 80 + anhydrous glycerol + water. Previous research work summarized in Table 4-1 shows that drug loading can influence the particle properties.

Table 4-1: The influence of drug loading on the system [52]

Composition	Drug loading (%)	Appearance	Emulsification time (min)	Particle fraction (%)	Mean particle size[a] (nm)
$O_{20}T_{80}$	0	Clear	3	100	12
	1	Cloudy	2	82	192
				10	10
				8	26
	2.5	Cloudy	3	75	209
				14	11
				11	47
	5	Cloudy	3	80	179
				12	14
				8	851
$O_{20}C_{80}$	0	Clear	50	100	13
	1	Slightly cloudy	52	56	164
				44	15
	2.5	Cloudy	58	55	242
				32	1000
				13	14
	5	Cloudy	62	95	236
				5	13
$O_{20}T_{40}C_{40}$	0	Clear	3	100	11
	1	Clear	4	100	11
	2.5	Clear	3	100	11
	5	Clear	4	100	10

[a] O for oil Capmul PG8 (propylene glycol monocaprylate), T for surfactant Tween 20 (polysorbate 20), C for Cremphor EL (polyoxyl 35 castor oil). For example, the $O_{20}T_{40}C_{40}$ indicates that the preconcentrate contained 20% Capmul PG8, 40% Tween 20, 40% Cremphor EL.

In this work, the effect of Vitamin E loading on the phase diagrams was investigated.

Vitamin E is considered as a model drug for the system soybean oil + Tween 80 + anhydrous glycerol + water. The loading of the drug can result in changes in the properties of the oil phase and can influence the ME region significantly. Other properties, such as the particle size, can also be affected because of the drug loading.

To study the influence of Vitamin E loading on the phase diagrams, the titration method discussed in chapter 3 was used. Water was employed as the aqueous phase. The surfactant phase consisted of Tween 80 and anhydrous glycerol in the ratio of 1:1 on the weight basis. The oil phase consisted of soybean oil loaded with different amounts of Vitamin E. The particle size was measured by DLS technique discussed in chapter 3.

4.2 Experimental Methods

The drug (vitamin E) was loaded in the oil phase and the influence of different drug loadings on the pseudo-ternary phase diagram was determined. The oil phase contained 10%w/w, 20%w/w, 30%w/w, 40%w/w and 50%w/w of vitamin E respectively. The ratio of surfactant (Tween 80) to co-surfactant (Anhydrous Glycerol) was fixed at 1:1. The systems investigated are listed as follow:

(1) Soybean oil (100%) + Tween 80 + Anhydrous Glycerol + Water

(2) Soybean oil, Vitamin E (90%/10%) + Tween 80 + Anhydrous Glycerol + Water

(3) Soybean oil, Vitamin E (80%/20%) + Tween 80 + Anhydrous Glycerol + Water

(4) Soybean oil, Vitamin E (70%/30%) + Tween 80 + Anhydrous Glycerol + Water

(5) Soybean oil, Vitamin E (60%/40%) + Tween 80 + Anhydrous Glycerol + Water

(6) Soybean oil, Vitamin E (50%/50%) + Tween 80 + Anhydrous Glycerol + Water

To construct the pseudo-ternary phase diagrams, the oil phase was prepared by mixing the soybean oil and Vitamin E together. The same titration method was applied for each of the systems listed above. The oil phase was mixed with the surfactant phase (Tween 80 + Anhydrous Glycerol) at ratios of 1/9, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2 and 9/1. Titration was carried out by adding a small amount of deionized water (less than 5%) at a time and mixing under gentle stirring for 2 to 3 minutes. The particle size was measured by DLS.

4.3 Experimental Results and Discussion

The pseudo-ternary phase diagrams for different loadings of Vitamin E in the system are shown in Figure 4-1 to 4-5.

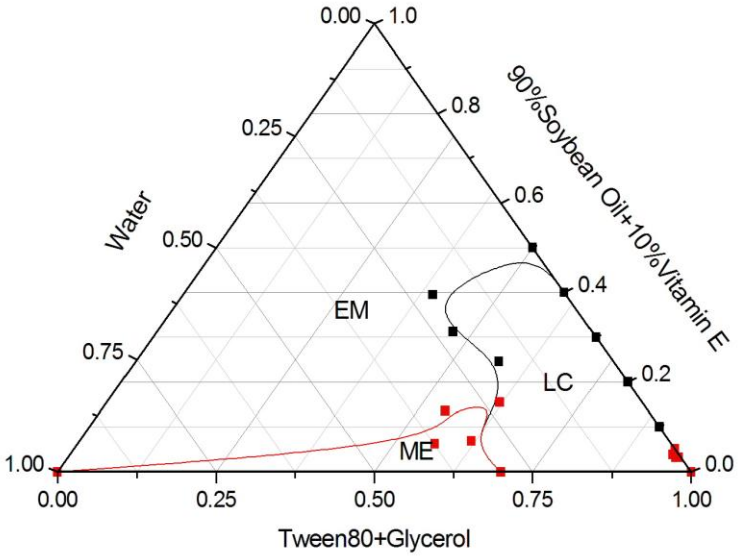


Figure 4-1: Pseudo-ternary phase diagram for the system: soybean oil (10% w/w Vitamin E loading in the oil phase) + Tween 80 + anhydrous glycerol + water

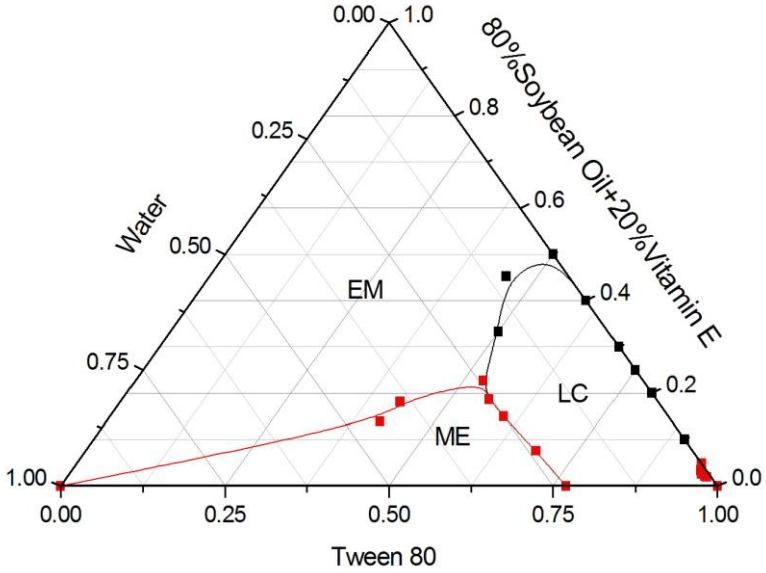


Figure 4-2: Pseudo-ternary phase diagram for the system: soybean oil (20% w/w Vitamin E loading in the oil phase) + Tween 80 + anhydrous glycerol + water

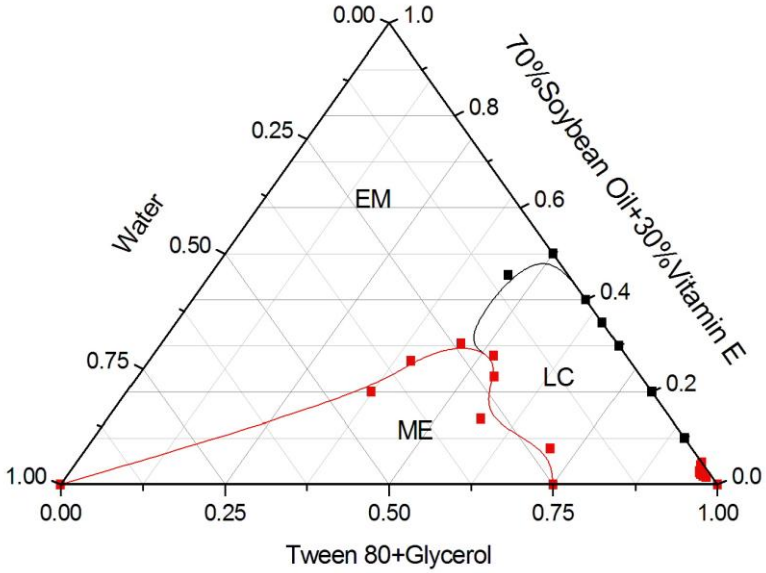


Figure 4-3: Pseudo-ternary phase diagram for the system: soybean oil (30% w/w Vitamin E loading in the oil phase) + Tween 80 + anhydrous glycerol + water

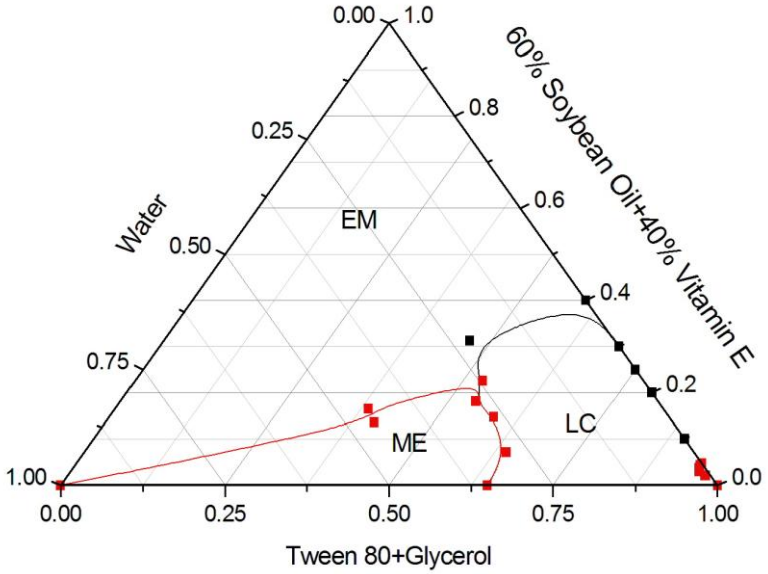


Figure 4-4: Pseudo-ternary phase diagram for the system: soybean oil (40% w/w Vitamin E loading in the oil phase) + Tween 80 + anhydrous glycerol + water

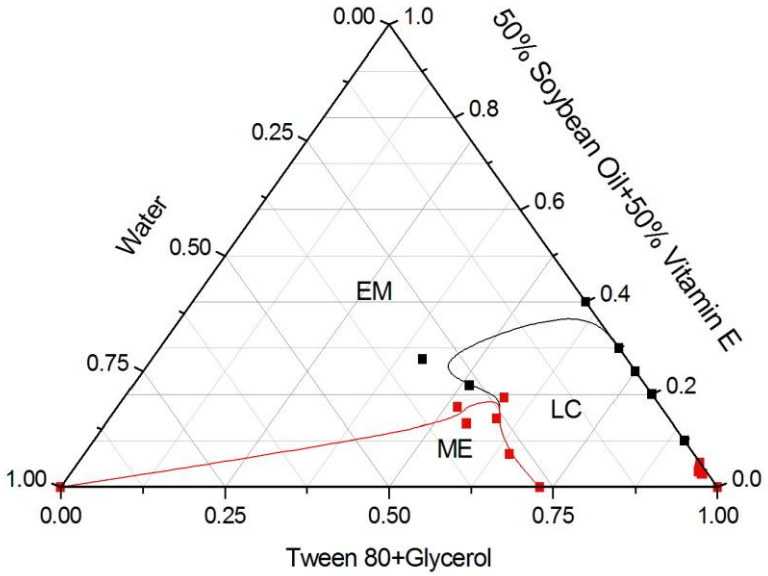


Figure 4-5: Pseudo-ternary phase diagram for the system: soybean oil (50% w/w Vitamin E loading in the oil phase) + Tween 80 + anhydrous glycerol + water

Comparing Figure 3-2 and 4-1 to 4-5, it is clear that the influence of drug loading on the phase diagrams (particularly the microemulsion region) is quite significant. The microemulsion (ME) region of the phase diagrams undergoes enlargement when the drug (Vitamin E) loading is increased from 0 to 30% w/w in the oil phase. With further increase in the drug loading, the microemulsion region tends to shrink. It should also be noted that for drug loading levels of 40% w/w and 50% w/w, the microemulsions were not very stable; the samples exhibited phase separation when left for a few days. Thus, the best loading level of vitamin E is 30% w/w based on the oil phase (Figure 4-3). At this level of drug loading, the microemulsion region is large enough to allow some flexibility in choosing an optimal composition for the SMEDDS (self-microemulsifying drug delivery system). One reason for the variations in the size of ME region with drug loading is the changes in the properties of the oil phase. The viscosity of the oil phase was increased significantly with Vitamin E loading. A gel-like product was formed at the beginning of the titration due to high viscosity of oil phase. The gentle shaking or stirring lost its effect when the Vitamin E loading reached 50% w/w (very high viscosity) in the oil phase. Only vigorous stirring lead to the formation of microemulsion for the system with Vitamin E loading of 50% w/w based on the oil phase. When pure Vitamin E was used as the oil phase, microemulsions could not be formed even with vigorous stirring.

The particle size of microemulsions also revealed useful information about the optimal Vitamin E loading level. The detailed results for different levels of Vitamin E

loading are given in appendix A. Table 4-2 presents the mean particle size for different compositions.

Table 4-2: Mean Particle size

Mean Particle Size (nm)	0% Vitamin E loading in the oil phase	10% Vitamin E loading in the oil phase	20% Vitamin E loading in the oil phase	30% Vitamin E loading in the oil phase	40% Vitamin E loading in the oil phase	50% Vitamin E loading in the oil phase
$O_5S_{45}W_{50}$	21.893	20.043	37.393	26.487	16.190	68.157
$O_{10}S_{40}W_{50}$	101.573	80.263	47.973	27.890	76.093	97.207
$O_{15}S_{35}W_{50}$	161.233*	117.333	107.433	79.397	93.917	116.233*
$O_{20}S_{30}W_{50}$	121.533*	140.200	130.800	109.577	110.867*	99.700*

The composition is specified using the notation $O_xS_yW_z$, where x is % w/w of oil phase, y is % w/w of surfactant phase, and z is % w/w of water phase. For example, $O_5S_{45}W_{50}$ represents the composition with 5% w/w of oil phase, 45% w/w of surfactant phase and 50% w/w of water phase. In any given row of the table, the effect of drug loading level on the mean droplet size (nm) is shown. The water content of all compositions shown in Table 4-2 is 50% w/w. The emulsifying time (stirring time) was kept the same (24 hours) for all the compositions. Phase separation occurred within a few days of storage in the case of data point with asterisk (see Table 4-2).

The measurements in such cases were biased because the particles did not distribute homogeneously in DLS instrument.

According to Table 4-2, the mean particle size in any column (fixed Vitamin E loading) increases when the oil concentration is increased and the surfactant concentration is decreased simultaneously. This is to be expected as less surfactant is used to emulsify more oil phase. More importantly, the mean particle size is minimum for 30% Vitamin E loading especially when the oil phase is more than 5% w/w. Thus, the optimum loading level of drug (Vitamin E) is 30% w/w based on the mean particle size information.

Figures 4-6 to 4-8 show the particle size distributions of microemulsions for different loading levels of Vitamin E. The composition of the microemulsions (other than Vitamin E loading) is the same, that is, 10% w/w oil phase, 20% w/w surfactant phase, and 50% w/w water phase. The microemulsion at 20% Vitamin E loading (Figure 4-6) has low polydispersity but the particle size is large. At 40% Vitamin E loading (Figure 4-8), the microemulsion has a high polydispersity. The best distribution in terms of low mean size and low polydispersity seems to be at 30% Vitamin E loading (Figure 4-7).

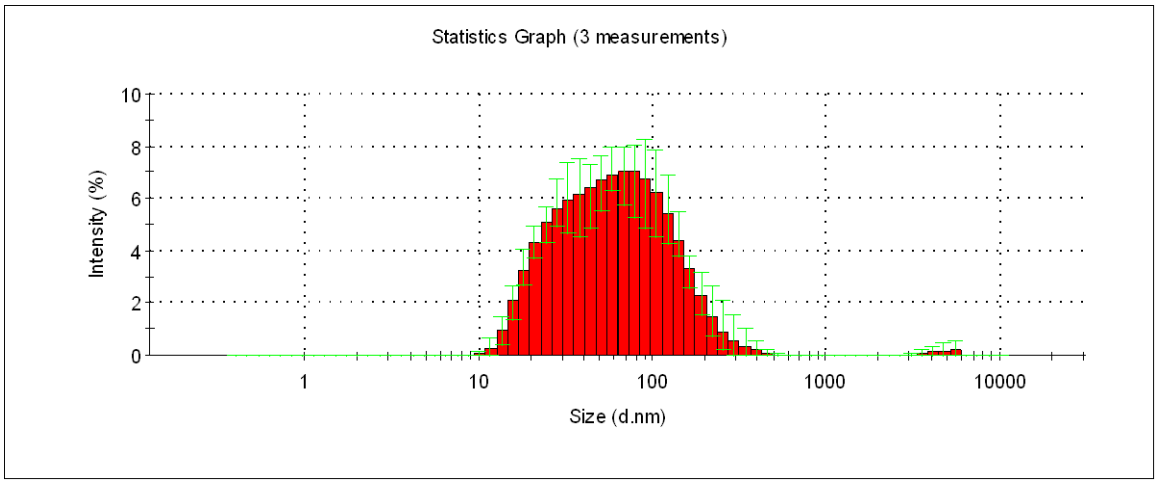


Figure 4-6: Particle size distribution by intensity for $O_{10}S_{40}W_{50}$ (oil phase/surfactant phase/water phase=10%/40%/50%), 20% w/w Vitamin E loading in the oil phase

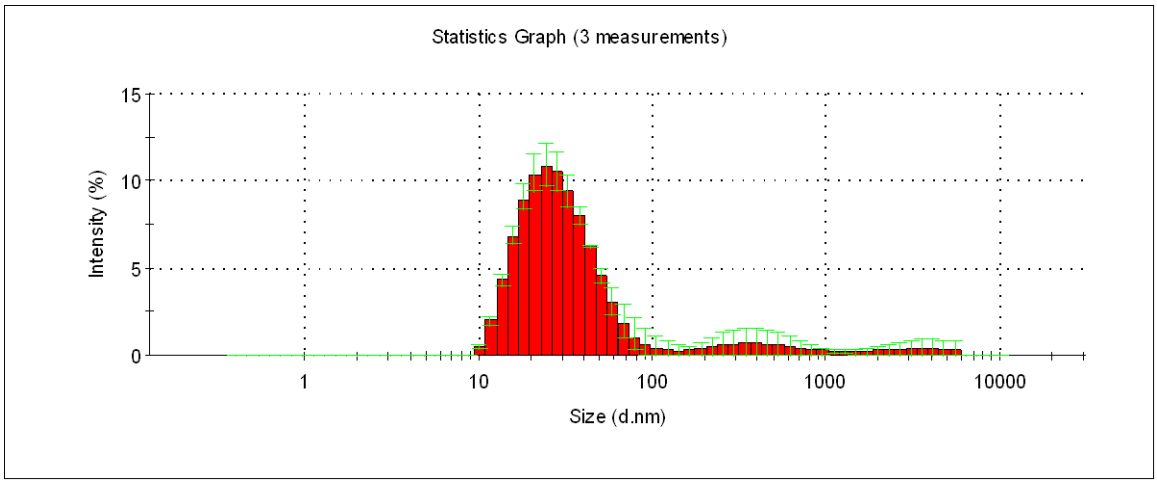


Figure 4-7: Particle size distribution by intensity for $O_{10}S_{40}W_{50}$ (oil phase/surfactant phase/water phase=10%/40%/50%), 30% w/w Vitamin E loading in the oil phase

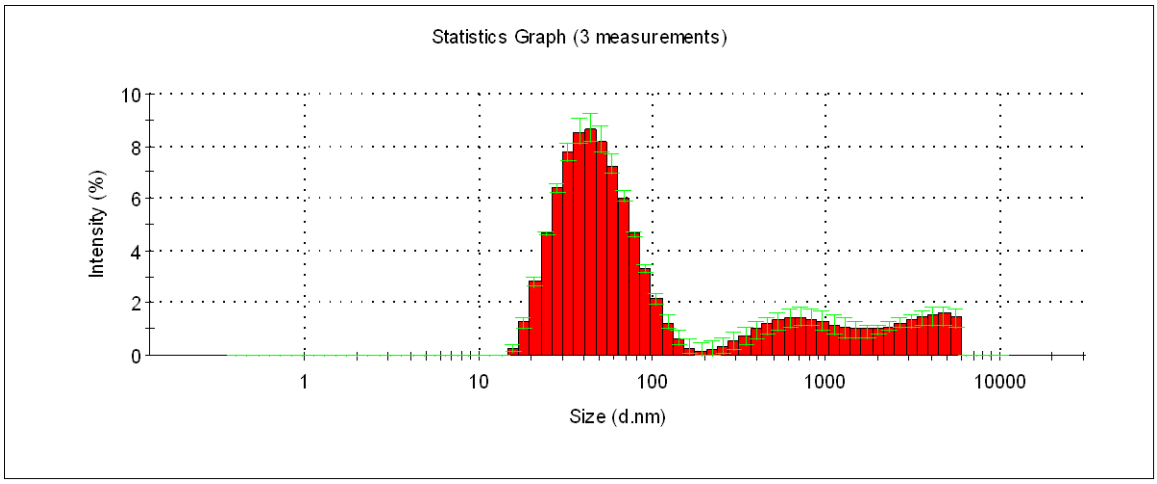


Figure 4-8: Particle size distribution by intensity for $O_{10}S_{40}W_{50}$ (oil phase/surfactant phase/water phase=10%/40%/50%), 40% w/w Vitamin E loading in the oil phase

Therefore, the best system is Soybean oil, Vitamin E (70%/30%) + Tween 80 + Anhydrous Glycerol + Water. The phase diagram of this system is given in Figure 4-3.

The next step is to select the optimal composition in the ME region for the system with 30% w/w Vitamin E loading in the oil phase. The main factor to be considered is the initial ratio of the oil phase to the surfactant phase. The target of this research is to use as little surfactant to emulsify as much drug as possible. Based on the phase diagram shown in Figure 4-3, five initial starting points are considered as alternative compositions; they are: oil phase/surfactant phase=10%/90%, 20%/80%, 30%/70%, 35%/65%, 40%/60%. Note that the oil phase of these compositions consisted of 30% w/w Vitamin E.

It is found that the optimal composition is about 30% w/w oil phase and 70% w/w

surfactant phase. The reason for choosing this composition as optimal composition is as follow: Firstly, the microemulsion formed with the addition of water to this composition had an acceptable particle size. For example, Figure 4-9 shows the particle size distribution for $O_{15}S_{35}W_{50}$ (Note that oil phase is 30% w/w based on oil + surfactant) with Vitamin E loading of 30% w/w in the oil phase. The average particle size is around 80-120 nm. This is an acceptable level of average particle size for delivering drugs using microemulsion. Secondly, the usage of surfactant is relatively smaller than other compositions such as 20% w/w oil phase + 80% w/w surfactant phase. This is helpful in reducing the toxicity of the drug with little negative impact on drug delivery. Thirdly, the selected composition is not too close to the boundary line, ensuring product stability.

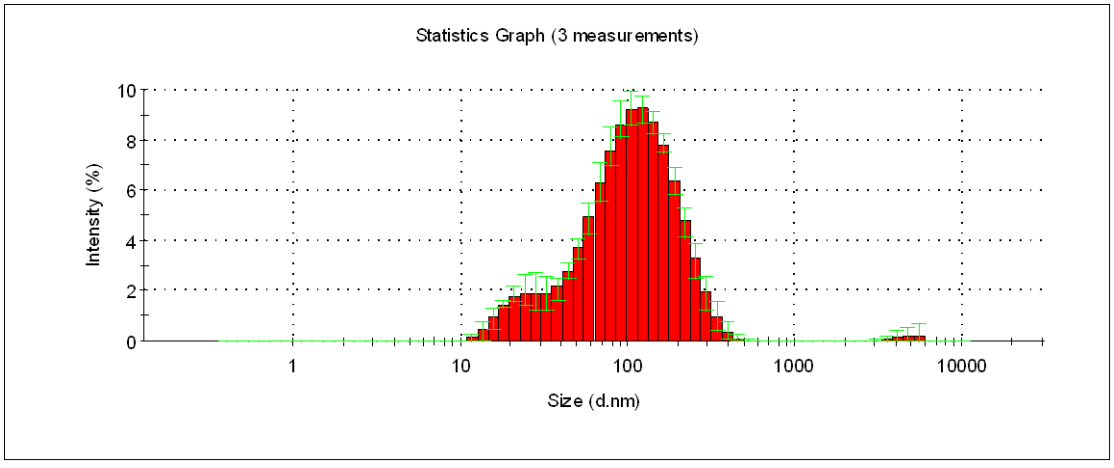


Figure 4-9: Particle size distribution by intensity for $O_{15}S_{35}W_{50}$ (oil phase/surfactant phase/water phase=15%/35%/50%), 30% w/w Vitamin E loading in the oil phase

In summary, a particular SMEDDS with Vitamin E as a model drug was investigated in this chapter. Based on the experimental data and figures, the optimal formulation of

the particular system investigated is: 21% w/w Soybean oil, 9% w/w Vitamin E, 35% w/w Tween 80 and 35% w/w anhydrous glycerol (this formulation corresponds to the oil phase to surfactant phase ratio of 30%/70%). The *in vitro* study reported has used 35% w/w Tween 80 to deliver 9% w/w Vitamin E. Thus, the amount of drug (Vitamin E) delivered relative to the amount of surfactant required is reasonably good.

Chapter 5 Phase Inversion Phenomenon in Self-microemulsifying Drug Delivery System (SMEDDS)

5.1 Introduction

Phase inversion phenomenon occurs during the titration process. The changes in viscosity can detect for this phenomenon [70]. Watanabe et al. [21] detected the appearance of phase inversion and formation of bicontinuous microemulsion structure (Figure 2-3) by monitoring the changes in viscosity. Their results are shown in Figure 5-1:

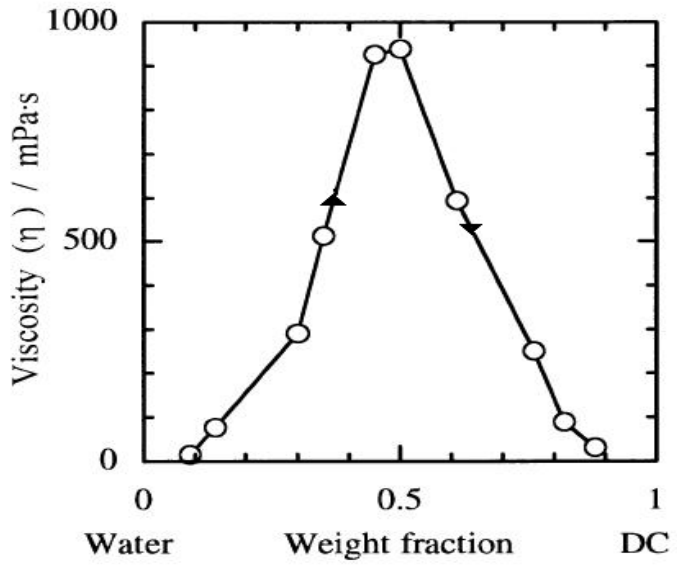


Figure 5-1: Zero shear viscosity of ME in DC (decamethyl cyclopentasiloxane)/CIO (oil cetyl isooctanoate)/PGMI (polyoxyethylene glyceryl monoisostearate)/15% Ethanol Aqueous Solution System [21]

The experiment was started with aqueous phase initially. Upon addition of oil phase (decamethyl cyclopentasiloxane), O/W microemulsion is formed. The viscosity of O/W microemulsion increases with the increase in oil concentration. The phase inversion occurs when the viscosity reaches the maximum value. After phase inversion, the microemulsion was W/O type. Near the phase inversion point, they also detected the bicontinuous microemulsion structure (Figure 2-3) using FF-TEM [21]. The viscosity of W/O microemulsion decreased with further addition of oil phase.

Other ways to detect phase inversion in emulsions are: conductivity measurement, dying method and dilution method.

5.2 Experimental Methods

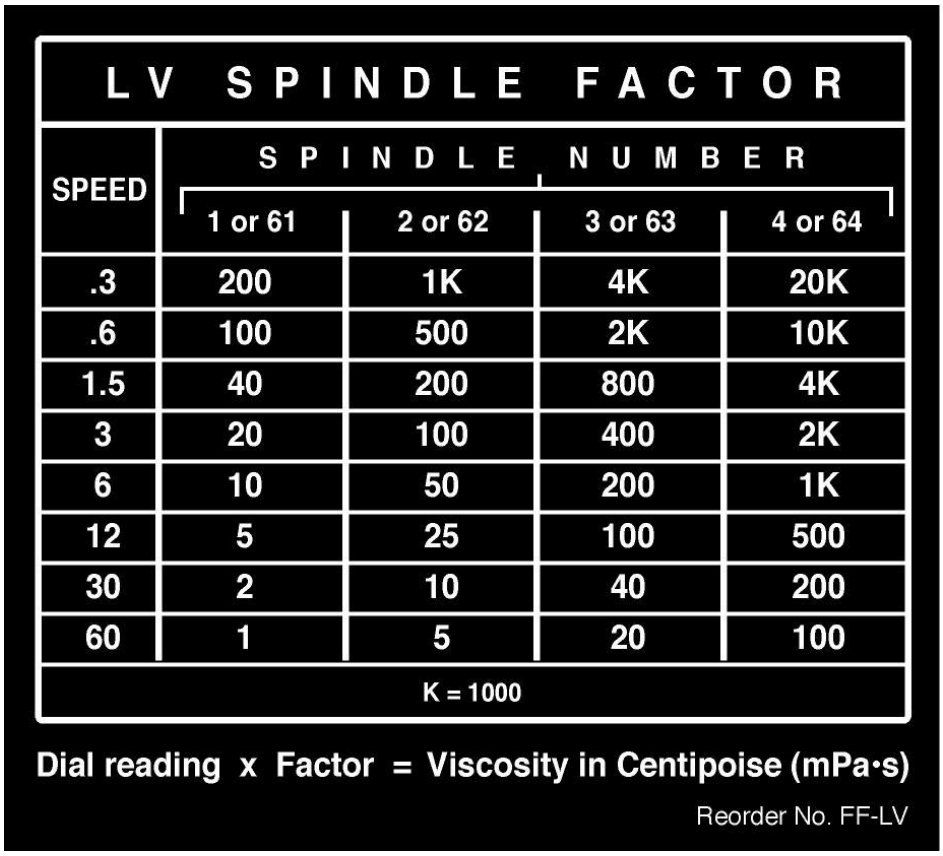
5.2.1 Sample Preparation

The objective of this part of our research is to demonstrate that phase inversion occurs during the titration process involved in the construction of phase diagrams. For this purposes, two systems (system without drug-Soybean oil (100%) + Tween 80 + Anhydrous Glycerol + Water and Soybean oil; system with drug-Vitamin E (70%/30%) + Tween 80 + Anhydrous Glycerol + Water) were selected. For a given system, two initial compositions (oil phase/surfactant phase=10%/90% and 20%/80% for both the systems) were selected. The viscosity of the system was measured progressively with incremental addition of water.

5.2.2 Viscosity Instrument

The viscosity was measured using Brookfield synchro-lectric Viscometer. A disc shaped spindle (LV 3, see Table 5-1) was used in the measurements. The rotation speed was fixed at 12 rpm. From Table 5-1, the spindle factor for LV 3 spindle is 100. The viscosity measurement was conducted after each titration run. The spindle was cleaned after every run.

Table 5-1: LV Spindle factor



LV SPINDLE FACTOR				
SPEED	SPINDLE NUMBER			
	1 or 61	2 or 62	3 or 63	4 or 64
.3	200	1K	4K	20K
.6	100	500	2K	10K
1.5	40	200	800	4K
3	20	100	400	2K
6	10	50	200	1K
12	5	25	100	500
30	2	10	40	200
60	1	5	20	100
K = 1000				

Dial reading x Factor = Viscosity in Centipoise (mPa·s)

Reorder No. FF-LV

5.3 Experimental Results and Discussion

Figures 5-2 and 5-3 show the viscometer dial reading as a function of water weight fraction for systems without drug (Vitamin E). Note that the dial reading can be converted to viscosity (cP) by multiplying with a factor of 100. For Figure 5-2, the ratio of oil phase to surfactant phase is 10%/90%. For Figure 5-3, the ratio of oil phase to surfactant phase is 20%/80%.

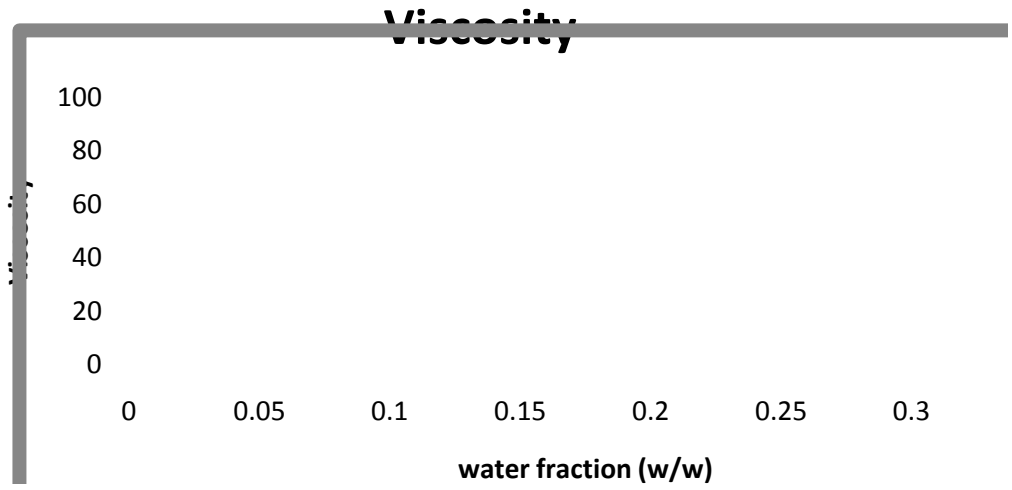


Figure 5-2: Viscosity for 10%O (0% Vitamin E loading in the oil phase)/90% S (the ratio of the oil phase to the surfactant phase is 10%/90% with 0% Vitamin E loading in the oil phase)

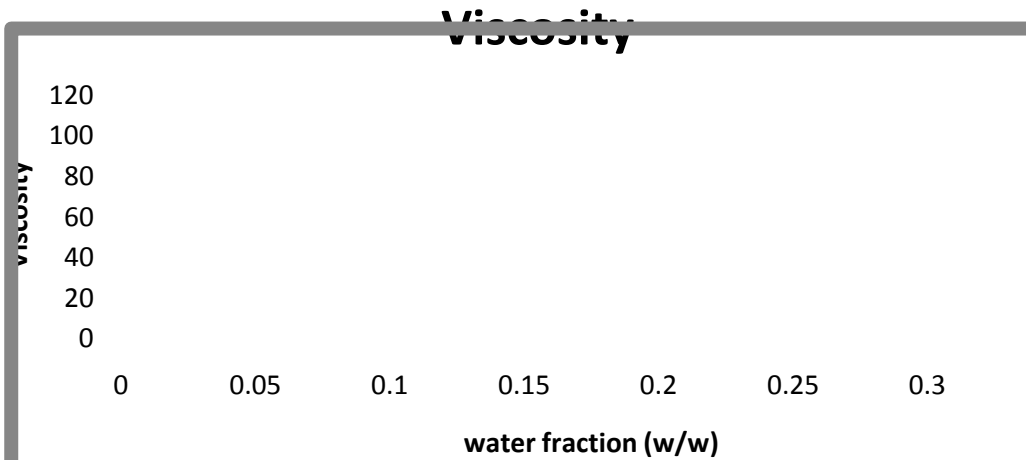


Figure 5-3: Viscosity for 20%O (0% Vitamin E loading in the oil phase)/80% S (the ratio of the oil phase to the surfactant phase is 20%/80% with 0% Vitamin E loading in the oil phase)

All the data are listed in Appendix B. The mixture was clear at the beginning of the titration. With the addition of water, the mixture turned cloudy. White precipitate can be visually detected under stirring. Both the figures (Figure 5-2 and 5-3) exhibit a drop in viscosity at the beginning of the titration. The mixture became clear and transparent when the viscosity declined to the lowest point (5%-9% water w/w). Streaks could also be detected under stirring.

With further addition of water the viscosity begins to increase due to the formation of W/O microemulsion. The viscosity reaches a maximum value at water fraction of about 0.167 (Figure 5-2) and 0.13 (Figure 5-3). Near the peak values of viscosity, phase inversion from W/O to O/W microemulsion occurs. The viscosity of O/W microemulsion decreases with further increase in water fraction. Also note that the phase inversion from W/O to O/W microemulsion is delayed in the case of higher

surfactant concentration (Figure 5-2, ratio of oil phase to surfactant phase 10%/90%).

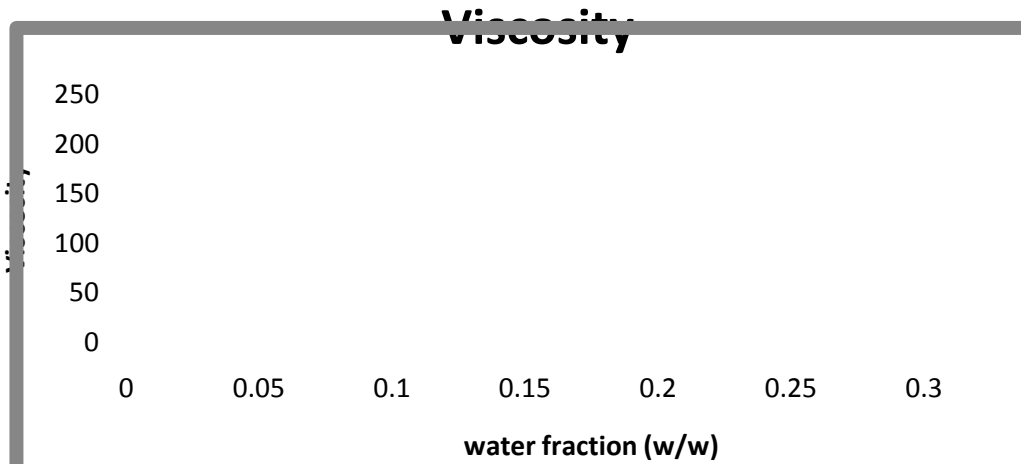


Figure 5-4: Viscosity for 10%O (30% Vitamin E loading in the oil phase)/90% S (the ratio of the oil phase to the surfactant phase is 10%/90% with 30% Vitamin E loading in the oil phase)

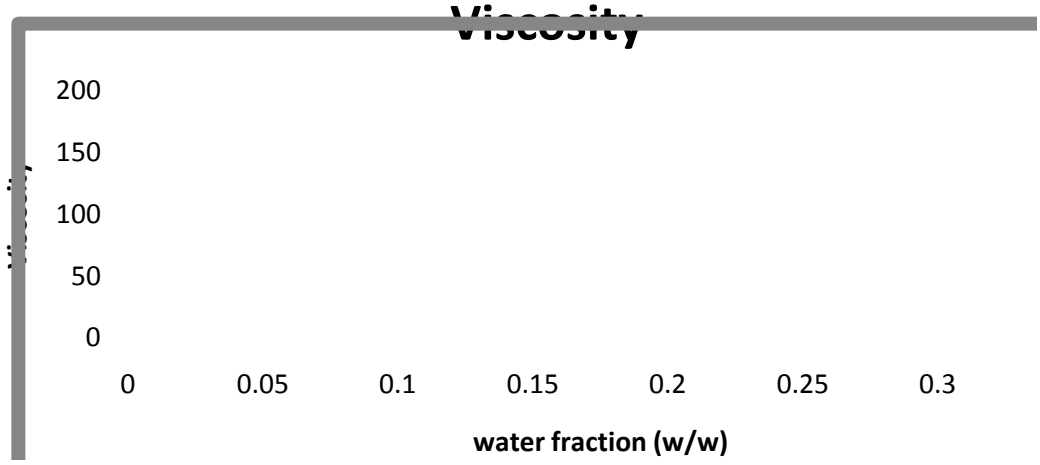


Figure 5-5: Viscosity for 20%O (30% Vitamin E loading in the oil phase)/80% S (the ratio of the oil phase to the surfactant phase is 20%/80% with 30% Vitamin E loading in the oil phase)

Figure 5-4 and 5-5 show the viscometer reading for systems with 30% drug (Vitamin

E) loading. The viscometer reading versus water weight fraction plots of systems with drug loading are generally similar to those of systems without drug loading. The initial range of water fraction where the viscosity is either constant or varies slightly corresponds to liquid crystal region. The region where viscosity reading increases corresponds to W/O microemulsion region. After the peak value of viscosity, the region where viscosity decreases with the increase in water fraction corresponds to O/W microemulsions.

In summary, phase inversion occurred in all water titrating systems. In the initial water fraction range of about 0 to 0.15, the system was either liquid crystal or W/O microemulsion. At higher water fraction, the W/O microemulsion inverted to O/W microemulsion. As the O/W microemulsions are more important from drug delivery point of view, they are shown as a separate region on the phase diagrams discussed in previous chapters. The initial water fraction region, where the system is either liquid crystal or W/O microemulsions, is shown only as one liquid crystal region studied in this work.

Chapter 6 Conclusions and Future Work

6.1 Conclusions

(1) The pseudo-ternary phase diagrams were successfully developed for the following system: Soybean oil (Vitamin E) + Surfactant + Anhydrous Glycerol + Water. Special attention was given to identify the microemulsion (ME) region of the pseudo-ternary phase diagram.

(2) The influence of different surfactants (Tween 20, Tween 80, Cremopher EL) and their mixtures on the microemulsion region (ME region) of the phase diagrams was studied. The system: Soybean oil + pure Tween 80 + Anhydrous Glycerol + Water showed the largest ME region. Consequently, Tween 80 was selected as the best surfactant for the system.

(3) The effect of drug (Vitamin E) loading on the selected system, Soybean oil + Tween 80 + Anhydrous Glycerol + Water, was also studied. The loading of different amounts of Vitamin E in the oil phase resulted in changes of the size of ME region of the pseudo-ternary phase diagram. The drug (Vitamin E) loading of 30% by weight in the oil phase (Soybean oil/Vitamin E=70%/30%) showed the largest ME region. Therefore, the system: Soybean oil, Vitamin E (70%/30%) + Tween 80 + Anhydrous Glycerol + Water was considered as the best system.

(4) The phase inversion phenomenon was investigated at the beginning of the titration process. The viscosity measurements showed an initial increase and then a large

drop with the increase in water content of the system. The W/O microemulsion inverted to an O/W microemulsion resulting in a large drop of viscosity.

Another conclusion was reached based on the measurement of particle size distribution for the selected best system (Soybean oil, Vitamin E (70%/30%) + Tween 80 + Anhydrous Glycerol + Water). The particle size was measured for several compositions within the microemulsion (ME) region of this system. The formulation consisting of 21% w/w Soybean oil, 9% w/w Vitamin E, 35% w/w Tween 80 and 35% w/w anhydrous glycerol was found to be optimal in that it required less surfactant for microemulsification and resulted in acceptable droplet size for drug delivery.

6.2 Future Work

This research was executed *in vitro* as a preliminary step to develop an optimal drug composition. *In vivo* research is needed in the future as it is critical to select an optimal composition for the drugs. Some other issues that need to be addressed are: The modeling of the phase diagrams and the investigation of the structure of bicontinuous microemulsions. These issues are discussed further in the following sections.

6.2.1 Mixture Design

The objective of modeling the phase diagrams is to quantify the effect of composition (different amounts of components) on the particle size. A successful D-optimal design shows the statistical approach to obtain the relationship between the particle size distribution and the amounts of various components [71]. Another method called "mixture design" can also be applied. In this method, the pseudo-ternary phase diagrams are plotted and several points are selected within the ME region for particle size measurement. The method can be explained briefly with the help of Figures 6-2 and 6-3.

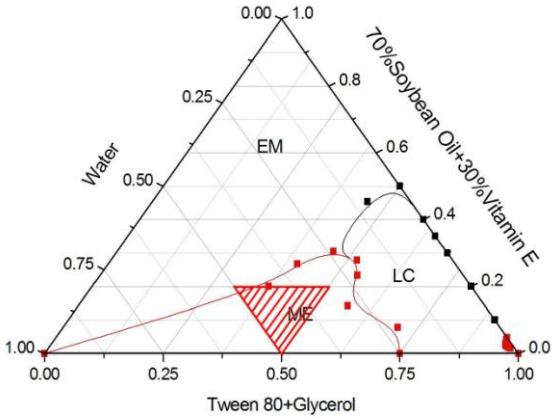


Figure 6-1: Sketch map for mixture design

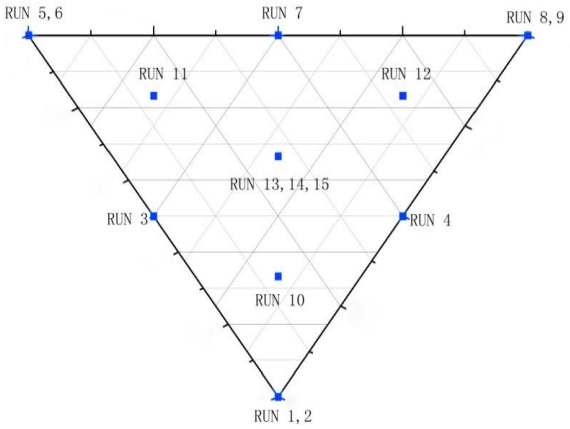


Figure 6-2: Distribution for each of the run

As shown in Figure 6-1, a triangular region (shaded area) is selected arbitrarily within the ME region. The constraint that the proportions of different components must sum to 100% should be satisfied. Note that in the selected triangular region, the oil phase is less than 20% w/w, the water phase is between 30% w/w and 50% w/w, and the surfactant phase is between 30% w/w and 50% w/w. Following all these constraints, the points (composition) can be selected according to Figure 6-2. The particle size

distribution can be obtained for each of the composition points. Then the regression models can be constructed using software called Statistica. The minimum particle size can be inferred from the regression model and an optimal composition can be calculated in a statistical way.

6.2.2 Observation of Bicontinuous Microemulsion

The structure of bicontinuous microemulsion is difficult to observe experimentally. Several instruments (Environmental Scanning Electron Microscope and Atomic Force Microscopy) were tried to observe the microemulsion but the results were unsuccessful. The fluidic (liquid) nature of the microemulsion complicated the matter. A method called freeze-fracture transmission electron microscopy (FF-TEM) has been used to observe the structure of bicontinuous microemulsion [21]. Generally, the sample is replicated on a golden disk at first. Then the replica is plunged into liquid nitrogen and is fractured in a special instrument. The surface structure is then observed [21]. This method has the limitation that it can be used to observe the structure of fractured-surface only.

Appendices

Appendix A Particle Size Distribution

Table A-0: Average particle size for 100S0VET80GW (100%w/w Soybean oil in the oil phase + 0% w/w Vitamin E in the oil phase + Tween 80 + anhydrous glycerol + water)

Sample Name	Measurement Date and Time	T (°C)	Z-Ave (d.nm)
Water 1	06/05/2008 15:21	25	0
*O/S=10%/90%	06/05/2008 15:29	25	21.92
O/S=10%/90%	06/05/2008 15:30	25	22.05
O/S=10%/90%	06/05/2008 15:31	25	21.71
Water 1	06/05/2008 15:40	25	0
O/S=20%/80% 1	06/05/2008 15:49	25	103.9
O/S=20%/80% 2	06/05/2008 15:50	25	101.2
O/S=20%/80% 3	06/05/2008 15:50	25	99.62
Water 1	06/05/2008 16:04	25	0
O/S=30%/70% 1	06/05/2008 16:11	25	158.4
O/S=30%/70% 2	06/05/2008 16:12	25	160.8
O/S=30%/70% 3	06/05/2008 16:13	25	164.5
Water 3	06/05/2008 16:21	25	0
O/S=40%/60% 1	06/05/2008 16:28	25	120.8
O/S=40%/60% 2	06/05/2008 16:29	25	122.2
O/S=40%/60% 3	06/05/2008 16:30	25	121.6
Water 1	06/05/2008 17:03	25	0
O/S=50%50% 1	06/05/2008 17:11	25	172.4
O/S=50%50% 2	06/05/2008 17:12	25	177.4
O/S=50%50% 3	06/05/2008 17:13	25	175

**O/S=10%/90% means the ratio of oil phase to the surfactant phase is 10%/90%*

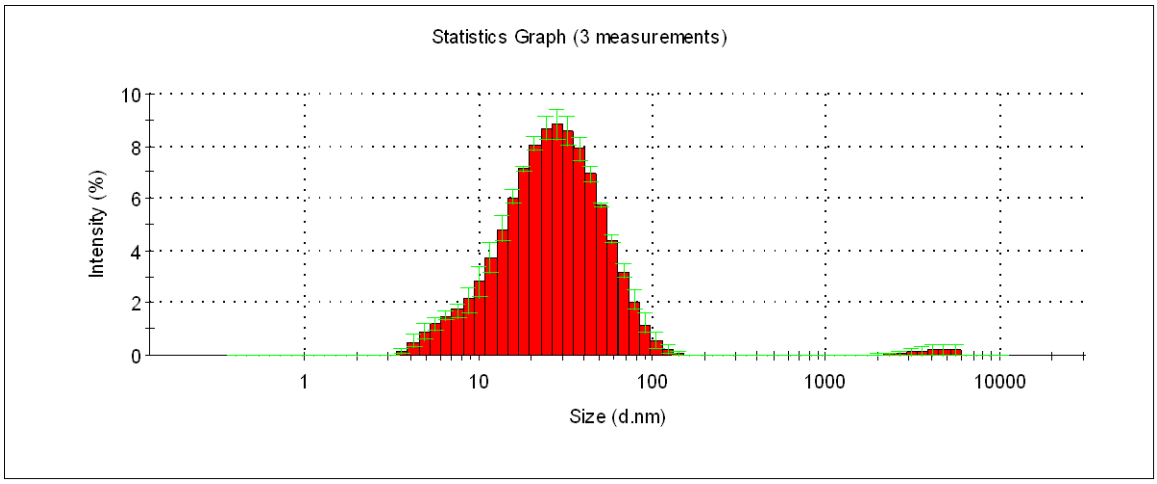


Figure A-0-1: Particle size distribution by intensity for O/S=10%/90% (0% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 10%/90% with 0% Vitamin E loading in the oil phase)

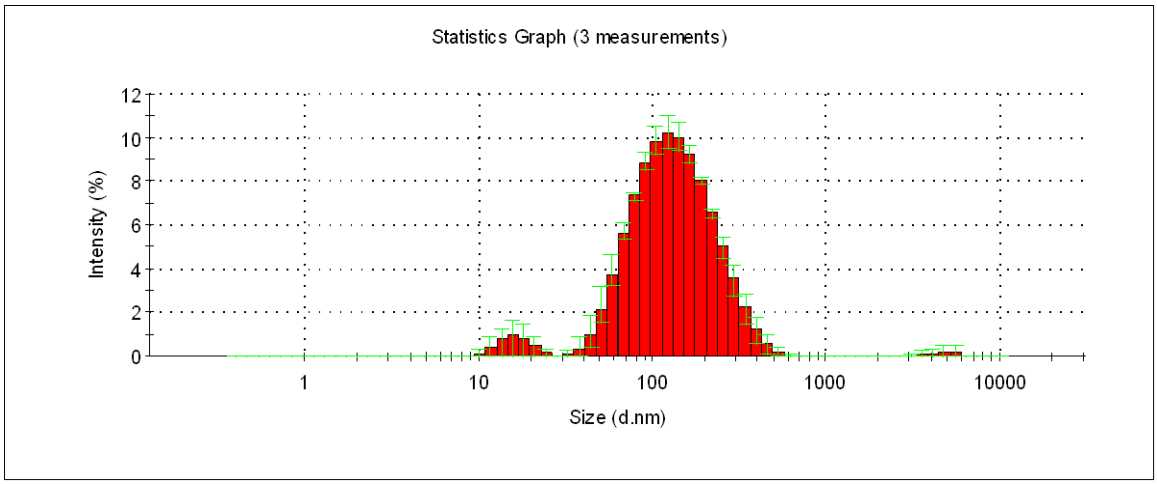


Figure A-0-2: Particle size distribution by intensity for O/S=20%/80% (0% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 20%/80% with 0% Vitamin E loading in the oil phase)

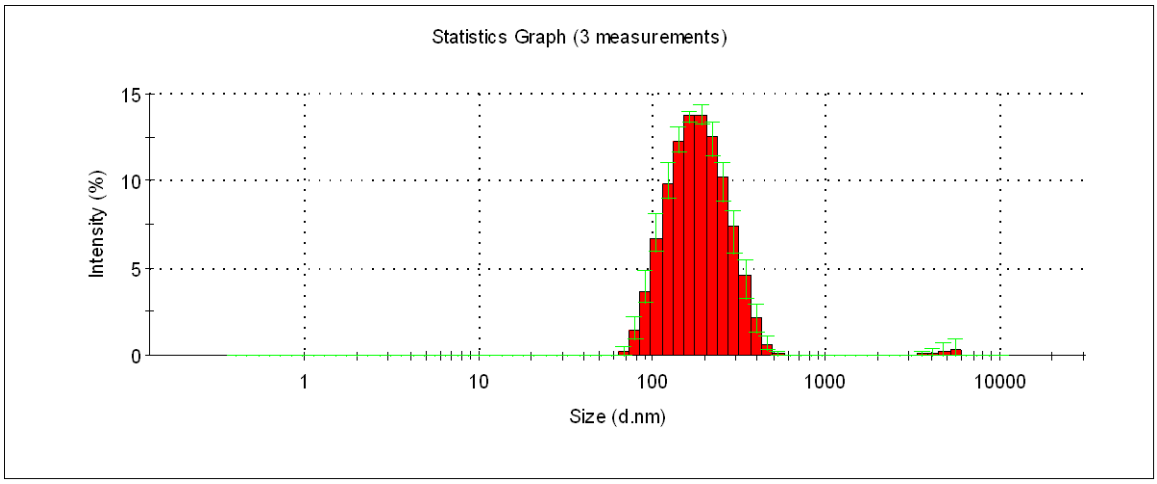


Figure A-0-3: Particle size distribution by intensity for O/S=30%/70% (0% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 30%/70% with 0% Vitamin E loading in the oil phase)

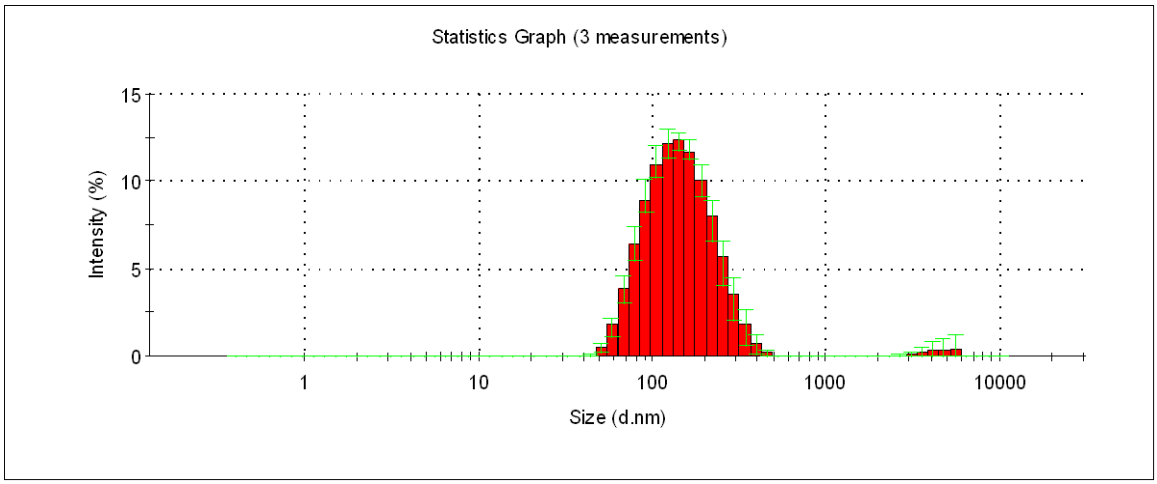


Figure A-0-4: Particle size distribution by intensity for O/S=40%/60% (0% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 40%/60% with 0% Vitamin E loading in the oil phase)

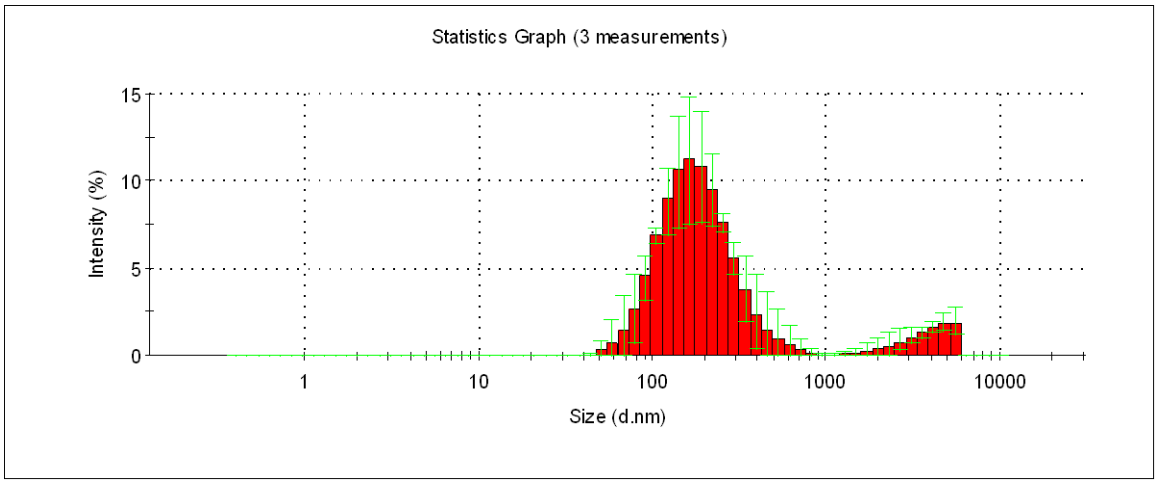


Figure A-0-5: Particle size distribution by intensity for O/S=50%/50% (0% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 50%/50% with 0% Vitamin E loading in the oil phase)

Table A-1: Average particle size for 90S10VET80GW (90%w/w Soybean oil in the oil phase + 10% w/w Vitamin E in the oil phase + Tween 80 + anhydrous glycerol + water)

Sample Name	Measurement Date and Time	T (°C)	Z-Ave (d.nm)
Water 1	08/05/2008 15:19	25	0
*O/S=10%/90% 1	08/05/2008 15:26	25	20.29
O/S=10%/90% 2	08/05/2008 15:27	25	20.06
O/S=10%/90% 3	08/05/2008 15:28	25	19.78
Water 1	08/05/2008 15:36	25	0
O/S=20%/80% 1	08/05/2008 15:42	25	81.44
O/S=20%/80% 2	08/05/2008 15:43	25	80.09
O/S=20%/80% 3	08/05/2008 15:44	25	79.26
Water 1	08/05/2008 16:14	25	0
O/S=30%/70% 1	08/05/2008 16:21	25	117.7
O/S=30%/70% 2	08/05/2008 16:22	25	116.8
O/S=30%/70% 3	08/05/2008 16:23	25	117.5
Water 1	08/05/2008 16:29	25	0
O/S=40%/60% 1	08/05/2008 16:36	25	140.2
O/S=40%/60% 2	08/05/2008 16:36	25	138.7
O/S=40%/60% 3	08/05/2008 16:37	25	141.7
Water 1	08/05/2008 16:43	25	0
O/S=50%/50% 1	08/05/2008 16:50	25	114.6
O/S=50%/50% 2	08/05/2008 16:51	25	112.4
O/S=50%/50% 3	08/05/2008 16:51	25	112.4

**O/S=10%/90% means the ratio of oil phase to the surfactant phase is 10%/90%*

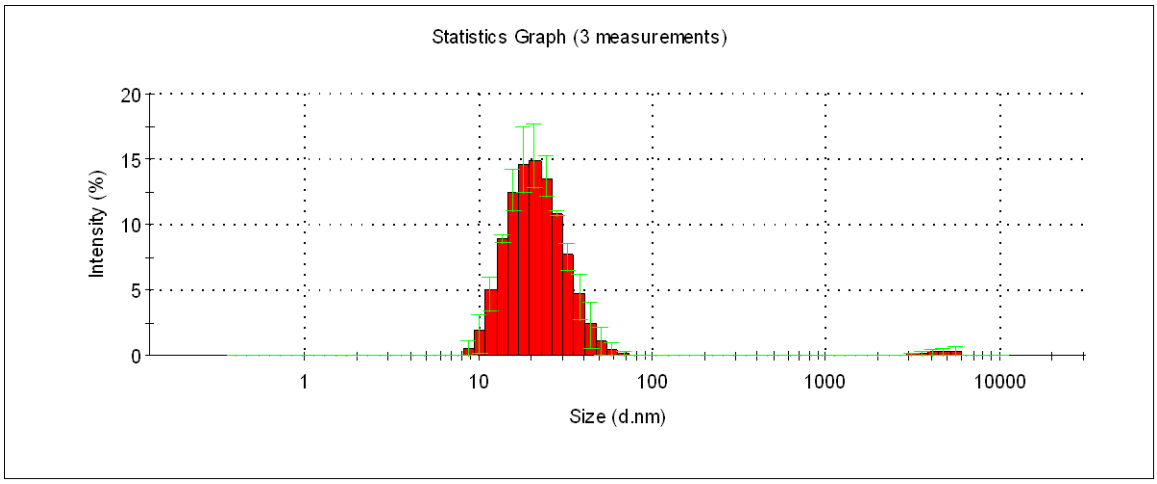


Figure A-1-1: Particle size distribution by intensity for O/S=10%/90% (10% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 10%/90% with 10% Vitamin E loading in the oil phase)

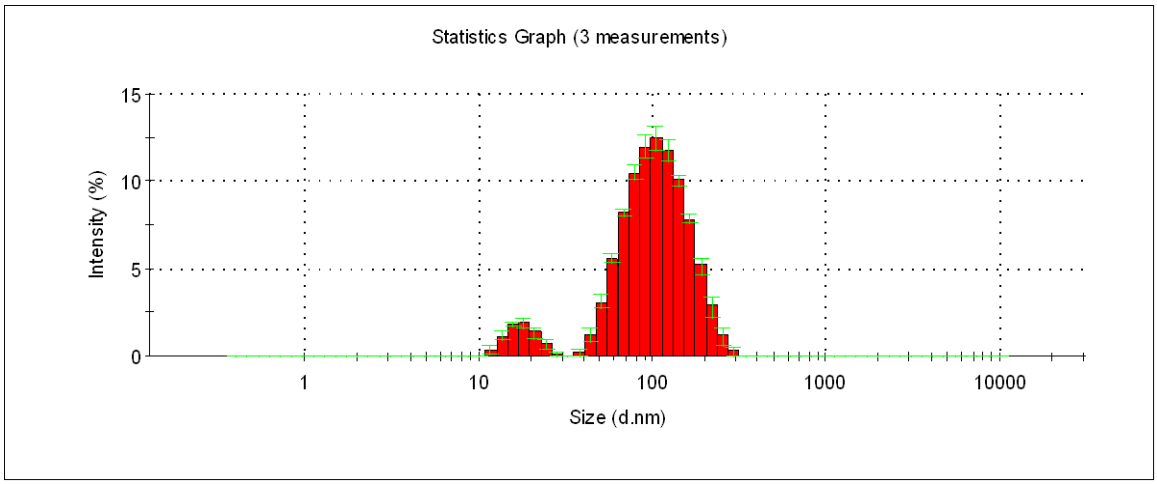


Figure A-1-2: Particle size distribution by intensity for O/S=20%/80% (10% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 20%/80% with 10% Vitamin E loading in the oil phase)

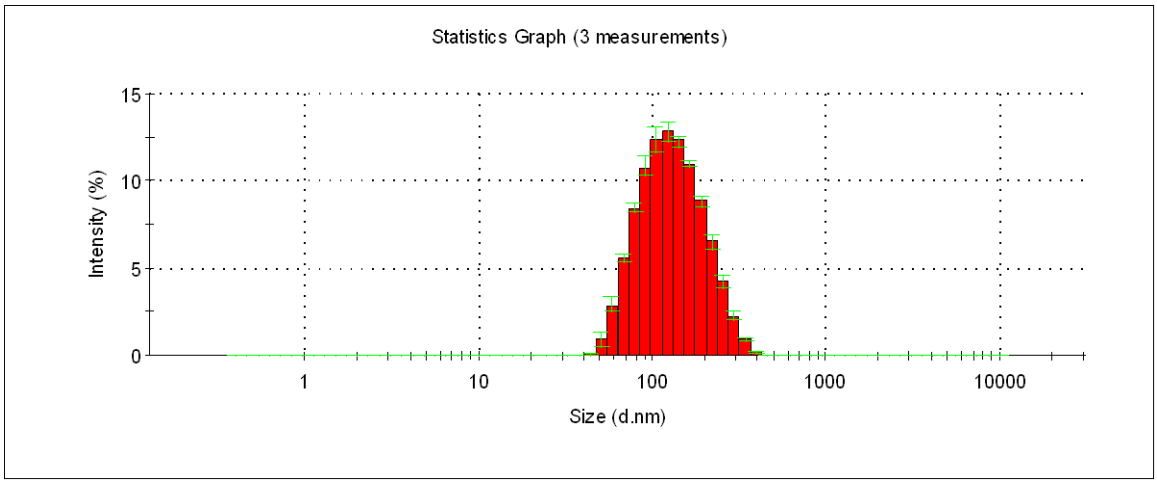


Figure A-1-3: Particle size distribution by intensity for O/S=30%/70% (10% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 30%/70% with 10% Vitamin E loading in the oil phase)

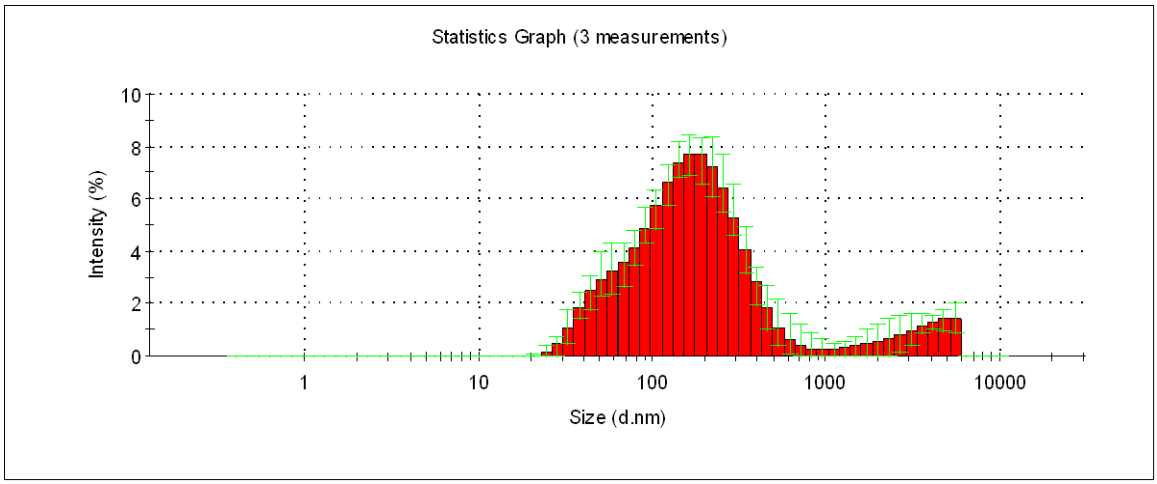


Figure A-1-4: Particle size distribution by intensity for O/S=40%/60% (10% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 40%/60% with 10% Vitamin E loading in the oil phase)

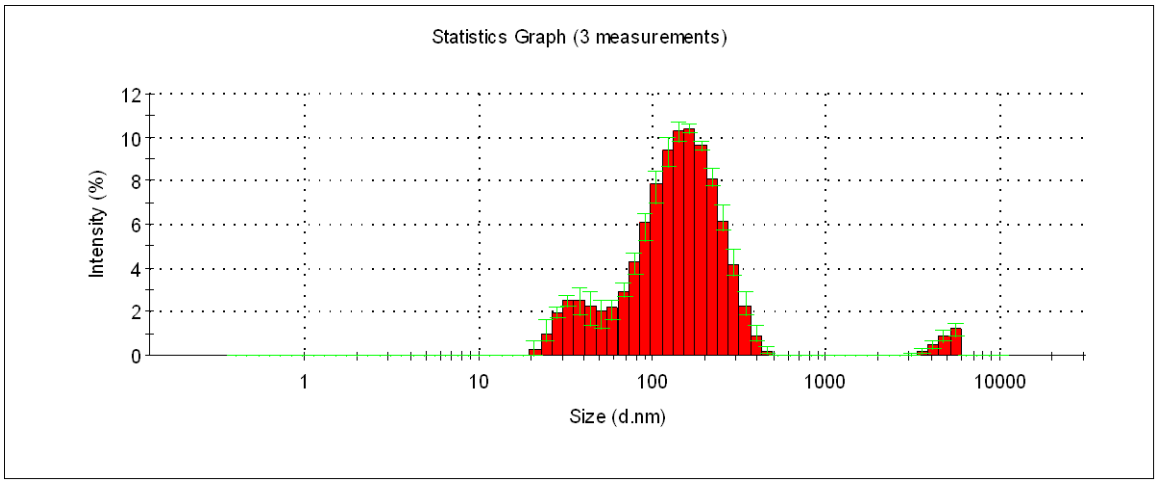


Figure A-1-5: Particle size distribution by intensity for O/S=50%/50% (10% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 50%/50% with 10% Vitamin E loading in the oil phase)

Table A-2: Average particle size for 80S20VET80GW (80%w/w Soybean oil in the oil phase + 20% w/w Vitamin E in the oil phase + Tween 80 + anhydrous glycerol + water)

Sample Name	Measurement Date and Time	T (°C)	Z-Ave (d.nm)
Water 1	15/05/2008 20:17	25	0
*O/S=10%/90%	15/05/2008 20:34	25	37.02
O/S=10%/90%	15/05/2008 20:34	25	36.46
O/S=10%/90%	15/05/2008 20:35	25	38.7
Water 1	15/05/2008 18:39	25	0
O/S=20%/80%	15/05/2008 18:55	25	48.22
O/S=20%/80%	15/05/2008 18:56	25	47.39
O/S=20%/80%	15/05/2008 18:57	25	48.31
Water 1	15/05/2008 19:03	25	0
O/S=30%/70% 1	15/05/2008 19:11	25	109.1
O/S=30%/70% 2	15/05/2008 19:12	25	107.2
O/S=30%/70% 3	15/05/2008 19:13	25	106
Water 1	15/05/2008 19:18	25	0
O/S=40%/60%	15/05/2008 19:32	25	131.4
O/S=40%/60%	15/05/2008 19:33	25	132.5
O/S=40%/60%	15/05/2008 19:34	25	128.5
Water 1	15/05/2008 19:40	25	0
O/S=50%/50% 1	15/05/2008 19:49	25	135.9
O/S=50%/50% 2	15/05/2008 19:50	25	125.7
O/S=50%/50% 3	15/05/2008 19:51	25	129.7

**O/S=10%/90% means the ratio of oil phase to the surfactant phase is 10%/90%*

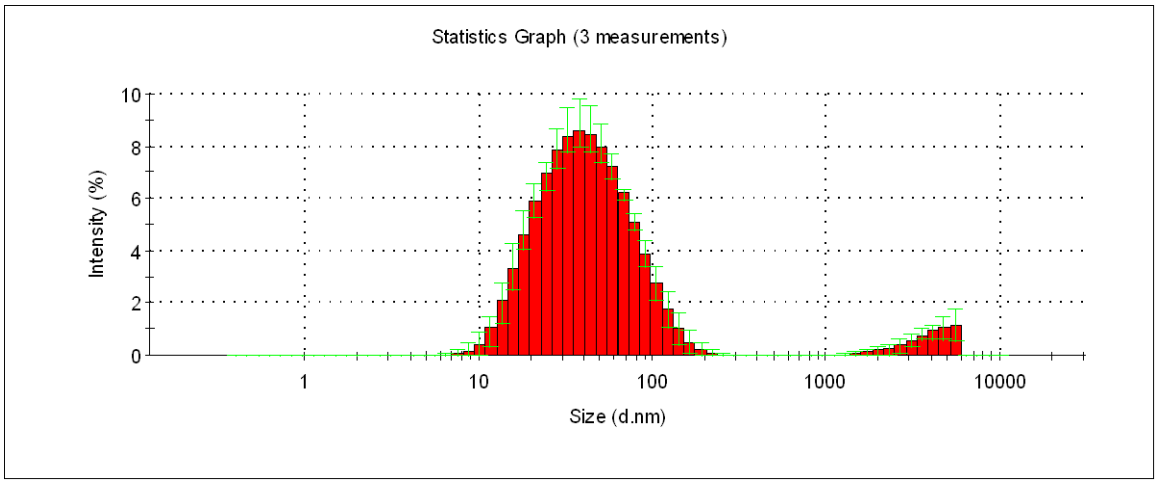


Figure A-2-1: Particle size distribution by intensity for O/S=10%/90% (20% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 10%/90% with 20% Vitamin E loading in the oil phase)

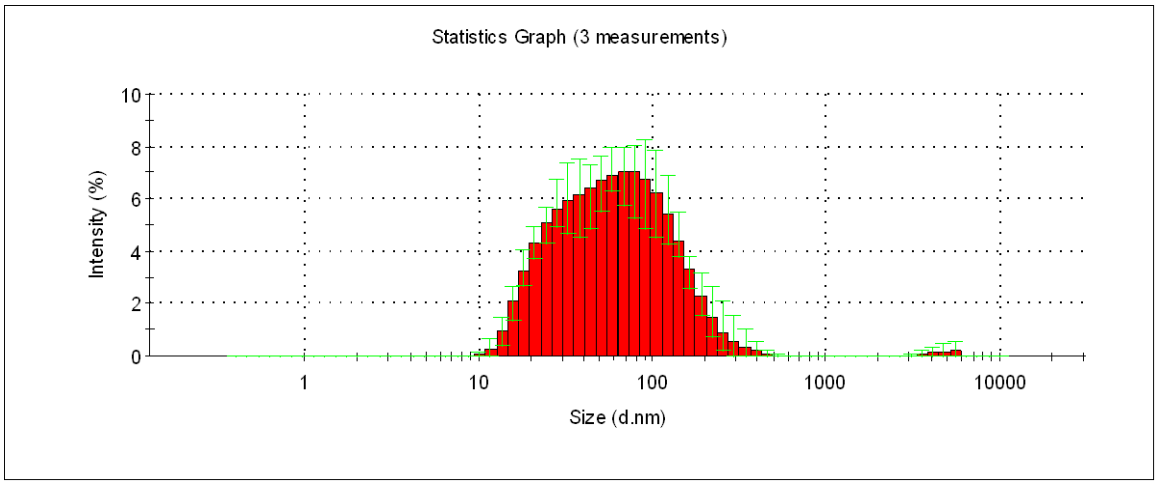


Figure A-2-2: Particle size distribution by intensity for O/S=20%/80% (20% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 20%/80% with 20% Vitamin E loading in the oil phase)

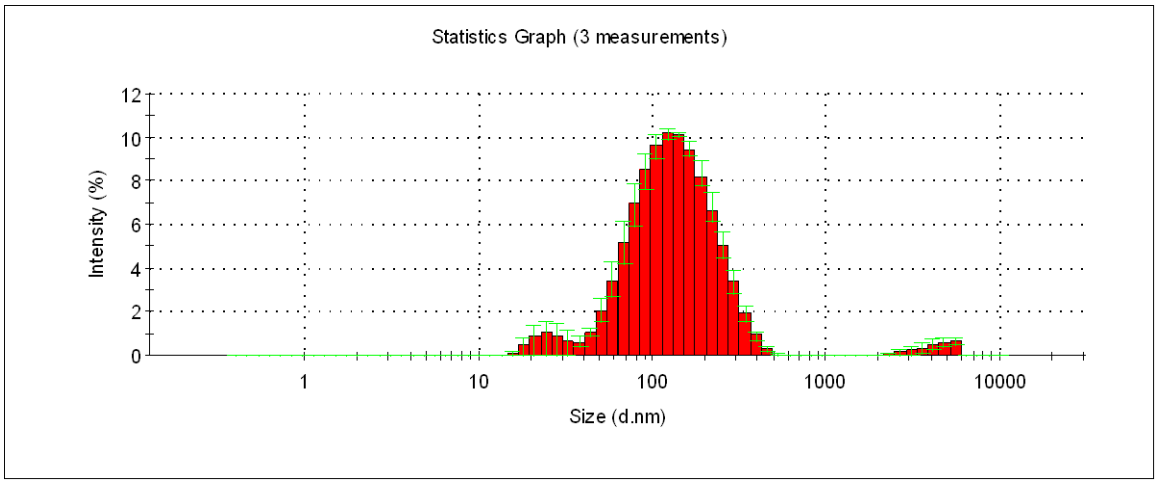


Figure A-2-3: Particle size distribution by intensity for O/S=30%/70% (20% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 30%/70% with 20% Vitamin E loading in the oil phase)

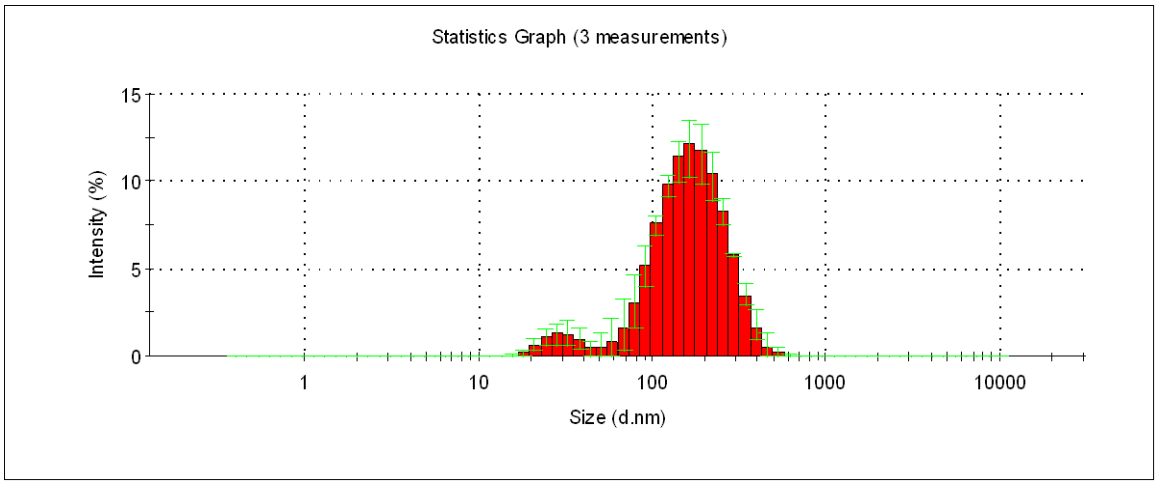


Figure A-2-4: Particle size distribution by intensity for O/S=40%/60% (20% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 40%/60% with 20% Vitamin E loading in the oil phase)

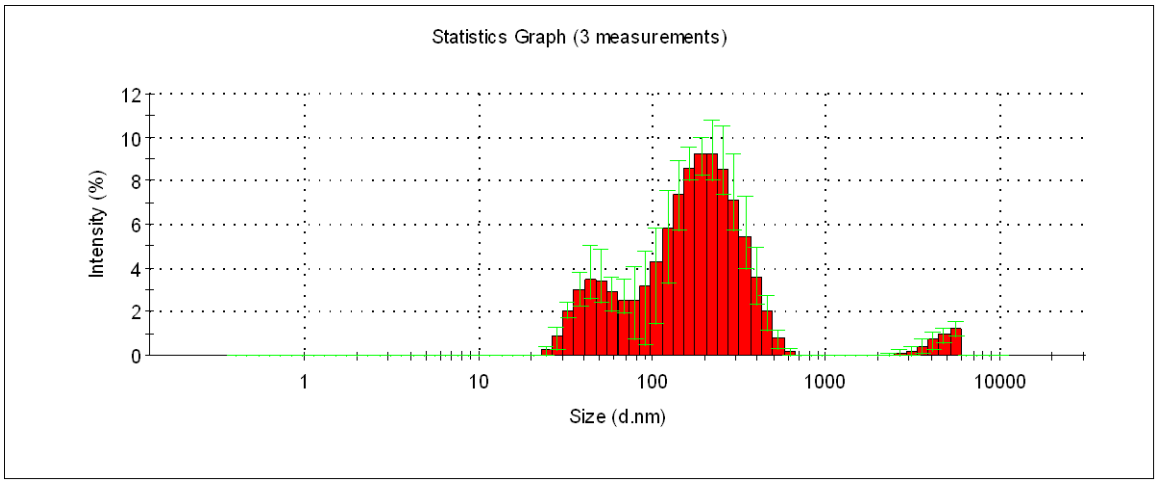


Figure A-2-5: Particle size distribution by intensity for O/S=50%/50% (20% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 50%/50% with 20% Vitamin E loading in the oil phase)

Table A-3: Average particle size for 70S30VET80GW (70%w/w Soybean oil in the oil phase + 30% w/w Vitamin E in the oil phase + Tween 80 + anhydrous glycerol + water)

Sample Name	Measurement Date and Time	T (°C)	Z-Ave (d.nm)
Water 1	16/05/2008 14:15	25	0
*O/S=10%/90% 1	16/05/2008 14:32	25	34.21
O/S=10%/90% 2	16/05/2008 14:33	25	18.35
O/S=10%/90% 3	16/05/2008 14:34	25	26.9
Water 1	07/02/2008 11:10	25	0
O/S=20%/80% 1	07/02/2008 11:51	25	26.02
O/S=20%/80% 2	07/02/2008 11:53	25	28.83
O/S=20%/80% 3	07/02/2008 11:55	25	28.82
Water 1	07/02/2008 13:36	25	0
O/S=30%/70% 1	07/02/2008 13:44	25	79.01
O/S=30%/70% 2	07/02/2008 13:46	25	78.37
O/S=30%/70% 3	07/02/2008 13:48	25	80.81
Water 1	07/02/2008 14:14	25	0
O/S=40%/60% 1	07/02/2008 14:21	25	98.83
O/S=40%/60% 2	07/02/2008 14:23	25	123.2
O/S=40%/60% 3	07/02/2008 14:25	25	106.7
Water 1	07/02/2008 14:32	25	0
O/S=50%/50% 1	07/02/2008 14:38	25	155.8
O/S=50%/50% 2	07/02/2008 14:40	25	151.6
O/S=50%/50% 3	07/02/2008 14:42	25	149.4

**O/S=10%/90% means the ratio of oil phase to the surfactant phase is 10%/90%*

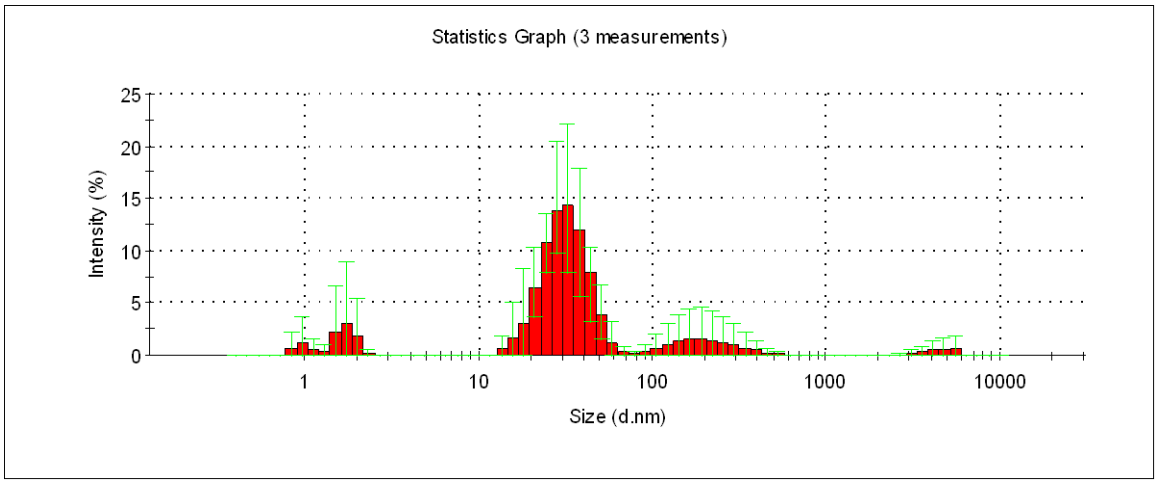


Figure A-3-1: Particle size distribution by intensity for O/S=10%/90% (30% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 10%/90% with 30% Vitamin E loading in the oil phase)

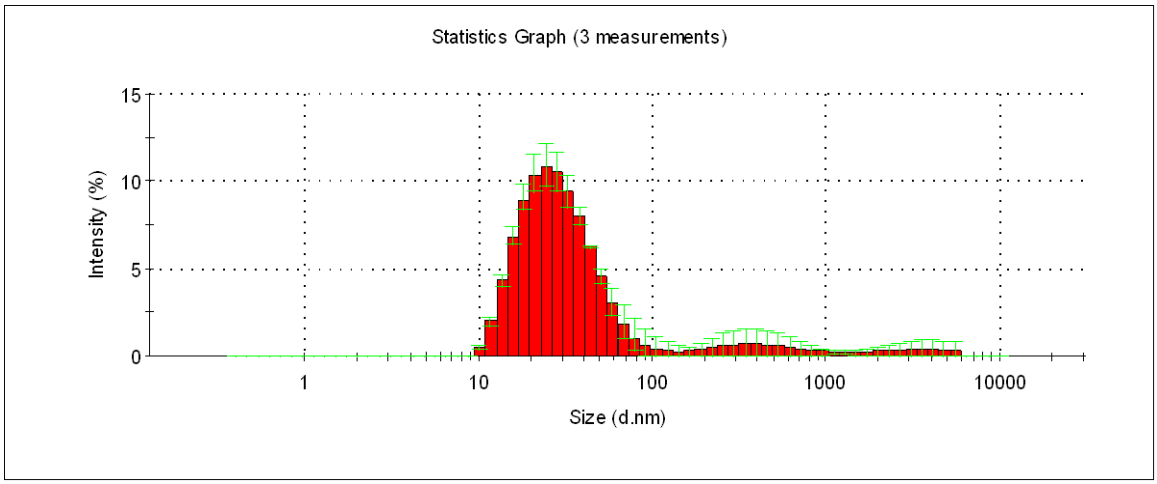


Figure A-3-2: Particle size distribution by intensity for O/S=20%/80% (30% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 20%/80% with 30% Vitamin E loading in the oil phase)

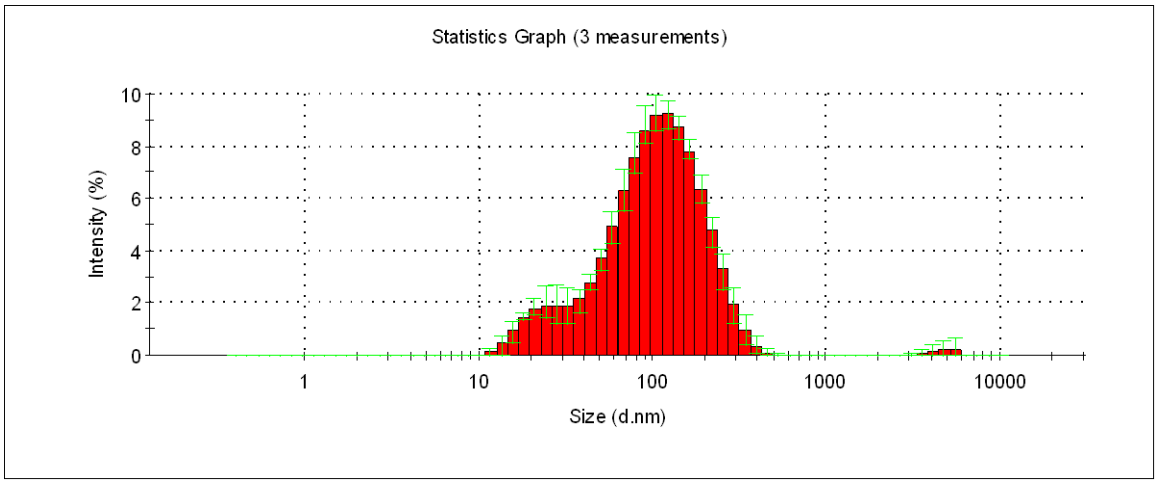


Figure A-3-3: Particle size distribution by intensity for O/S=30%/70% (30% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 30%/70% with 30% Vitamin E loading in the oil phase)

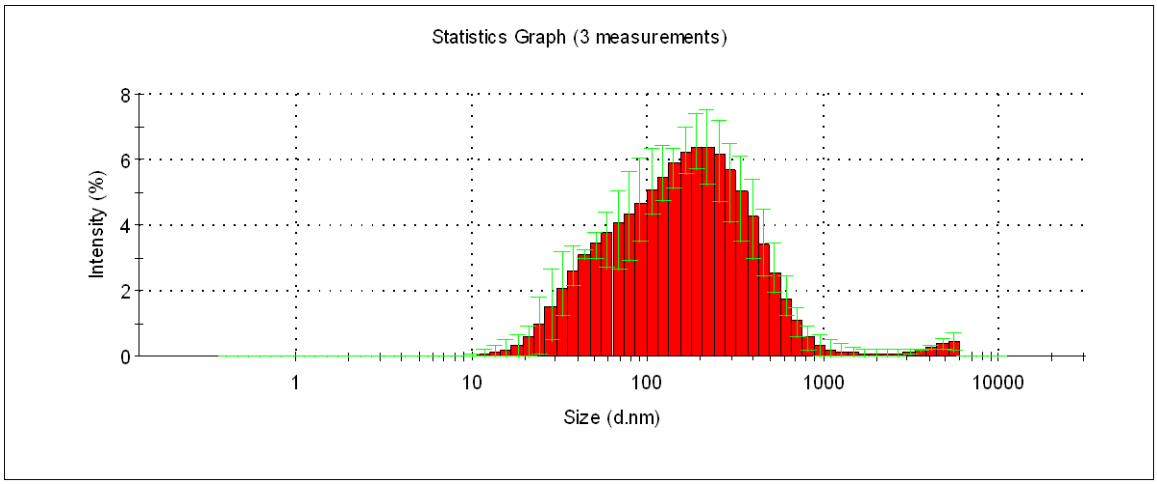


Figure A-3-4: Particle size distribution by intensity for O/S=40%/60% (30% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 40%/60% with 30% Vitamin E loading in the oil phase)

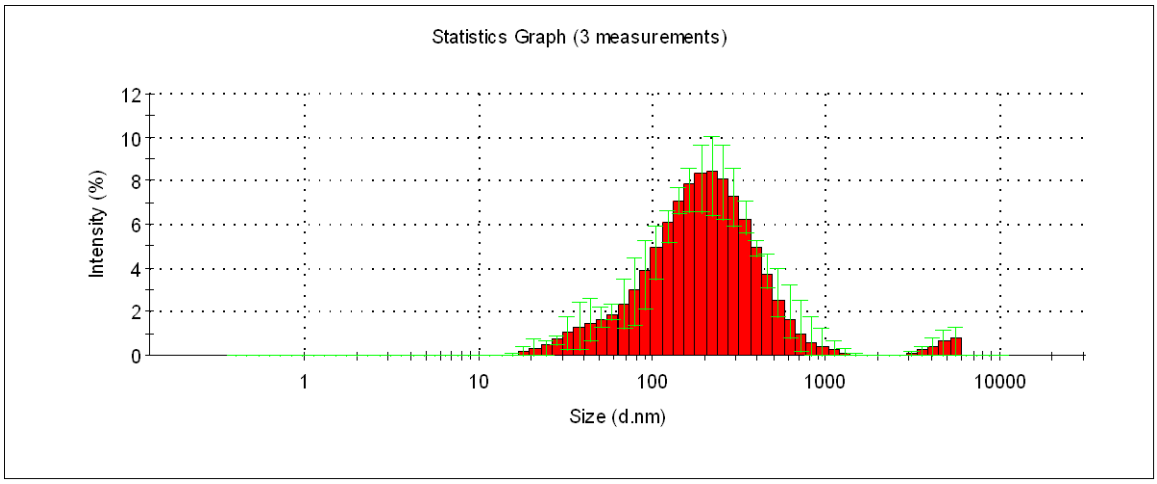


Figure A-3-5: Particle size distribution by intensity for O/S=50%/50% (30% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 50%/50% with 30% Vitamin E loading in the oil phase)

Table A-4: Average particle size for 60S40VET80GW (60%w/w Soybean oil in the oil phase + 40% w/w Vitamin E in the oil phase + Tween 80 + anhydrous glycerol + water)

Sample Name	Measurement Date and Time	T (°C)	Z-Ave (d.nm)
Water 1	20/10/2008 14:44	25	0
*O/S=10%/90% 1	20/10/2008 14:56	25	16.42
O/S=10%/90% 2	20/10/2008 14:58	25	15.78
O/S=10%/90% 3	20/10/2008 15:00	25	16.37
Water 1	19/05/2008 14:15	25	0
O/S=20%/80% 1	19/05/2008 14:22	25	73.83
O/S=20%/80% 2	19/05/2008 14:23	25	77.2
O/S=20%/80% 3	19/05/2008 14:24	25	77.25
Water 1	28/02/2008 15:00	25	0
O/S=30%/70% 1	28/02/2008 15:08	25	94.36
O/S=30%/70% 2	28/02/2008 15:10	25	93.62
O/S=30%/70% 3	28/02/2008 15:12	25	93.77
Water 1	28/02/2008 15:41	25	0
O/S=40%/60% 1	28/02/2008 15:48	25	115
O/S=40%/60% 2	28/02/2008 15:50	25	111.6
O/S=40%/60% 3	28/02/2008 15:52	25	106
Water 1	28/02/2008 15:59	25	0
O/S=50%/50% 1	28/02/2008 16:06	25	126.2
O/S=50%/50% 2	28/02/2008 16:08	25	124.1
O/S=50%/50% 3	28/02/2008 16:10	25	122.9

**O/S=10%/90% means the ratio of oil phase to the surfactant phase is 10%/90%*

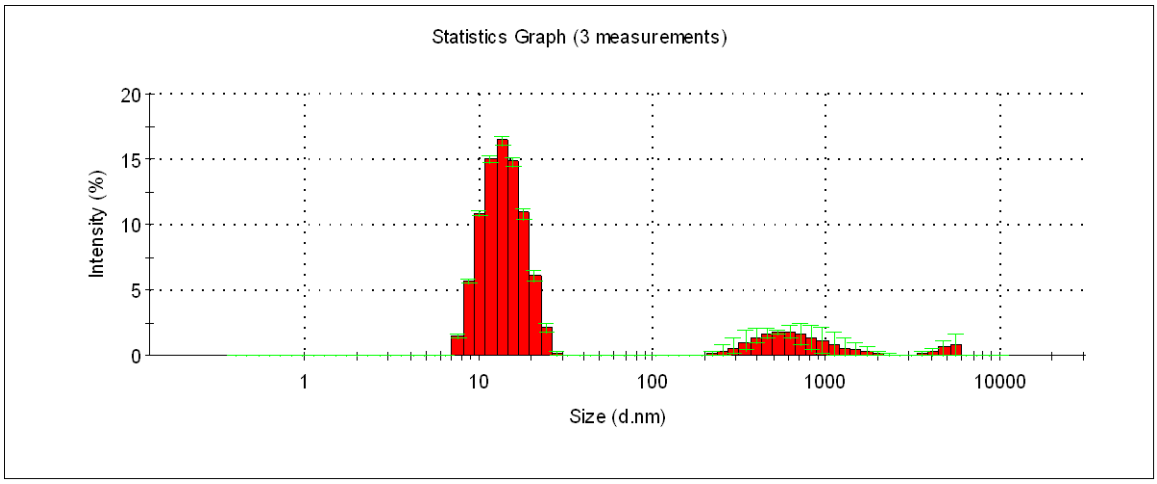


Figure A-4-1: Particle size distribution by intensity for O/S=10%/90% (40% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 10%/90% with 40% Vitamin E loading in the oil phase)

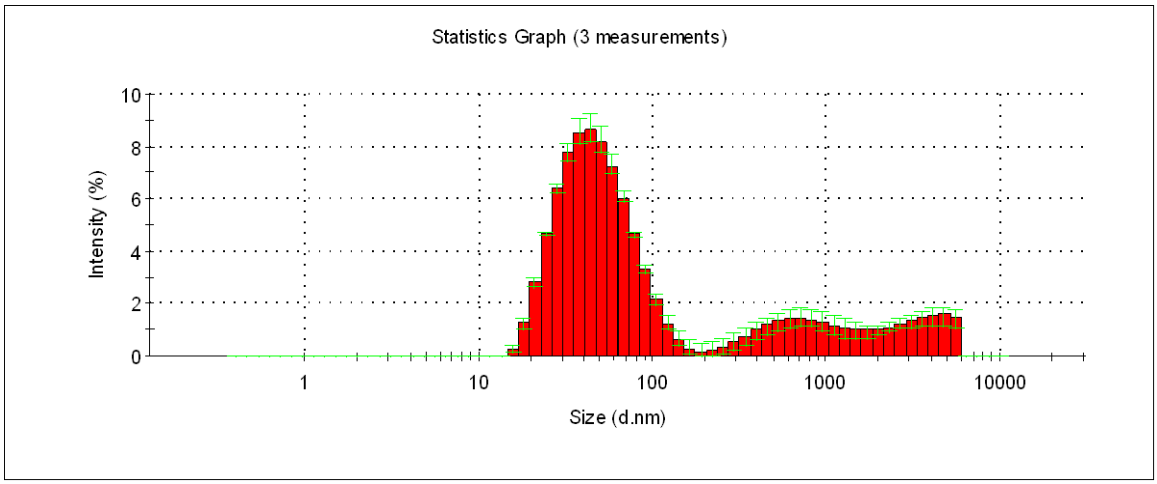


Figure A-4-2: Particle size distribution by intensity for O/S=20%/80% (40% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 20%/80% with 40% Vitamin E loading in the oil phase)

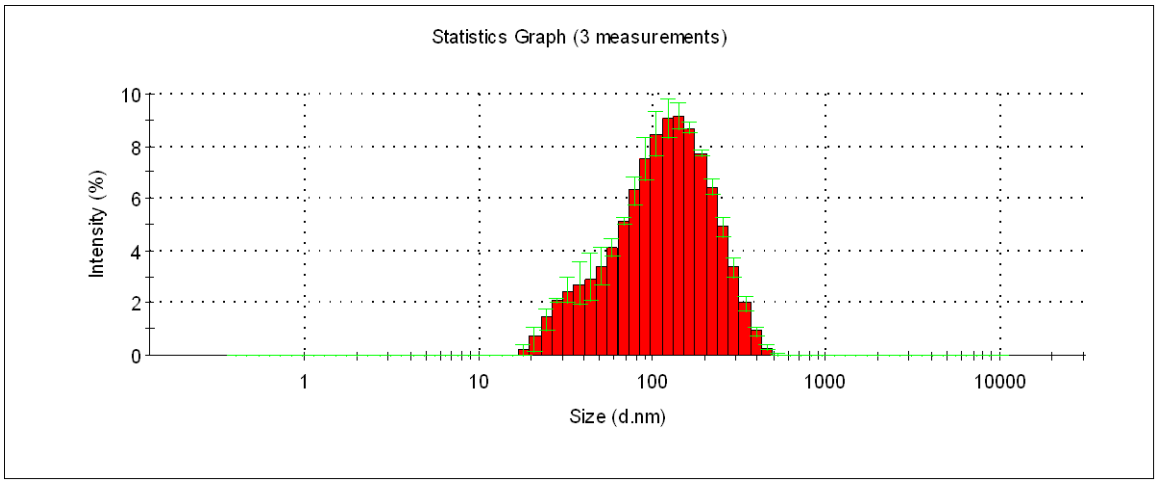


Figure A-4-3: Particle size distribution by intensity for O/S=30%/70% (40% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 30%/70% with 40% Vitamin E loading in the oil phase)

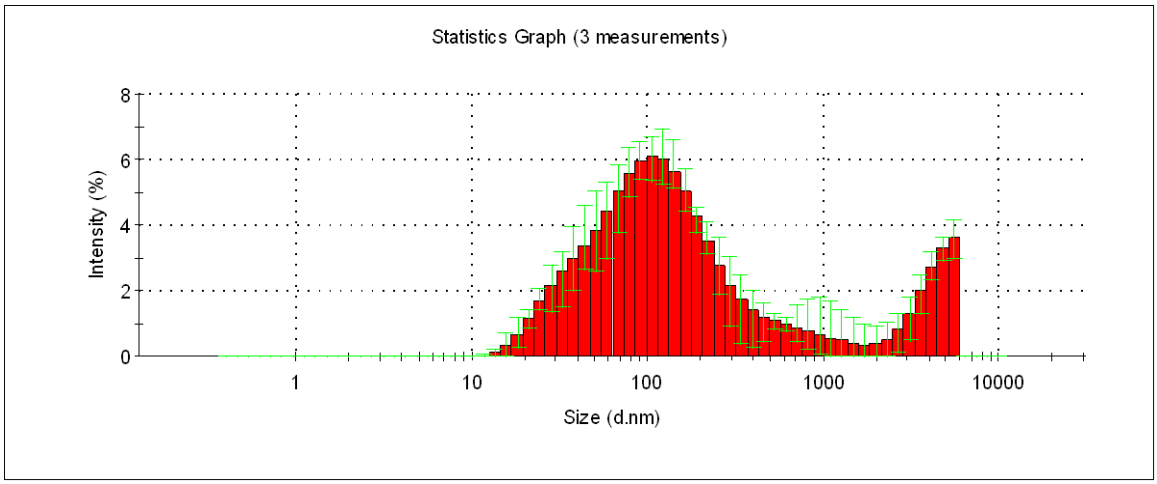


Figure A-4-4: Particle size distribution by intensity for O/S=40%/60% (40% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 40%/60% with 40% Vitamin E loading in the oil phase)

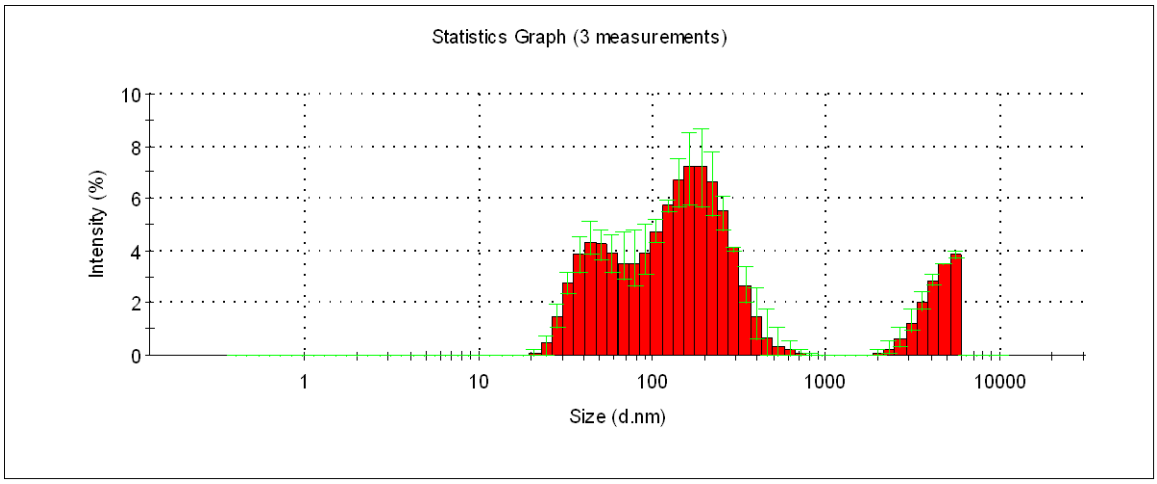


Figure A-4-5: Particle size distribution by intensity for O/S=50%/50% (40% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 50%/50% with 40% Vitamin E loading in the oil phase)

Table A-5: Average particle size for 50S50VET80GW (50%w/w Soybean oil in the oil phase + 50% w/w Vitamin E in the oil phase + Tween 80 + anhydrous glycerol + water)

Sample Name	Measurement Date and Time	T (°C)	Z-Ave (d.nm)
Water 1	22/02/2008 13:13	25	0
*O/S=10%/90%	22/02/2008 13:20	25	58.58
O/S=10%/90%	22/02/2008 13:22	25	81.11
O/S=10%/90%	22/02/2008 13:24	25	64.78
Water 1	22/02/2008 13:34	25	0
O/S=20%/80% 1	22/02/2008 13:40	25	83.62
O/S=20%/80% 2	22/02/2008 13:42	25	84.1
O/S=20%/80% 3	22/02/2008 13:44	25	123.9
Water 1	22/02/2008 14:15	25	0
O/S=30%70% 1	22/02/2008 14:22	25	119.9
O/S=30%70% 2	22/02/2008 14:24	25	117.7
O/S=30%70% 3	22/02/2008 14:26	25	111.1
Water 1	22/02/2008 14:31	25	0
O/S=40%/60% 1	22/02/2008 14:37	25	109.1
O/S=40%/60% 2	22/02/2008 14:39	25	96.5
O/S=40%/60% 3	22/02/2008 14:41	25	93.5
Water 1	22/02/2008 14:57	25	0
O/S=50%/50% 1	22/02/2008 15:03	25	127.4
O/S=50%/50% 2	22/02/2008 15:05	25	120
O/S=50%/50% 3	22/02/2008 15:07	25	124.5

**O/S=10%/90% means the ratio of oil phase to the surfactant phase is 10%/90%*

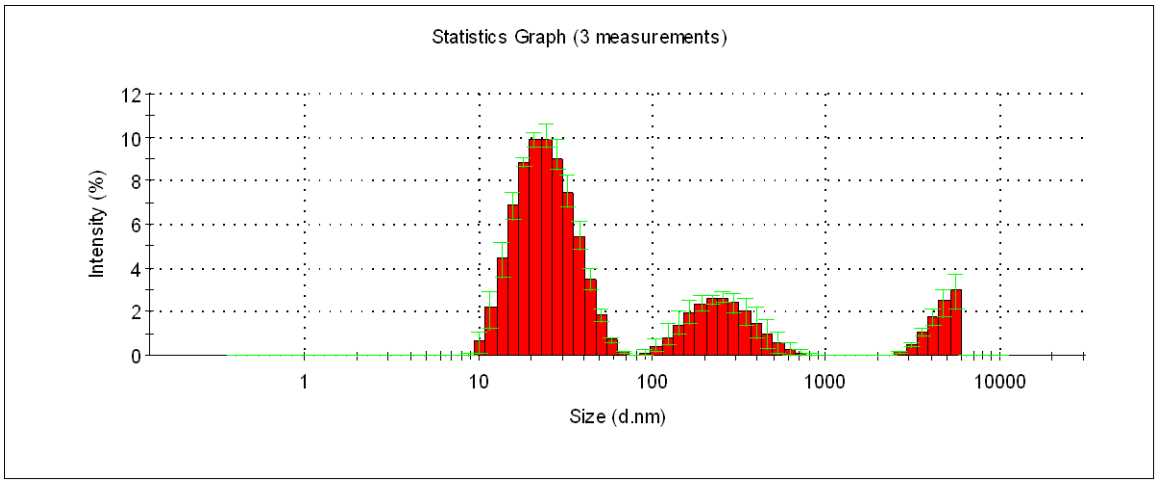


Figure A-5-1: Particle size distribution by intensity for O/S=10%/90% (50% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 10%/90% with 50% Vitamin E loading in the oil phase)

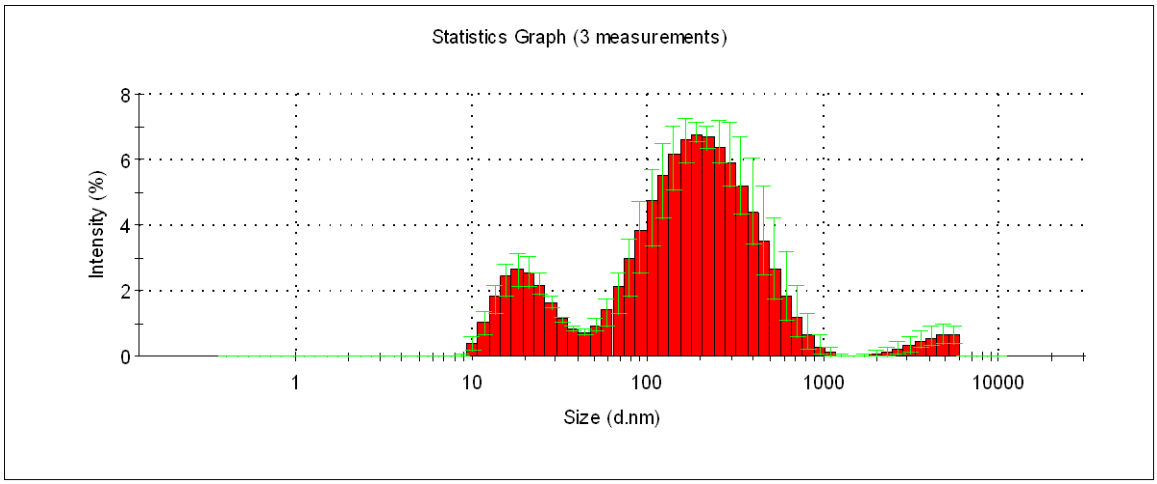


Figure A-5-2: Particle size distribution by intensity for O/S=20%/80% (50% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 20%/80% with 50% Vitamin E loading in the oil phase)

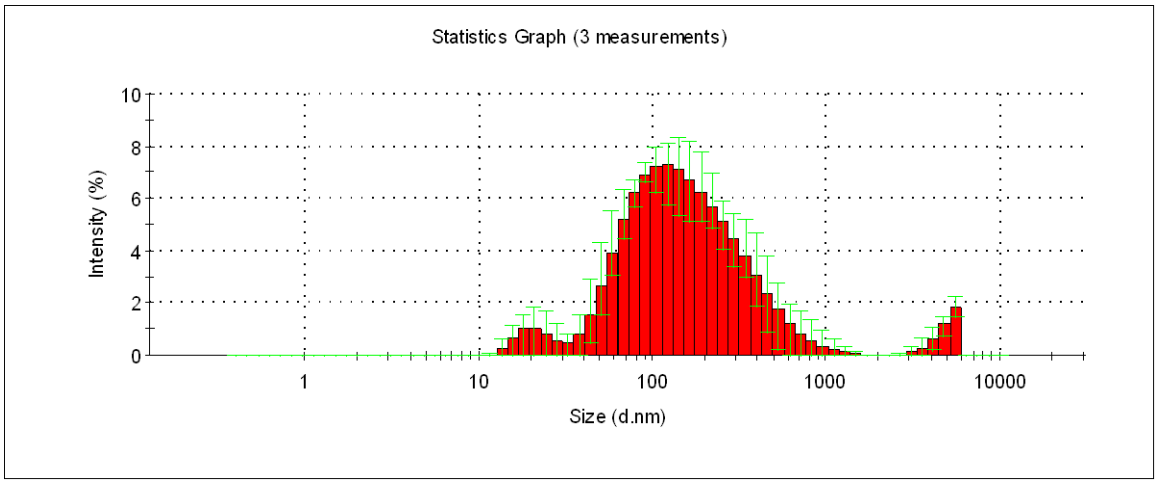


Figure A-5-3: Particle size distribution by intensity for O/S=30%/70% (50% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 30%/70% with 50% Vitamin E loading in the oil phase)

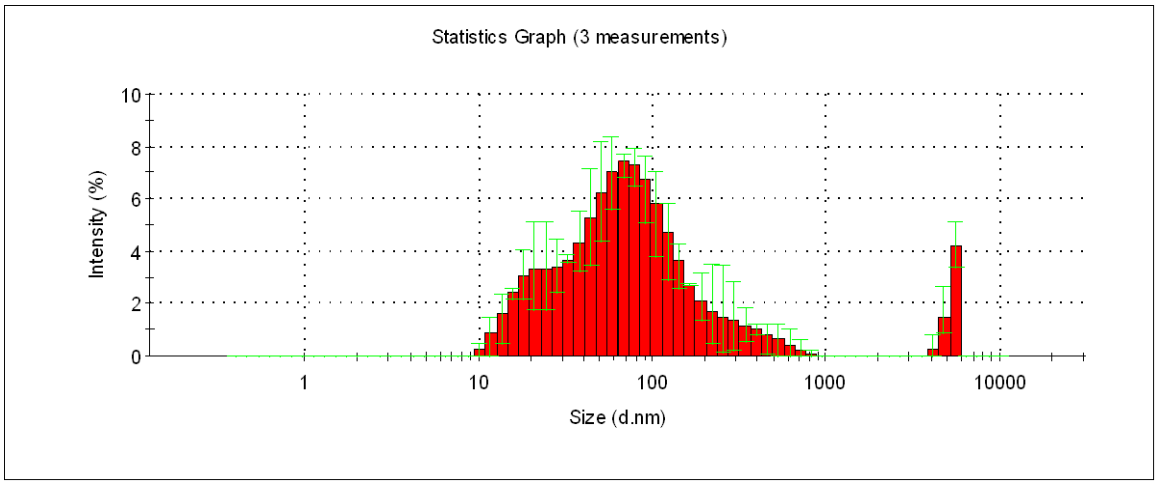


Figure A-5-4: Particle size distribution by intensity for O/S=40%/60% (50% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 40%/60% with 50% Vitamin E loading in the oil phase)

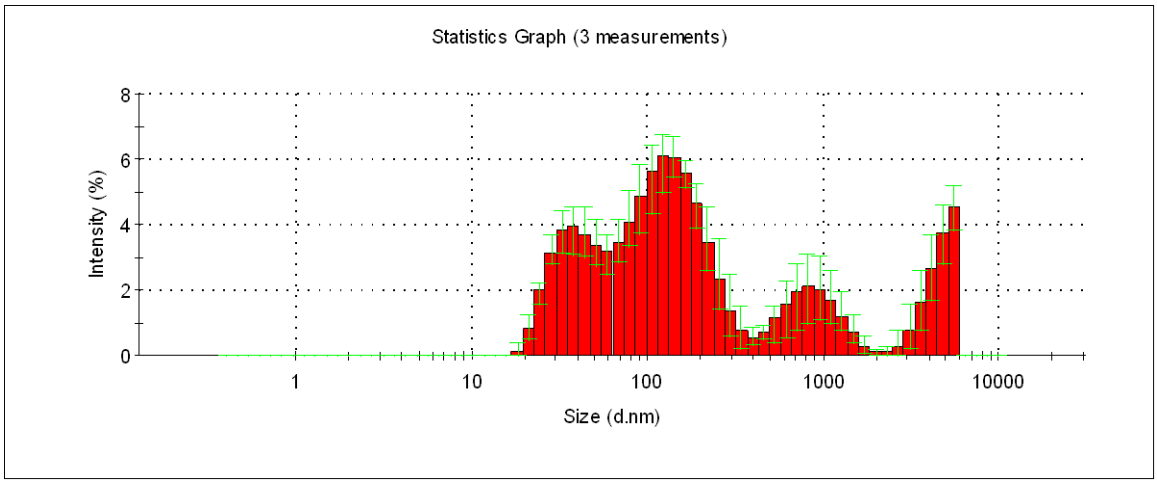


Figure A-5-5: Particle size distribution by intensity for O/S=50%/50% (50% w/w Vitamin E loading) (the ratio of the oil phase to the surfactant phase is 50%/50% with 50% Vitamin E loading in the oil phase)

Appendix B Data for the Phase Inversion

Table B-1-1: Dial reading value for 10%O (0% Vitamin E loading)/90%S (the ratio of oil phase to the surfactant phase is 10%/90% with 0% w/w Vitamin E loading in the oil phase)

Water (%)	Dial Reading	Water(g)
0	84	0
0.032258	62	1
0.062793	43	2.01
0.091460	30	3.02
0.118166	40	4.02
0.143347	52	5.02
0.167129	56	6.02
0.189627	44	7.02
0.211149	34	8.03
0.231361	28	9.03
0.250562	22	10.03
0.269362	16	11.06
0.286733	10	12.06

Table B-1-2: Dial reading value for 20%O (0% Vitamin E loading)/80%S (the ratio of oil phase to the surfactant phase is 20%/80% with 0% w/w Vitamin E loading in the oil phase)

Water (%)	Dial reading	Water(g)
0	47	0
0.047619	28	1
0.090909	42	2
0.130435	96	3
0.167707	56	4.03
0.202234	36	5.07
0.232835	28	6.07
0.261175	20	7.07
0.287496	10	8.07

Table B-2-1: Dial reading value for 10%O (30% Vitamin E loading)/90%S (the ratio of oil phase to the surfactant phase is 10%/90% with 30% w/w Vitamin E loading in the oil phase)

Water (%)	Dial Reading	Water (g)
0	84	0
0.032258	64	1
0.062500	57	2
0.090909	52	3
0.117647	95	4
0.143591	50	5.03
0.166667	208	6
0.189189	152	7
0.210526	62	8
0.231360	38	9.03
0.250562	24	10.03
0.268828	18	11.03
0.303783	14	13.09
0.319574	10	14.09

Table B-1-2: Dial reading value for 20%O (30% Vitamin E loading)/80%S (the ratio of oil phase to the surfactant phase is 20%/80% with 30% w/w Vitamin E loading in the oil phase)

Water (%)	Dial Reading	Water (g)
0	48	0
0.048525	52	1.02
0.090909	54	2
0.130435	70	3
0.166667	148	4
0.200000	78	5
0.230769	68	6
0.259259	52	7
0.285714	26	8
0.310345	18	9
0.333333	10	10

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