UNIVERSITI PUTRA MALAYSIA

FORMULATION AND PARAMETRIC STUDY OF LOW ENERGY PROCESS FOR PASSION FRUIT OIL NANOEMULSION

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ABSTRACT

In the growing demand for products with natural active ingredients, passion fruit seed oil has been selected for producing nanoemulsion using low energy spontaneous emulsification approach in this study. Previous research has shown that long chain triglycerides are difficult to produce nanoemulsion using low energy method and the application of different types of food grade surfactants is less explored. In order to successfully produce nanoemulsion with low energy approach, the balance between surfactants, water and oil are important to allow low interfacial tension of oil-water interface that promote the formation of small particle size. The study examined the used of combinations of food-grade surfactants namely Tween 80 (T80), sucrose ester (SE), lecithin (LCT) and polyglycerol polyricinoleate (PGPR) in lowering the interfacial tension (IFT) and in producing nanoemulsions of passion fruit seed oil. It was found out that IFT at ratio of 100:0 to 80:20 for combination of SE:LCT and SE:PGPR with concentration up to 1% is the lowest and hence is more likely to result in nanoemulsion. Then, ratio between surfactants that result is smallest particle size was found to be 336 nm for SE:LCT at 75:25 and SE:PGPR at 90:10 result in 493.4nm. Although the combination between SE:LCT and SE:PGPR result in similar HLB, the particle size formed is different possibly due to different packing geometry with different combination of surfactants. Further investigation is carried out on the effect of surfactants concentration and oil concentration on the emulsion produced. The optimum concentration for both surfactants and oil is important in order to achieve nano size. Thus, 5% concentration of surfactants found to be most optimum for SE:LCT and 2.5% concentration for SE:PGPR. Concentration of PGPR required is lesser compared to that of lecithin due to the fact that PGPR is bigger

molecule. Variation of process parameter including mixing temperature, stirring speed and oil dropping rate showed less significant effect in reducing the particle size of the emulsion produced. It is being concluded that focus should be given on chemical formulation instead of process parameters in low energy spontaneous emulsification approach. Overall, the smallest particle size was found to be 246.4nm on the formulation of 5% concentration of sucrose ester:lecithin at ratio 75:25 with 5% passion fruit oil at 50°C mixing temperature along with 500rpm stirring speed and 1drop/20seconds oil dropping rate.

ABSTRAK

Permintaan produck kosmetik yang mengandungi bahan aktif semula jadi semakin meningkat. Oleh itu, minyak biji buah markisa telah dipilih untuk menghasiklan nanoemulsi menggunakan pendekatan tenaga rendah iaitu pengemulsi spontan dalam kajian ini. Penyelidikan yang lepas telah menunjukkan bahawa trigliserida rantai panjang sukar digunakan untuk menghasilkan nanoemulsi dengan kaedah tenaga rendah. Selain itu, kajian mengenai pengguna pelbagai jenis surfaktan kelas makanan adalah terhad. Keseimbangan antara surfaktan, air dan minyak sangat penting untuk merendahkan ketegangan antara permukaan minyak-air bagi mendorong penghasilan nanoemulsi yang berjaya dengan zarah yang kecil. Kajian ini mengaji pengunna surfaktan dalam menurunkan ketegangan antara permukaan air dan minyak pada kepekatan dan nisbah gabungan surfaktan yang berbeza. Dengan kepakatan lebih daripada 1%, nisbah 100:0 hingga 80:20 didapati lebih cenderung dalam pengahsilan naoemulsi. Dengan ini, zarah yang terkecil didapati adalah 336nm utuk SE:LCT dalam nisbah 75:25 dan 493.4nm bagi SE:PGPR dalam 90:10. Walaupun gabungan antara SE:LCT dan SE:PGPR menghasiklan HLB yang serupa, saiz zarah yang dihasilkan adalh berbeza disebabkan oleh geometri pengaturan yang berbeza bagi kombinasi masing-masing. Penyelidikan selanjutnya dijalankan pada pengaruh kepekatan surfaktan and kepekatan munyak pada emulsi yang dihasilakn. Kepakatan optimum harus dikenalpasti bagi menjayakan hasilan nanoemulsi. Dengan ini, kepekatan surfaktan yang paling optimum bagi SE:LCT adalah 5% dan 2.5% bagi SE:PGPR. Adalah didapati bahawa kepekatan PGPR yang diperlukan lebih rendah berbanding dengan LCT disebabkan PGPR adalh molekul yang lebih besar daripada LCT.

Pengoptimuman parameter proses termasuk suhu pencampuran, kecepatan pengadukan dan kadar penurunan minyak adalah kurang ketara terhadap penurunan saiz zarah emulsi. Oleh itu, fokus harus diberikan pada formulasi kimia bukannya parameter proses dalam aplikasi pengemulsi spontan tenaga rendah. Secara keseluruhannya, saiz zarah terkecil didapati adalah 246.4nm dengan menggunakan formulasi 5% kepekatan sucrose ester:lecithin pada nisbah 75:25 serta 5% minyak buah markisa pada suhu pencampuran 50°C selari dengan kelajuan pengadukan 500rpm dan kadar penurunan minyak pada 1titik dalam 20 saat.

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LIST OF ABBREVIATIONS

VO	Vegetable Oil
PFO	Passion Fruit Oil
HLB	Hydrophilic Lipophilic Balance
CMC	Critical Micelle Concentration
IFT	Interfacial Tension
SE	Sucrose Ester
T80	Tween 80
LCT	Lecithin
PGPR	Polyglycerol Polyricinoleate
PDI	Polydispersity Index
SOW	Surfactant to Oil to Water
Conc.	Concentration
Avg.	Average

CHAPTER 1

INTRODUCTION

1.1 Background

Passion fruits seed oil are one of the popular raw materials for natural consumer products giving important sources of vitamins, minerals, essential fatty acids, antioxidants and UV protection efficacy. The use of fruit seed oils in food and personal care products is of significance to both their function and image. The seed of passion fruit has been widely used as a multifunctional ingredient in certain industries including cosmetics owing to its potent antioxidant activity and UV protection efficacy, which are synergistic in photoaging protection. The antioxidant activity of the seed extract is comparable to that of ascorbic acid, owing to a high content of health beneficial phenolic. Moreover, passion fruit seed extract has been used as a natural skin-lightening agent owing to its tyrosinase inhibitory effect (Leão, Sampaio, Pagani, & Da Silva, 2014). Passion fruit seed oil rich in essential fatty acids and nutrients that are vital for general health and makes it a valuable source products that aim on nourishing and improving skin conditions.

Nanoemulsion have been extensively studied on its perspective as delivery system for pharmaceutical and cosmetic products. Nanoemulsion are colloidal dispersions made up of two phases, (an oil phase and an aqueous phase) with the help of surfactants at accurate proportions result in droplet size at the range of 5 to 200nm. It is kinetically stable, optically clear and transparent (Azmi et al., 2019a). Nanoemulsion when compared to microemulsion has reduced gravity force and Brownian motion averted the destabilization of the system hence contribute to zero sedimentation during storage. Other

destabilization factors such as flocculation and Ostwald ripening can be prevented by the minuscule size of nanoemulsion help in prolong shelf life of products (Tadros, Izquierdo, Esquena, & Solans, 2004). In fact, two main uses of nanoparticles in cosmetic products have been focused on UV filtering and delivery of active ingredients, through encapsulation technology to transport a wide range of beneficial ingredients.

Nanoemulsion offers many advantages for the food, pharmaceutical and cosmetic industry including a high surface-to-volume ratio that promote penetration of the products and protect it from chemical and physical instability Nanoemulsion exhibits better penetration efficacy due to its nano size allowing efficient absorption of the products. There is an increasing demand for skin care nanoemulsion result from the ability in controlling the delivery and dispersion of active ingredients to deeper layers of skin. The major benefits of using nanotechnology are the enhanced stability of various ingredients, unsaturated fatty acids, vitamins, or antioxidants encapsulated within the nanoparticles, improved penetration rate of certain ingredients, such as vitamins and other antioxidants, improved aesthetics of the product (Mu & Sprando, 2010).

There are two methods to prepare nanoemulsion which are high energy method and low energy method. High energy method use mechanical devices such as high pressure homogenizer that generate immense forces in forming very fine droplets while low energy method involve complex interfacial hydrodynamic phenomena and depends on the composition properties (Hadžiabdiü Jasmina, Orman Džana, Elezoviü Alisa & Vraniü Edina, 2017). High energy method is unfavorable to be used for thermolabile drugs and macromolecules such as protein, enzymes, retinoid, peptides and nucleic acids due to high temperature and pressure used cause damage on the composition in the

components. Low energy methods are more favorable due to lower cost and ease of implementation. Therefore, in this study, low energy spontaneous emulsification method was studied for the nanoemulsion of passion fruit seed oil.

Low energy method utilizes the low interfacial tension property in the system to reduce droplet size with energy input by a magnetic stirrer and provide an easy yet scalable route to make nanoemulsion without excess shear (Gupta, Badruddoza, & Doyle, 2017). However, due to its bulky properties, the use of low energy method to produce nanoemulsion from the triglyceride of passion fruit seed oil was very difficult. Previous research have shown that the success to produce nanoemulsion from triglycerides is highly dependent on the correct surfactant structure that allows the interface to achieve an ultralow interfacial tension (IFT) values (Witthayapanyanon, Acosta, Harwell, & Sabatini, 2006). One of the successful surfactant is an extended surfactant type which generally has much lower critical micelle concentration (CMC) value than conventional type surfactants and large solubilization parameters associated help in produce ultralow IFT (Miñana-Perez, Graciaa, Lachaise, & Salager, 1995). However, this surfactant is not suitable for food and cosmetics application due to its toxicity. Another probable method is to combine surfactant with high HLB (hydrophilic lipophilic balance) with that of a low HLB. HLB is an indicator that quantifies the balance between the hydrophilic group's capacity of attracting water and the lipophilic group's capacity of attracting oil (Nakama, 2017). Several researchers found that surfactant mixtures could provide more stable emulsion with minimum size compared to one surfactant (Traynor et al., 2013). The combination of nonionic emulsifier is expected to increase the accessibility of the emulsifier into the non-polar and polar region. However, combination based on HLB alone will still not

guarantee an ultra-low IFT since the combined surfactant should also have the correct packing parameter, ρ. The packing parameter determines the optimum packing of surfactants when assemble into monolayers and so on the optimum curvature (Komaiko & Mcclements, 2016). Surfactant combination that allow for bicontinuous structure as shown in the diagram below has the best potential to form nanoemulsion using the low energy method.

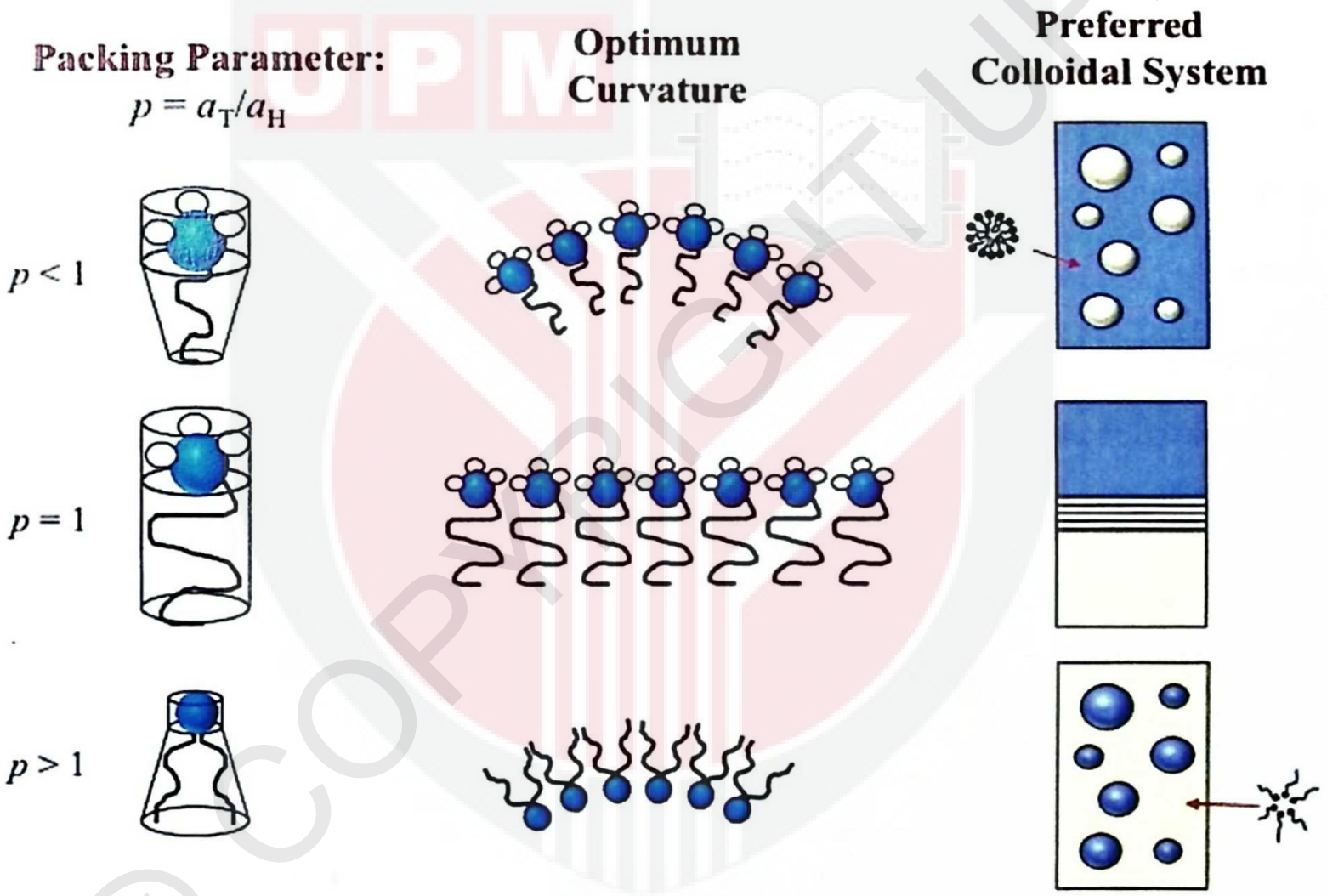


Figure 1-1: Packing geometry of surfactants

In this study, several food-grade surfactants were explored including sucrose ester (SE), Tween 80 (T80), lecithin (LCT) and polyglycerol polyricinoleate (PGPR) to produce nanoemulsion from passion fruit seed oil using low energy method. Sucrose esters is a high HLB food grade surfactant that has been proven to be non-irritant, skin moisturizing, provide silky feeling to skin and reduce irritant properties of other substances present in

cosmetic formulations. Tween 80 is also a high HLB surfactant commonly used as an emulsifier in food, cosmetic and pharmaceutical fields due to its low toxicity and irritation potential, less viscous and having lower CMC. Lecithin and PGPG on the other hand are low HLB surfactant that will be combined with Tween 80 and sucrose ester. In cosmetic applications, lecithin is commonly used in milky lotion and creams due to its refreshing feeling and softening properties when apply to skin (Mitsui, 1997). PGPR has been found to be used as a skin conditioning agent as well as an emulsifier in cosmetic products.

Other than the types of surfactant used, the parameters such as temperature, stirring speed and oil dropping rate will affect the process of forming nanoemulsion too. Raising temperature to higher value can cause decrease in the solubility of hydrophilic surfactant that lead to increase in the droplet size. In general, slow dropping rate is important to ensure the formation of oil water phase can achieve while increasing stirring speed can reduce droplet size due to the mechanical energy applied that broken up the interface tension and ensure an even distribution (Su et al., 2017). Thus, in this study, passion fruit seed oil is employed to undergo nanoemulsion with appropriate concentration of each phases and varies process parameters.

1.2 Problem Statement

In this study, the formation of nanoemulsion from passion fruit oil which is a type of long triglyceride oil will be explored using low energy method. There is limited choices of surfactants that can be used in producing the emulsion system that is safe in the food industry. In this case, the selection of food grade surfactants suitable for producing stable nanoemulsion is the key point in the study. Emulsifying agents are well known to play an

important role in the stability of an emulsion and proper selection of a combination of surfactants gives a synergistic effect. Single surfactant used in the emulsion system is usually unable to produce stable emulsion where the interfacial tension between the oil and water interface could not be lowered. This is because single surfactant is generally facing difficulty in balancing between the head and tail group of the system causing the poor packing geometry thus lead to failure in formation of emulsion.

Packing geometry is one of the parameters which determine the ability on the formation of nanoemulsion. The packing geometry correlate to the ratio of head to tail group cross sectional area should be similar to result in monolayer which tend to be planar favor for the formation if bilayers and vesicles (Komaiko & Mcclements, 2016). Combination of surfactants are found to be provide more stable emulsion with minimum size as compared to use of single surfactants. In fact, combination of surfactants in terms of achieving the HLB required for the oil phase is more accessible with proper concentration used in combination. Besides that, the concentration of oil, water and surfactants also affect the production of nanoemulsion. A proper concentration between these three phases are important in giving small particles size. This is because high concentration of surfactants used can lead to formation of micelles while too low concentration might be unable to form the bilayer in the emulsion system. Therefore, combination of surfactants are used in this study to lower the interfacial tension between oil and water for producing nanoemulsion with small particle size.

Furthermore, problem arise when the process parameters such as temperature, oil dropping rate and stirring speed varied. In producing a nanoemulsion, the process parameters has an effect towards the stability of the emulsion produced. As instance, high

temperature can lead to increase in the particles size due to dehydration of hydrophilic groups causing the imbalance between head and tail groups. Oil dropping rate also vital to be determined as one of the factor that can lead to big particle size in the emulsion system. It is being noticed that different combination of SOW system can lead to variation in the result of the emulsion produced. Stirring provide the mechanical energy in breaking down the interface tension is one of the parameters can affect the resulting particle size of the emulsion produced. Since an increase in the stirring energy is not expected to have exactly the same effect on these opposite phenomena, it is likely to shift the dynamic equilibrium. Therefore, it is necessary to investigate the optimum stirring speed for each formulation.

1.3 Objectives

This study is carried out on the formulation and parametric study of low energy process for passion fruit oil nanoemulsion using by the combination of surfactants at different process parameters. The objectives of this study are:

1. To identify the concentration of combined surfactants and the ratio of surfactants to surfactants that result in lower possible interfacial tension.

2. To develop passion fruit oil nanoemulsion by identifying the optimum ratio of surfactant to oil to water (SOW) in comprise with surfactant to surfactant ratio on the nanoemulsion produced.

3. To study the effect of process parameters (mixing temperature, dropping rate, stirring rate) for producing small particle size and stable nanoemulsion.

1.4 Scope of the Study

This research scope is divided into two main parts. First part is on the development of passion fruit oil nanoemulsion. At this part, the focus is to identify the concentration of combined surfactants and the ratio of surfactants to surfactants that result in lowest possible interfacial tension. At this section, different concentration of surfactants (0%, 0.5%, 1%, 1.5% and 2%) are examined on its interfacial tension value. The preparation is done by firstly dissolved the surfactants in aqueous solution then cooled down to room temperature. After that, a drop of passion fruit oil is dropped on the solution and tested with its interfacial tension value. Then, the results obtained from this stage on the concentration that give lowest interfacial tension will be further examine on the ratio of surfactant to surfactant. The ratio varied for few set (100:0; 95:5; 90:10; 80:20; 50:50; 20:80; and 0:100). After that, the nanoemulsion is produced by varying the ratio of surfactant to oil to water (SOW) in order to obtain the nanoemulsion produced with lowest possible particles size.

The second part of this research is on the variation of process parameters of the passion fruit oil nanoemulsion. In this research, the process parameters that are being examined are temperature; stirring speed; and oil dropping rate. In this section, temperature is the main parameter that is being varied followed by varying the stirring speed and oil dropping rate to identify the pairs of parameters that result in smallest particle size of the nanoemulsion produced.

CHAPTER 2

LITERATURE REVIEW

2.1 Vegetable Oil (VO)

Vegetable oils are a group of fats that are derived from seeds, nuts, cereal grains and fruits. Vegetable oils, in food, are comprised of complex mixtures of triacylglycerol with some minor amounts of diacyglycerols, tocopherols and phytosterol esters (Hammond, 2003). Due to the fact that VO is a renewable resources and environmental benefits, it has becoming more attractive to be used in different products at the recent decades. VO are considered non polar and lipophilic systems whose composition is highly variable and complex and having high flash point. VO plays an important role in our diet and it can also be utilized in cosmetics, nutraceuticals, paints, lubricants and biodiesel. The characteristics of triglycerides are determined by types, proportions and positions of fatty acids on the glycerol backbone (Yara-Varón et al., 2017). VO contains substance with antioxidant properties that is beneficial for preventing lipid oxidative deterioration and act as protective on aging related diseases (Malacrida & Jorge, 2012). Common types of VO from seeds are coconut oil, cottonseed oil, palm oil, rapeseed oil, soybean oil and sunflower oil. In this study, the VO used is passion fruit seed oil.

2.2 Passion Fruit Seed Oil (PFO)

Passion fruit (*Passiflora edulis*) belongs to the genus *Passiflora*. Passion fruit is an exotic climbing vine originating in South America and grown worldwide nowadays. The other name given to passion fruit is known as maracuja. Passion fruit can be grouped into two which are the purple, *P. edulis f. edulis* Sims and the yellow, *P. edulis f. flavicarpa*

Degener. The purple passion fruit is a native of southern Brazil and has been cultivated in the tropics, subtropics and temperature region in large scale (Zibadi & Watson, 2004). In the juice industry, extraction of passion fruit juice yields thousands of tons of seeds as agricultural by-products in which the seeds contain large amounts of fiber and oil that add value to this agro-industrial waste (Malacrida & Jorge, 2012). Seeds content in passion fruits is approximately 4% to 12% with oil content of about 30%. The high content of linoleic acid, an unsaturated fatty acids enables it to be utilized in food, pharmaceutical and cosmetics industries. PFO is said to be as a potential source of bioactive compounds containing antioxidants, antimicrobials, antitumor compounds, phenolic, carotenoids and flavonoids (Oliveira, Angonese, Ferreira, & Gomes, 2017). The main fatty acid present in the oil including stearic, palmitic, oleic and linoleic. Among this fatty acids, linoleic contain about 70%, oleic about 18%, palmitic about 10% and stearic about 2%. The average content of unsaturated fatty acids was higher than the content of saturated fatty acids (Regis, Resende, & Antoniassi, 2015). Passion fruit seeds also content high percentage of carbohydrate and fiber which is about 48% making it a great source of fiber. The total tocopherol content in PFO is approximately 500 mg/kg while total phenolic content is about 1315 mg/kg. Passion fruit seeds provide protection against damage caused by free radicals that can lead to chronic diseases such as diabetes, heart disease arthritis and Alzheimer's diseases. The ability to destroy free radicals making it beauty benefits by removing free radicals from body and hence preventing wrinkling of skin (Wijeratnam, 2015). PFO with high content of polyunsaturated fatty acid is greatly used as skin and hair conditioning agents as well as ingredients for make-up products (Malacrida & Jorge, 2012).

2.3 Nanoemulsion

Nanoemulsion are colloidal dispersions made up of two phases, (an oil phase and an aqueous phase) with the help of surfactants at accurate proportions result in droplet size at the range of 5 to 200nm. It is kinetically stable, optically clear and transparent (Azmi et al., 2019). It is generally being used for improving bioavailability of bioactive (such as drugs, vitamins, supplements), developing cosmetic products and functional food, and crystallizing active pharmaceutical ingredients for formation of drug nanocrystals (Gupta et al., 2017). Nanoemulsion can be classified into three types (i) oil-in-water (O/W) emulsion; (ii) water-in-oil (W/O) emulsion, and (iii) bi-continuous emulsions. O/W is which it has water as continuous phase and oil as dispersed phase and vice versa for W/O emulsion while for bi-continuous is where oil and water are interspersed within the system (Ronak P. Patel, 2012). The free energy of colloidal dispersion is higher than that of separate phases that causes nanoemulsion to be thermodynamically unstable. Adequate energy barrier between two states in nanoemulsion is important to ensure it present in kinetically stable or metastable state. When particle size decrease, the interfacial free energy getting positive which indicated that it is becoming more unfavorable to form colloidal dispersion due to increase in interfacial area (McClements, 2012). Nanoemulsion is prone to Oswald ripening that lead to creaming, flocculation and other instability problems. With adequate surfactants used, the small particles size of nanoemulsion is achieved and hence it is in metastable state for long period (Simonazzi et al., 2018). There are two techniques used to produce nanoemulsion: high energy method and low energy method. Advantages of nanoemulsion including increase rate of absorption, eliminate variability in adsorption, increase bioavailability of active drug,

rapid and efficient penetration, improve efficacy of drug and hence reduce the required dose, and improve taste (Sharma et al., 2013).

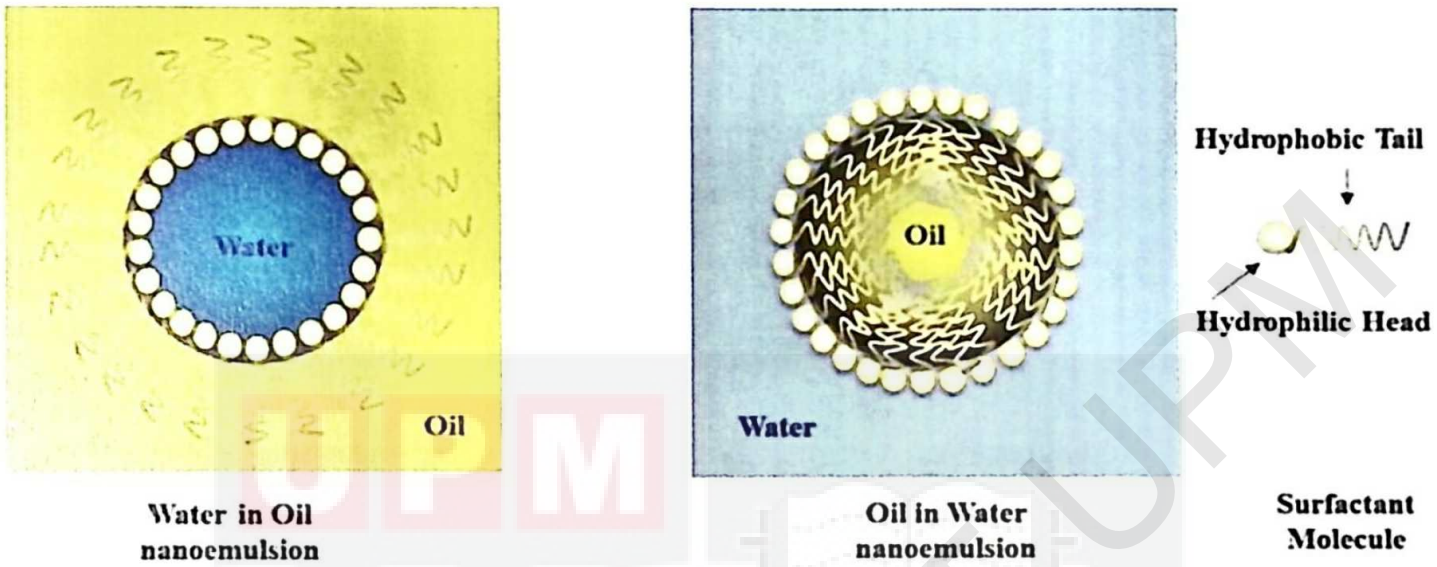


Figure 2-1: Schematic diagram of water in oil and oil in water nanoemulsion.

2.3.1 High Energy Method

High energy method used mechanical devices to produce intense disruptive forces to break the oil and water phase that can then achieve small droplets size. High energy method provides a robust way in producing nanoemulsion with dispersed phase volume fraction as high as 40%. However, this method is less efficient and susceptible to heat effects due to excess shear in producing nanoemulsion (Gupta et al., 2017). Examples of high energy methods including high pressure homogenization, microfluidization, sonication and high amplitude ultrasound. The size of droplet is governed by few factors: operating condition, environmental condition, oil type, type and concentration of surfactants used and intensity of energy. To conclude, the droplet size decreases with increase in intensity of energy, decrease in interfacial tension and increase in surfactant adsorption rate (Mohamed A. Salem, 2016). High energy required to carry out this approach make it limit their utility at industrial scale and the equipment required is higher

in cost too. Although the particles size able to decrease efficiently with high energy method, it is unfavorable to be used for thermolabile drugs and macromolecules such as protein, enzymes, retinoid, peptides and nucleic acids due to high temperature and pressure used cause damage on the composition in the components (Azmi et al., 2019b).

2.3.2 Low Energy Method

Low energy method make use of low interfacial tension property to reduce droplet size with aid of magnetic stirrer to provide low amount of energy. It is an easy and scalable approach to produce nanoemulsion without use of excess shear (Gupta et al., 2017). Low energy method is dependent on the internal chemical energy of the system and nanoemulsion formed spontaneously in the concept of phase transitions due to change in temperature or composition (Maali & Mosavian, 2013). The droplet size is affected by the ratio of surfactant to oil to water, surfactant type, temperature, stirring speed and oil dropping speed. Low energy method is said to be able to produce smaller droplet size compared to high energy method (Mohamed A. Salem, 2016). Low energy methods can be classified into two groups: isothermal methods and thermal methods. Isothermal methods are the approaches that do not require any temperature change to form nanoemulsion. Nanoemulsion with isothermal method is achieved by spontaneous formation of small droplets at the interface between organic and aqueous phase at specific composition. Spontaneous emulsification, emulsion phase inversion (EPI) and phase inversion composition (PIC) are the examples of isothermal methods. Thermal method required change in temperature to achieve nanoemulsion which the method use is known as emulsion phase inversion temperature (PIT). To carry out isothermal methods, it can be done by various method: (a) simply mixing oil, water and water-miscible solvent

together; (b) contact of an oil, hydrophobic surfactant and water miscible solvent mixture with an aqueous phase; and (c) addition of oil and hydrophilic surfactant mixture into an aqueous phase (Komaiko & McClements, 2015). Low energy methods are more favorable due to lower cost and ease of implementation. However, higher concentrations of surfactants is required to produce nanoemulsion using low energy method and it is more complex to be carried out for long chain triglyceride vegetable oil. Besides that, due to limited food grade surfactants that can be applied, it increases the difficulties to achieve success nanoemulsion.

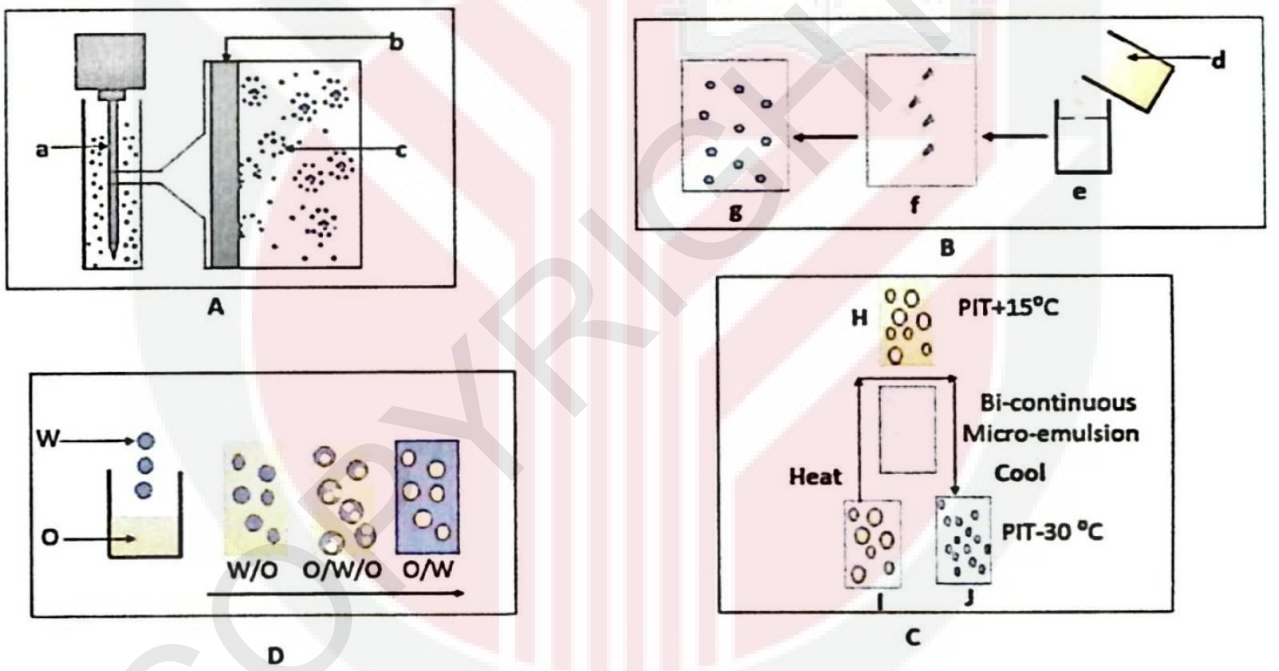


Figure 2-2: Schematic for four devices utilized in low energy method to product nanoemulsion. (A) membrane emulsification method; (B) spontaneous emulsification method; (C) phase inversion temperature method; and (D) emulsion inversion point method. (Mohamed A. Salem, 2016)

2.3.2.1 Spontaneous Emulsification

Spontaneous emulsification is a type of low energy isothermal approach used to produce nanoemulsion. Spontaneous emulsification or known as self-emulsification occur

when immiscible liquids in non-equilibrium conditions are in contact without the need of external energy input (Solans, Morales, & Homs, 2016). Generally, to carry out spontaneous emulsification, there are three main steps to be followed: (1) preparation of homogeneous organic solution composed of oil and lipophilic surfactant in water miscible solvent and hydrophilic surfactant; (2) injecting the organic phase to the aqueous phase and stir with magnetic stirrer to form emulsion; and (3) evaporation of the water-miscible solvent (Ronak P. Patel, 2012). Implementation of spontaneous emulsification to produce nanoemulsion can be varied in three main ways: (1) the composition of the organic and aqueous phases; (2) the environmental conditions (temperature); and (3) the mixing condition (stirring speed, rate of addition and order of addition) (McClements, 2011). Types of surfactants, amount of surfactant to dispersed phase, additive in the dispersed phase, type of phases used and viscosity of dispersed and continuous phase are some of the factors that affect the droplet size of spontaneous emulsification. In practice, spontaneous emulsification is carried out using titration of organic phase into aqueous phase and achieve the desired nanoemulsion result with optimum system composition and process parameters. In spontaneous emulsification method, bicontinuous microemulsion will initially formed when organic and aqueous phase are in contact and eventually formed small droplet spontaneously when the bicontinuous microemulsion phase are broken up by applying stirring to aid in the breaking action on movement of surfactant, oil and water molecules (Komaiko & Mcclements, 2016). Spontaneous emulsification is very advantageous in encapsulating bioactive compounds, protecting sensitive compounds against severe condition (high temperature and pressure), reducing surfactants needed and providing better thermal stability (Mohamed A. Salem, 2016).

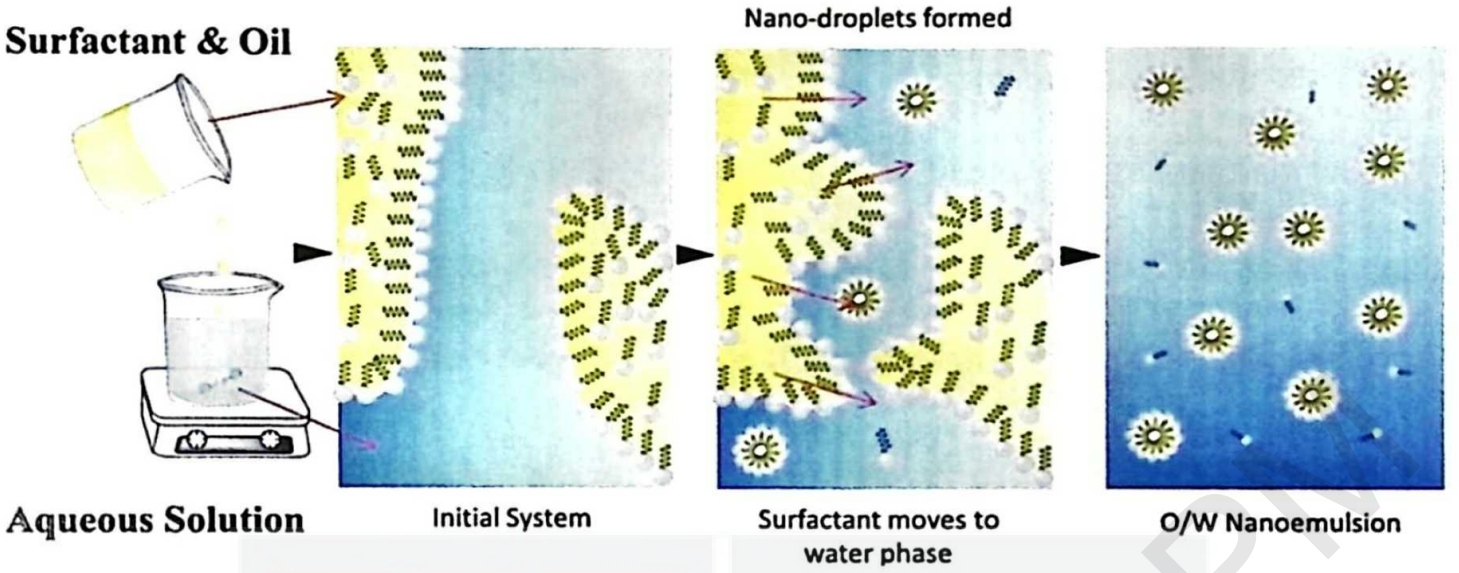


Figure 2-3: Schematic represent mechanism for spontaneous emulsification. (McClements, 2011)

2.3.2.2 Phase Inversion Composition (PIC)

Phase inversion composition (PIC) is an isothermal low energy method which involve changing in the composition of the system at a constant temperature. In PIC, fine dispersion are formed by the chemical energy from the reaction of components obtaining from phase transitions in emulsification (Azmi et al., 2019). Catastrophic phase inversion is required to change from water-in-oil to oil-in-water or vice versa. Catastrophic phase inversion is defined as the changing on the ratio of oil to water phases while the surfactant properties remain constant (Mohamed A. Salem, 2016). In conducting with this method, the dispersed phase must be thoughtfully mix with the continuous phase before more continuous phase is added until the nanoemulsion is reached (Che Marzuki, Wahab, & Abdul Hamid, 2019). As instance, W/O emulsion with high oil-to-water ratio is formed with addition of surfactant and then increasing amount of water added to the system by continuous stirring. Above a critical water content, the water droplet concentration is high and the droplets will pack tightly and emulsion formed at a phase inversion point where

W/O change to O/W. During the emulsification, a bi-continuous phase exists has to be determined to cross as to favor the formation of small and uniform droplets (Maali & Mosavian, 2013). For instance, an O/W emulsion stabilized by an ionic surfactant can experience phase inversion to W/O emulsion by addition of salt. This is because the packing parameter is adjusted from $\rho < 1$ to $\rho > 1$ due to the ability of salt ions to screen the electrical charge on the surfactant head groups (McClements, 2011). Factors affecting the droplet size formed are the stirring speed and the rate of water added to the system. The disadvantage from this method is where it required use of larger amounts of surfactants and a full control of the physicochemical parameter due to the possibility of prone to coalescence and creaming (Che Marzuki et al., 2019).

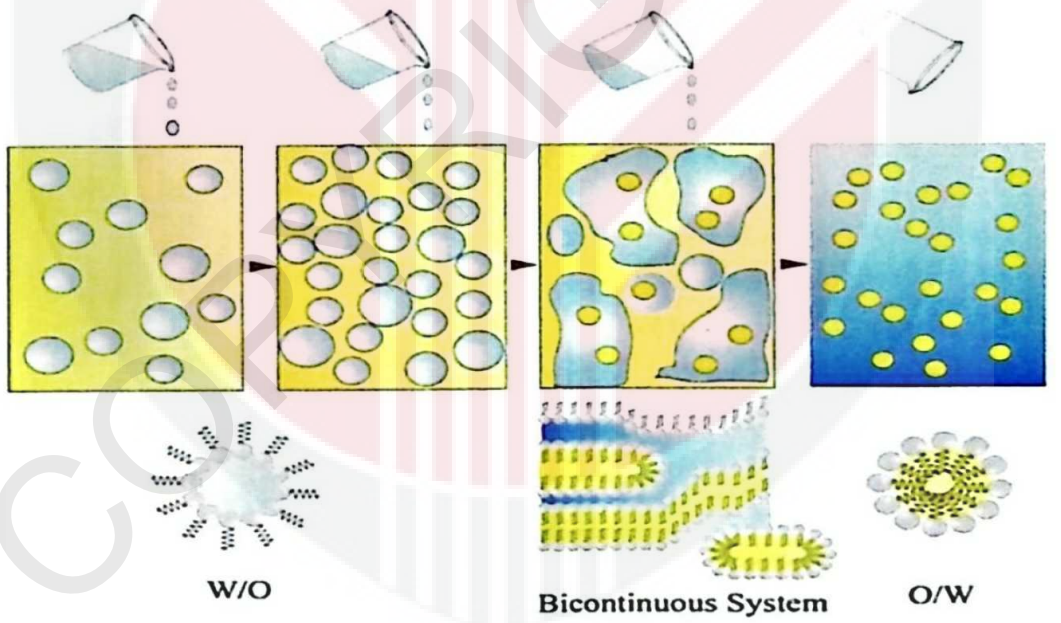


Figure 2-4: Schematic represent proposed mechanism for emulsification inversion point method. (McClements, 2011)

2.3.2.3 Phase Inversion Temperature (PIT)

Phase inversion temperature (PIT) is a thermal low energy method used to produce nanoemulsion. PIT is a temperature dependent process that allow flexibility in temperature changing to achieve nanoemulsion. It is an approach that utilized the principle of spontaneous formation of nanoemulsion by changing temperature and prompt temperature hinder the occurrence of coalescence which contribute to stable nanoemulsion (Azmi et al., 2019). PIT method involves a transitional phase inversion. At low temperature, the surfactant monolayer tend to favor in positive spontaneous curvature and it becomes more hydrophilic that results in O/W emulsion formed. In contrast, at high temperature, dehydration occur at the hydrophilic tail on the nonionic surfactant and the curvature become negative that makes the surfactant becomes lipophilic and promote the production of W/O emulsion. At intermediate temperature, the spontaneous curvature is near to zero and a bi-continuous phases exist (Maali & Mosavian, 2013). At the PIT, the packing parameter equal to unity (ρ=1) and the emulsion is broken down because of the droplets is having an ultralow interfacial tension and is readily coalesce with each other (McClements, 2011). The disadvantages of PIT method are it is limited to nonionic surfactants and required thermal energy for the presence of phases. A new approach has been developed to make nanoemulsion with nonionic surfactants with lower phase inversion temperature with dilution on solution containing higher phase inversion temperature so that the possibility of coalescence to occur during the preparation of nanoemulsion can be reduced (Hadžiabdiü Jasmina, Orman Džana , Elezoviü Alisa, Vraniü Edina, 2017).

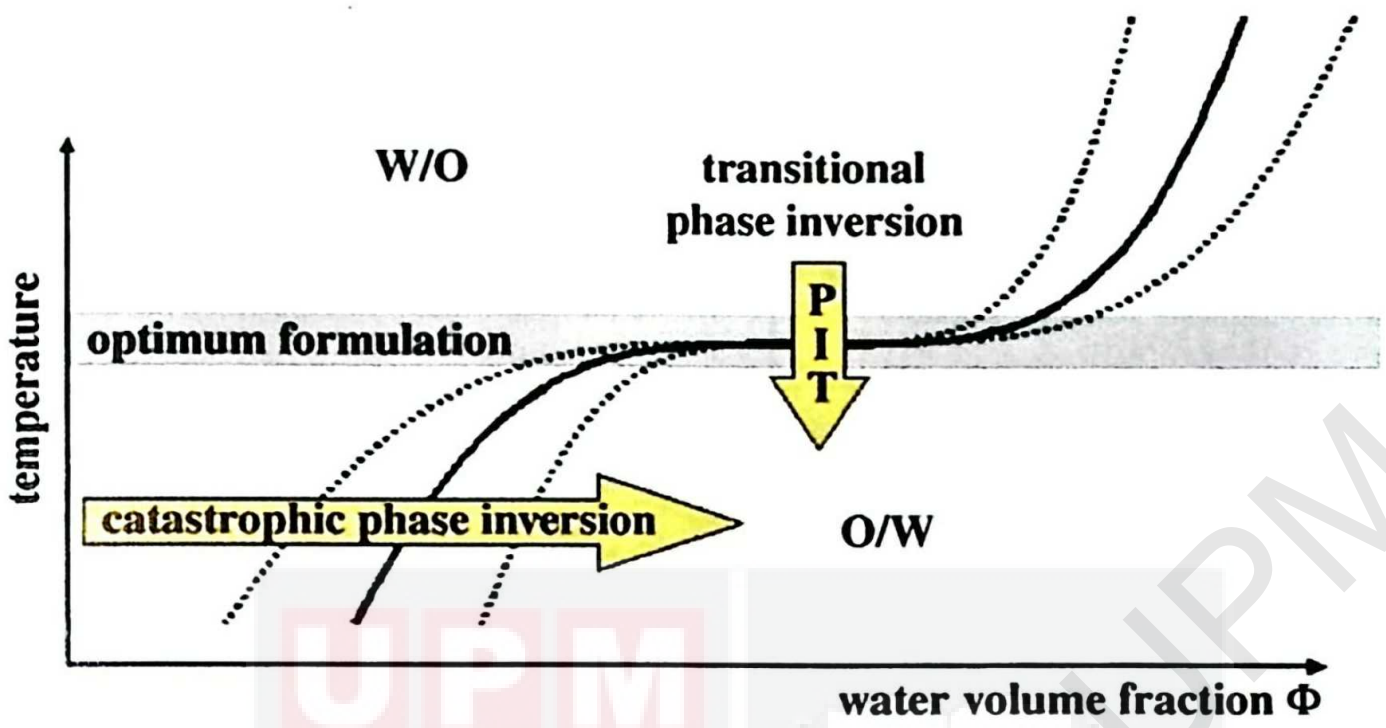


Figure 2-5: Phase inversion of emulsification

2.3.2.4 Previous Study on Low Energy Method Nanoemulsion of Long Chain Triglycerides Oil.

The title of the study was Development and Stability Evaluation of Olive Oil Nanoemulsion using Sucrose Monoester Laurate. Olive oil is a type of long chain triglyceride and it has been chosen in the study due to its efficiency in treating skin problem, it is also rich in monounsaturated fats of which high oleic acid content that is a good antioxidant substances. In the study, olive oil formulations were performed through various ratio of olive oil, sucrose laurate and glycerin. The aim of the study was to prepare olive oil nanoemulsion using low energy emulsification method. In the study, the analysis were made on the particle size, zeta potential and stability study. Particle size and zeta potential analysis were carried out by measuring with zetasizer while for stability study was conducted to measure the droplet size over storage for three months at three different temperatures. Based on the result, the mean droplet size was decrease with an increase in surfactant concentration with the ratio of olive oil at 50% to surfactant at 25%. It has been

found out that the droplet size decreased from 180nm to 38nm on the increased of surfactants from 30% to 75% due to the presence of more surfactants at the oil-water interface that contributed to stabilize nanoemulsion. For the stability study, it was found out that the best storage temperature of the study was at 4°C since there is no change in the droplets size and polydispersity (PDI) value. For the storage at 25°C and 40°C, there were increased in the droplet size and PDI after 3 months. In conclusion, from the study, stabile olive oil nanoemulsion with small droplets size and high zeta potential were obtained by low energy technique with preparation of 50% to 60% olive oil and 25% of sucrose laurate (Ahmad M.M. Eid, Baie, & Arafat, 2012).

2.4 Surfactant

A surfactant is a chemical substance that alters interfacial properties by absorbing to the boundary between two immiscible phases. Surfactant is an active agent that contain hydrophilic and lipophilic groups. Hydrophobic interaction is where the lipophilic group tends to move away from water molecules. Surfactant will spontaneously absorb to the interface when in contact in an emulsion system. Surfactant molecules will self-assemble in water forming micelles beyond a critical concentration as a result of the hydrophobic interaction (Yamashita, Miyahara, & Sakamoto, 2017).

Surfactants are substance that create self-assembled molecular clusters called micelles in a solution and adsorb to the interface between a solution and a different phase. It can be categorized into two groups which are ionic surfactants and nonionic surfactant. There are 3 types of ionic surfactants namely anionic surfactants, cationic surfactants and amphoteric surfactants. Anionic surfactants is which the hydrophilic groups dissociates

into anions in aqueous phase; cationic surfactants are those dissociate into cations; and amphoteric surfactants are those that dissociate into anions and cations depending on the pH. Nonionic surfactants are that do not dissociate into ions in aqueous phase and they are sub-categorized depending on the type of their hydrophilic group (Nakama, 2017).

Classification of surfactants can be done based on the solubility too. This is meant by which hydrophilic surfactants are those that soluble in water while lipophilic surfactant are those that soluble in lipids. Ionic surfactants are commonly hydrophilic surfactants while nonionic surfactants are either hydrophilic or lipophilic that depends on the balance between hydrophilic and lipophilic groups (Nakama, 2017).

Surfactants have been commonly used in food industry such as lecithin from egg yolk and various proteins from milk used for preparation of food products such as mayonnaise, salad creams, dressings, desserts and other (Kralova & Sjöblom, 2009). The surfactant molecules can arrange themselves to form aggregates with different shapes and the nature of the head group and hydrophobic chain will determine the type of aggregates formed. There are few type of surfactant aggregates namely regular micelles, reverse micelles, cylindrical, planar-lamellar, onion-like lamellar and interconnected cylinders (Vaidya & Ganguli, 2019).

Surfactants are very important in formulating emulsion because of the ability in reducing the interfacial tension between water and oil and absorb at the interface to stabilize emulsions. The main function of surfactants is to generate small drops in the emulsification step and provide long term stability after preparation. Surfactants have effects on the permeability characteristics thereby it has the potential to solubilize lipid

within the stratum corneum. It induced a concentration dependent biphasic action to alter the skin permeability. Therefore, it is widely used in therapeutic and cosmetic products (Som, Bhatia, & Yasir, 2012).

Increasing the surfactant concentration would result in a greater number of surfactant molecules emigrating from the oil phase to the aqueous phase of the nanoemulsion forming nano size droplets (Hasani, Pezeshki, & Hamishehkar, 2015). However, the use of the surfactants must be appropriate as an excess amount of surfactants does not result in a better or stable emulsion. When excessive surfactants are applied, it will cause the product to become sticky, unpleasant to use, irritating to skin, making product less stable and shorten the shelf life of products (Joseph Lin, 2017). Looking onto the particle size, the increase in surfactant concentration will lead to formation of micelle in continuous phase instead of oriented on the interface thus causing the increase in particle size (Hasani et al., 2015).

Surfactant help in reducing the interfacial tension thus lowering the free energy penalty associated with droplet formation. It acts as a protective coating around droplets that prevent droplets from aggregation during and after emulsification. Small molecule surfactants are more effective for producing nanoemulsion using low energy method (Komaiko & Mcclements, 2016). In producing nanoemulsion, the factors that must be considered are (1) surfactant must be selected carefully such that an ultralow interfacial tension may be achieved; (2) concentration of surfactant must be high enough to stabilize the microdroplets to produce nanoemulsion; and (3) the surfactants used must be flexible or fluid enough to promote the formation of nanoemulsion (Jaiswal, Dudhe, & Sharma, 2015). There are three parameters that will affect the type of surfactant aggregates which

are hydrophilic lipophilic balance (HLB), critical micelle concentration (CMC) and surfactant packing parameter (Vaidya & Ganguli, 2019).

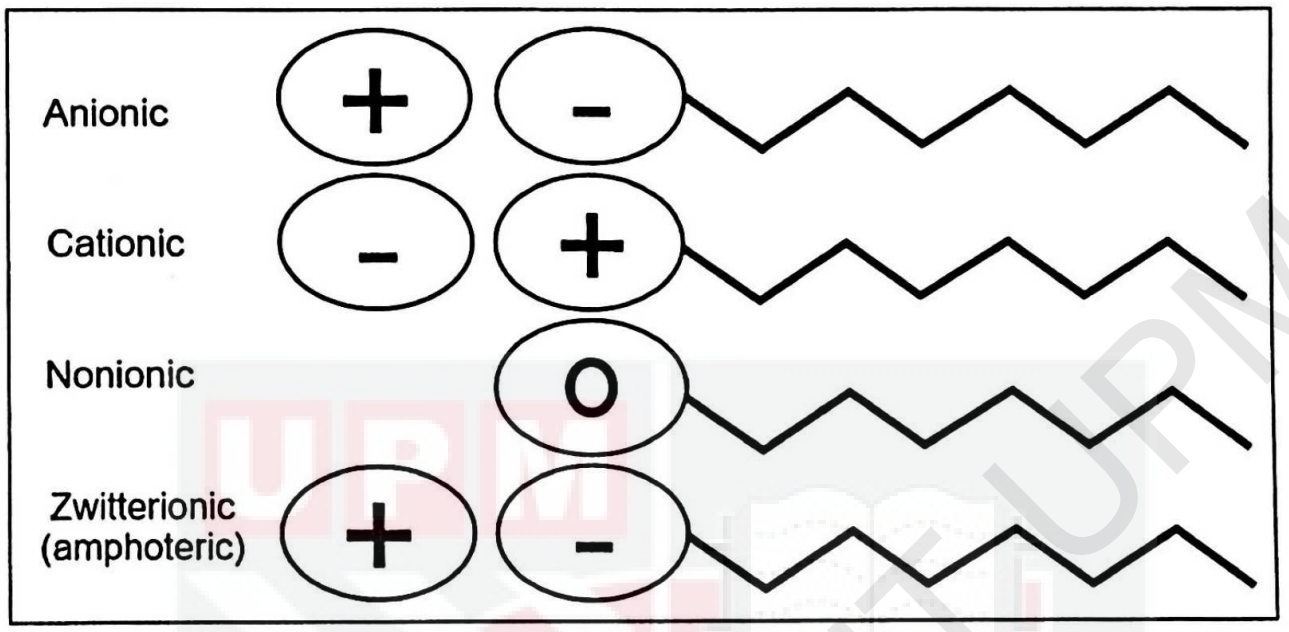


Figure 2-6: Schematic diagram of various types of surfactants. (Som et al., 2012)

2.4.1 Hydrophilic Lipophilic Balance (HLB)

Hydrophilic lipophilic balance (HLB) is the balance of the size and strength of the hydrophilic and lipophilic groups of a surfactant molecule. It is also defined as an index of the affinity of a surfactant towards a solvent. As instance, high HLB value indicated that the surfactant is more attracted to water or water soluble. Generally, the HLB scale range from 0 to 20. At lower range of 3 to 6, the surfactant are known to be lipophilic surfactants with longer tail and shorter head groups which are more suitable for use in W/O emulsion while surfactant with high HLB value (8 to 18) are hydrophilic surfactant that consist of shorter tail and longer head that being used for O/W emulsion (Zheng et al., 2015).

HLB value is useful for the selection of appropriate surfactant to emulsify O/W solution. Bancroft's rule has stated that oil-soluble surfactant preferably for W/O emulsion

while water-soluble surfactant preferably to O/W emulsion (Yamashita et al., 2017). Figure 2-7 representing suitable selection on the surfactant corresponding to the application depending on the range of HLB value.

HLB must be considered in any preparation of emulsion. It is a semi-empirical scales that aids formulators to select surfactants. HLB value provides the guideline on the ratio of the hydrophilic portion of the nonionic surfactant to the lipophilic portion to yield the best emulsion without experiencing flocculation or coalescence (Che Marzuki et al., 2019). The nature of emulsion which are either oil-water or water-oil can be easily predicted with the known of HLB value of surfactants.

Increasing in the HLB value has found to be able to decrease the mean droplet size and hence it is considered as a useful parameter for controlling the droplet size (Che Marzuki et al., 2019). However, HLB is not a universal property due to the fact that it is based exclusively on the weight percent of polyoxyethylene or polyol in the surfactant molecule while discounting its molecular weight, the chemical nature of its hydrophilic and lipophilic moieties and the structural features. Different categories of surfactants such as for sorbitan monoesters or polyoxyethylates aliphatic alcohols tend to exhibit different relations between HLB and its general property (Schott, 1995).

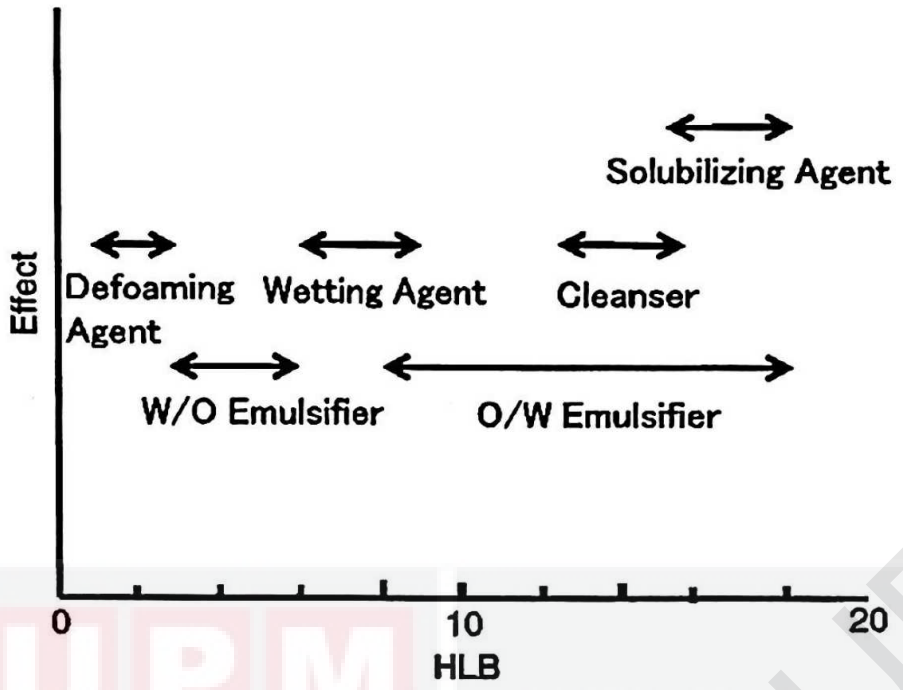


Figure 2-7: Index for choosing surfactant. (Nakama, 2017)

2.4.2 Critical Micelle Concentration (CMC)

Critical micelle concentration (CMC) is one of the parameter used for determination of the formation on type of surfactant aggregated. CMC is known as the minimum concentration of the surfactant that micelle formed. Micellization formed when the concentration of the surfactant has reached the CMC (Vaidya & Ganguli, 2019). CMC is depending on the surfactant structure and experimental condition where below the CMC value, the surfactants are solubilized as monomers in the solution and increasing the amount of surfactants after CMC reached will lead to formation of new micelles that promoting growth of aggregates. One of the factors that will be affecting the CMC is the alkyl chain length of the surfactant where the longer the alkyl chain or the surfactant chain, the lower the CMC and hence the surfactant are more prone to be in micellar state in higher concentration. Besides that, temperature can affect the micellar formation of which increase in temperature can lead to increase in the micelle sizes and lowering the CMC (Zheng et al., 2015).

The working principle of CMC can be explained as where the addition of surfactant that initially reducing the interface energy and removing the hydrophobic group of surfactants from in contact with water leading to decrease in surface free energy (surface tension). The continual addition of surfactant to the system will cause the molecules to start aggregating and form micelles that reduce the free energy in the system drastically due to the decrease in the contact between the hydrophobic groups and water until CMC reached. Further increase the amount of surfactant after CMC reached will lead to more formation of micelles but hardly reduce the free energy or in another word the surface tension stay more or less in constant (Sheng, 2013).

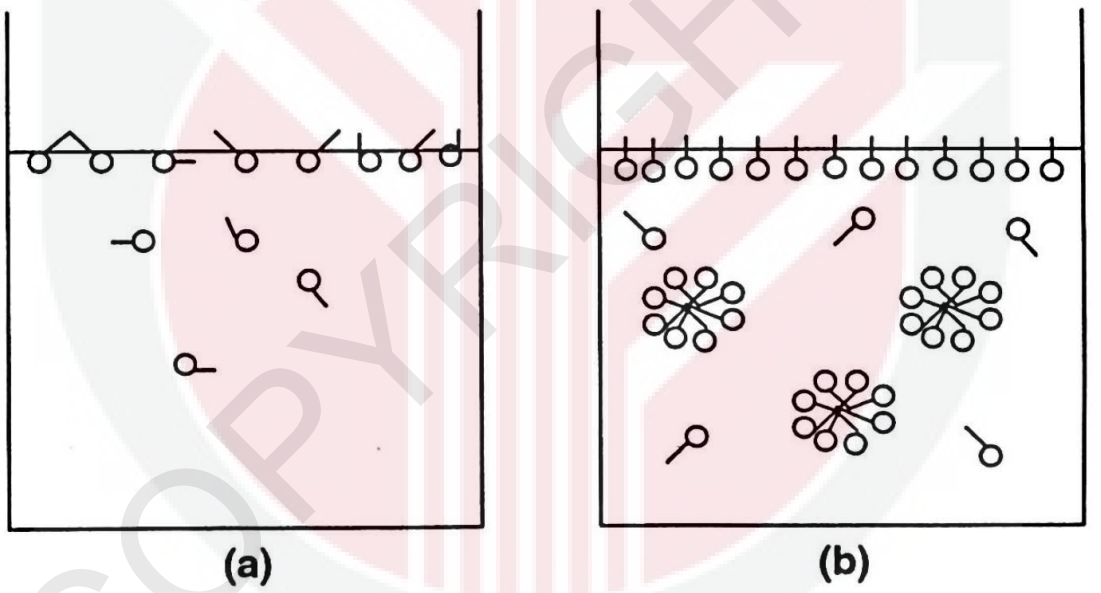


Figure 2-8: Distribution of surfactant molecules in solution at concentrations (a) below and (b) above CMC. (Sheng, 2013)

2.4.3 Surfactant Packing Parameter

Packing parameter is one of the important factors that will influence the type of surfactant aggregates formed. This is because in the structure of the molecule, there are packing constraints associated with it and in the absence of the constraint, surfactants will assemble to form spherical micelles (Vaidya & Ganguli, 2019). The packing parameter is

used to determine the optimum packing of surfactant when they assemble into monolayer and the optimum curvature on the surfactants. Packing parameter can be simplified to the meaning of ratio of the tail group to head group cross-sectional areas, $\rho=a_T/a_H$. (Komaiko & Mcclements, 2016).

A specific shape and size of the equilibrium aggregate of surfactants can be known from the translation of a particular value of the packing parameter (Nagarajan, 2002). As instance, the value of packing parameter at < 1/3 will form spherical micelles; 1/3 to 1/2 forming worm-like micelles; 1/2 to 1 is vesicles; 1 gives cylindrical or planar bilayers and >1 will cause inverted micelles (Stuart & Boekema, 2007).

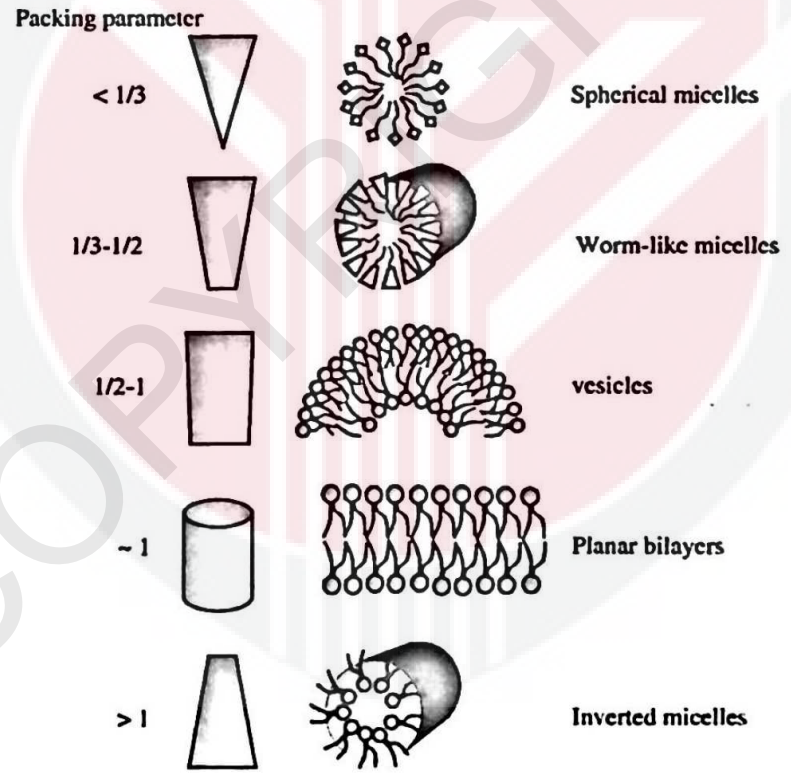


Figure 2-9: Packing parameter value correspond to the shape of molecule and aggregated structures formed. (Stuart & Boekema, 2007)

When the tail group is larger than the head group, it will result in packing parameter, ρ greater than 1 causing the monolayer having curvature where tail groups pointing outwards which in turn favor for formation of reverse micelles and W/O

emulsion. In contrast, with head group greater than tail group, ρ will be smaller than 1 leading to monolayer that giving curvature where head groups pointing outward that favor in the formation of micelles and O/W emulsion. All in all, to have an efficient emulsion, the ratio of head group to tail group shall be equal that give ρ=1 forming planar bilayer (Komaiko & Mcclements, 2016).

Comparing between ionic and nonionic surfactants, nonionic surfactants having smaller interaction in their head groups compared to ionic surfactant. Thus, the head group ratio will be smaller leading to greater value of the packing parameter and forming larger aggregation number compared to ionic surfactant of same tail length. Other than that, when looking on the number of tail on surfactant, double tail molecule will be having packing parameter twice as large as single tail molecule. In this case, the double tail surfactants can self-assemble to form bilayer while single tail surfactant tend to form spherical micelles (Nagarajan, 2002).

2.4.4 Interfacial Tension (IFT)

Interfacial tension (IFT) is known as the force of attraction between the molecules at the interface of two fluids. Oil/water interfacial tension can be used to calculate a spreading coefficient that give an idea on the tendency for the oil to spread (Speight, 2016). IFT is being defined as the accumulation of energy and the imbalance force at the interface of two different phases such as liquid-solid, gas-solid, liquid-liquid and gas/liquid. IFT also used to describe the surface free energy that exists between two fluid phases that preventing one liquid from emulsifying into another (Meckel, 2010).

The minimum interfacial tension is obtained when the equilibrated surfactant aqueous phase is at CMC and when the partition coefficient of the surfactant between oil and water phase is unity. In order to obtain minimum interfacial tension in the system, the partition coefficient and molar absorptivity ratio of oil to aqueous phase for surfactant should be unity too. Partition coefficient and the structure of surfactant aggregation are the main considerations in obtaining low interfacial tension. (Hou, Li, & Wang, 2001).

Surfactants having the ability to lower the interfacial tension between oil and water efficiently and formed stable emulsion. The lower the interfacial tension, the lesser the separation between water and oil from the emulsion and hence resulting in smaller diameter of the particles size. The interfacial tension can be affected by the concentration of surfactant, temperature and pressure. Theoretically, increase in the concentration of surfactant tend to lower the interfacial tension and same goes to temperature where increase in temperature has the ability to reduce the interfacial tension while increase the pressure will lead to increase in the interfacial tension (Al-Sahhaf, Elkamel, Ahmed, & Khan, 2005). However, further finding must be done to identify the other factors that will be in fact affecting the interfacial tension at the same time and an optimum parameters shall be determined to ensure the lower interfacial tension can achieve for producing a stable emulsion.

2.5 Combined of Surfactants

2.5.1 Sucrose Ester (SE)

Sucrose Ester (SE) is a nonionic surface active agents having sucrose as the hydrophilic group with a maximum of eight fatty acid per molecule as lipophilic groups.

The most common fatty acid used in SE are lauric, myristic, palmitic, stearic, oleic, behenic and erucic acids. A wide range of HLB value can be obtained through change in the nature or the number of fatty acid groups (Szuts & Szabó-Révész, 2012). SE is commonly used as emulsifier or known as surfactant for both O/W and W/O emulsions. SE is very beneficial in the used of solubilizing, foaming, anti-bacterial and releasing agents, enhancing or inhibiting crystal growth in fats and lubrication. When dispersed in a solvent at particular concentration and composition, SE has the ability to self-organize into micelles structures which in turn reaching the CMC leading to formation of spherical micelles (Bin Sintang et al., 2017).

Sucrose ester acting as a surfactant that is important in solubilization or stabilization of drugs and also used in modification in bioavailability of drugs. SE with high monoester contents are more hydrophilic while high esterification degree tend to form SE with lipophilic characteristics. SE with different HLB value are used in different applications (Szuts & Szabó-Révész, 2012). With the natural and biodegradable characteristics as well as the emulsifying and solubilizing behavior, SE is widely used in the food, cosmetic and pharmaceuticals industries (Youan, Hussain, & Nguyen, 2003). Monoester is a sucrose ester with 1 mol of fatty acid and 1 mol of sucrose while di-ester having 2 mol of fatty acid and tri-ester consist of 3 mol of fatty acid (Youan et al., 2003).

In this study, the sucrose ester used was DK Ester F-160 and the chemical formula given is $C_{33}H_{62}O_{12}$ with molecular weight of 650.84 g/mol. The SE used contained 70% of mono-ester and 30% di-ester, tri-ester and poly-ester with HLB value of 16 that favor oil-in-water emulsion.

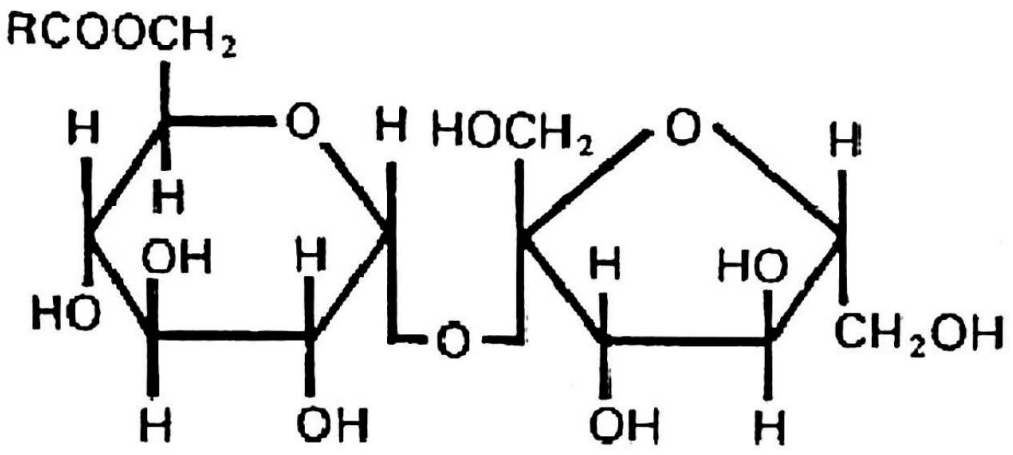


Figure 2-10: Chemical structure of sucrose ester. (Youan et al., 2003)

Application	Recommended HLB of SEs
Emulsification	Low to high
Solubilization	High
Dissolution improvement	High
Controlled/sustained release	Low to high
Absorption/penetration enhancement	High (especially with C12–C14 fatty acids)
Lubrication	Low to medium
Disintegrant	High

Figure 2-11: Recommended HLB values for different applications. (Szuts & Szabó-Révész, 2012)

2.5.2 Tween 80 (T80)

Tween 80 or known as polysorbate 80 is a nonionic surfactant that is widely used as an emulsifier in cosmetic, pharmaceuticals and food products. The structure of Tween 80 is in comprise of polyxyethylene and sorbitan monooleate. Tween 80 having single unsaturated tail with molecular formula of $C_{64}H_{124}O_{26}$ and molecular weight at 1309.65g/mol (Rabiee et al., 2016). It is low in toxicity and irritation potential and therefore suitable for utilize in food, cosmetic and pharmaceutical fields. When comparing with other surfactants in the Tweens family, Tween 80 is less viscous and having lower

CMC. Low CMC indicated that less surfactants is needed to achieve the minimum IFT in the system (Ramly, Zakaria, & Naim, 2017).

Tween 80 is known to be one of the most commonly used emulsifier in producing emulsion owing good emulsifying properties and having more important in hydrophilic groups than lipophilic groups as the HLB value of 15 that is very effective for O/W emulsion (Arancibia, Riquelme, Zúñiga, & Matiacevich, 2017). Tween 80 has been proven to have the ability to stabilize emulsion and having smaller particle diameter compared to that with Span 20. This is due to that the use of Tween 80 help in optimizing the balance of solubility, molecular geometry and HLB values (Komaiko & Mcclements, 2016).

Temperature can be one of the factors affecting the phase behavior of Tween 80. Increase in the temperature tend to cause dehydration of the oil chain thus increasing lipophilic character and improving the interaction between lipophilic chain of Tween 80 with triglyceride that lead to more oil soluble into the micelles (Ramly et al., 2017). Other than that, the concentration of Tween used in emulsion must be appropriate where excessive amount of Tween 80 can lead to increase in the particles size due to aggregation of Tween 80 in the aqueous phase (Chu et al., 2019).

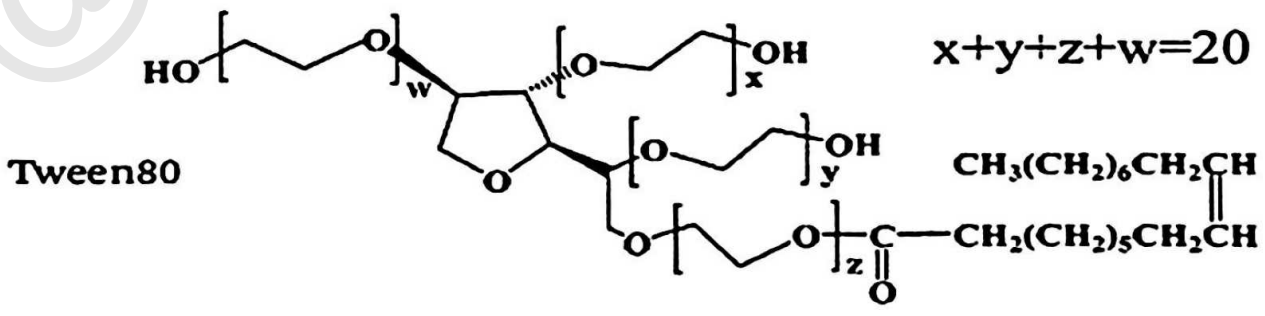


Figure 2-12: Chemical structure of Tween 80. (Rabiee et al., 2016)

2.5.3 Lecithin (LCT)

Lecithin is a complex mixture of acetone-insoluble phosphatide that consists of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine and phosphatidyl inositol combined with different amounts of triglycerides and fatty acids. The main sources of lecithin are soya beans and egg yolk. The chemical name given to lecithin is 1,2-diacyl-*sn*-glycero-3-phophatidylcholine (Raut et al., 2012). The molecular formula for lecithin is $C_{42}H_{80}NO_8P$ with molecular weight of 758.060 g/mol (ChemSrc, 2019). In the lecithin molecules, there is two portions namely fatty acids portion and phosphoric portion. Fatty acid portion function as to attract lipophilic drugs while phosphoric acid portion work to attract to hydrophilic drugs. With this properties, lecithin is able to arrange itself at the boundary between immiscible liquids of oil and water that lead to reduction in interfacial tension between oil and water promoting greater stability on emulsion produced (Raut et al., 2012).

Lecithin is a surfactant that featured with high biocompatibility and great emulsifying properties. LCT is commonly used as emulsifier or stabilizer, solubilizer, wetting agent and liposome former in different type of applications. It is widely used as natural surface active agent in food, cosmetic and pharmaceutical industries due to its greatest advantages such as owing its excellent toxicity and tolerability profile as well as produced using ecofriendly processes from renewable sources that contribute to lower in cost (Milić, Čalija, & Dordević, 2017).

In cosmetic applications, lecithin is commonly used in milky lotion and creams due to its refreshing feeling and softening properties when apply to skin (Mitsui, 1997).

Lecithin is beneficial in enhancing the skin lipid fluidity that enable the drug molecules from nanoemulsion to penetrate through deeper layers of skin more efficiently. The stability of lecithin-based nanoemulsion is derived from the formation of multilamellar shell around each droplet that act as effective mechanical barrier for droplet coalescence (Milić et al., 2017).

The HLB value of lecithin is 7 this indicated that lecithin is having hydrophobic properties and its molecular geometry is not perfectly suited for the formation of curved surfaces and therefore, in producing nanoemulsion, adjustment on the HLB value is needed to modify the packing characteristic of lecithin. Thus, combination with such as Tween 80, sucrose ester, poloxamer or short-chain alcohols can be performed to obtain minimal interfacial tension and stabilizing the emulsion made (Milić et al., 2017).

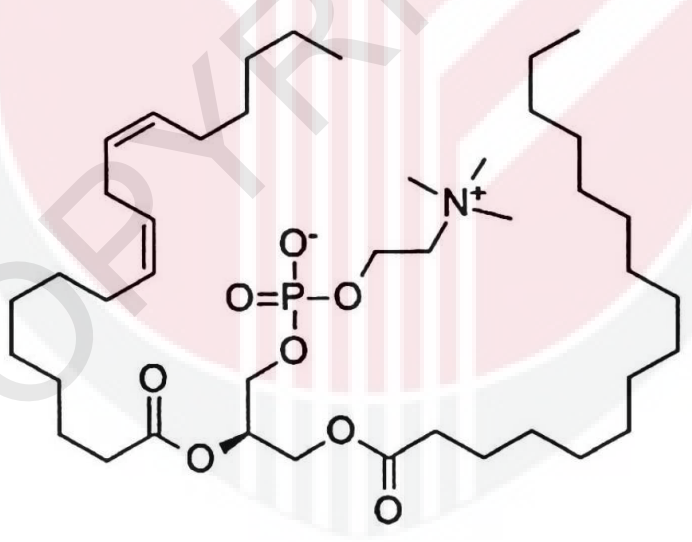


Figure 2-13: Chemical structure of lecithin. (ChemSrc, 2019)

2.5.4 Polyglycerol Polyricinoleate (PGPR)

Polyglycerol Polyricinoleate (PGPR) is a nonionic emulsifier comprised with hydrophilic polyglycerol and lipophilic ricinoleate. PGPR is formed by esterification of polyglycerol with condensed castor oil fatty acids (polyricinoleate part). Polyglycerol

moiety is composed off di-, tri- and tetraglycerol with not more than 10% heptaglycerol while the castor oil fatty acids are composed of ricinoleic acid at 80% to 90% (Mortensen, Aguilar, Crebelli, & Domenico, 2017). Polyglycerol can be prepared by heating glycerol under vacuum with potassium hydroxide as catalyst. The condensed fatty acids are prepared by heating castor oil fatty acids in the absence of oxygen (Wilson, Van Schie, & Howes, 1998). The molecular formula of PGPR used in this study is $C_{27}H_{52}O_9$ with molecular weight of 520.696 g/mol.

PGPR is a lipophilic emulsifier with HLB value at 4 that favor W/O emulsion. PGPR is an excellent W/O emulsifier due to its ability in forming stable emulsion even with high water content (Bastida-Rodríguez, 2013). PGPR is a polymeric emulsifiers that able to stretch across the interface to create a physical barrier against coalescence (Beri, Norton, & Norton, 2013). PGPR can also be used as an emulsifier and viscosity controller in cosmetics. It is advantageous in creating stable pharmaceutical or cosmetic system for complex and multiphase of which oxidation usually presence as an interface phenomena that influenced the nature of interface. PGPR has also been proven to result in smaller droplets in the formulation of cosmetics due to its ability in forming elastic interfaces that slowing the coalescence forming between droplets during producing the emulsions (Norn, 2015).

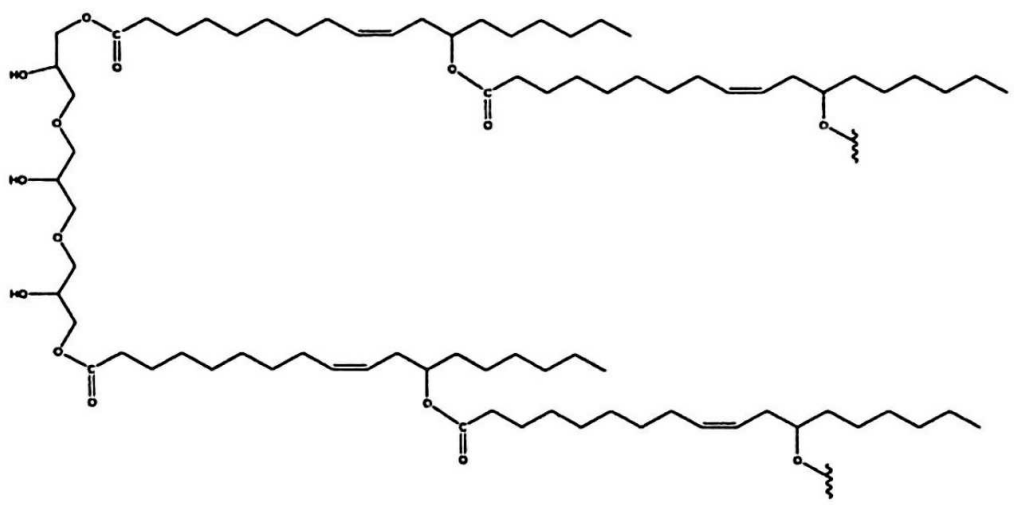


Figure 2-14: Chemical structure of polyglycerol polyricinoleate. (Mortensen et al., 2017)

2.6 Particle Size

Particle size or droplet size is a key factor in understanding the efficiency of the nanoemulsion produced which it determines the rate and extent of release and the absorption of active substances (Che Marzuki et al., 2019). Droplet size can be measured using different methods such as photon correlation spectroscopy (PCS) and light scattering techniques: static light scattering (SLS) or dynamic light scattering (DLS). Smaller droplet size can help in preventing flocculation due to high curvature and Laplace pressure that oppose the deformation of large droplets. Coalescence of droplets can also be prevented by a thick multilamelar surfactant film adsorbed over the interface of droplets. Destabilization phenomenon known as Ostwald ripening will happen when small droplets with high radius curvature are converted into large droplets with low radius of curvature or which two droplets diffuse to become one large droplets can result in failure of nanoemulsion produced (Che Marzuki et al., 2019). Constant or no change in particle size is very important to maintain the physical stability of the nanoemulsion throughout their shelf life. A large droplet size enhance the occurrence of Ostwald ripening that lead to forming of coalescence and creaming (De Azevedo Ribeiro et al., 2015).

2.7 Polydispersity Index (PDI)

Polydispersity index (PDI) is a parameter used to define the size range of lipidic nanocarrier system that is in regards to particle size distribution. PDI can also act as a parameter of quality measure on the stability, uniformity and dispersibility of nanoemulsion. Polydispersity in nanoemulsion is meant by the ratio of standard deviation to mean droplet size that reflecting the uniformity of droplet size (Che Marzuki et al., 2019). The value of PDI ranging from 0 to 1 where 0 indicating that a monodisperse droplet is formed while 1 indicating a wide ranging droplet size and it is less uniform in droplet size. It is being suggested that PDI value lower than 0.2 is desirable for nanoemulsion which means that the emulsion produced is well dispersed or homogenous in its droplet population and free from aggregation (De Azevedo Ribeiro et al., 2015). High value of PDI can lead to instability of the emulsion produced upon storage which is possibly due to lower amount of surfactant used that is inadequate to stabilize the emulsion (Ahmad, Elmarzugi, & El-Enshasy, 2013).

2.8 Zeta Potential

Zeta potential is a parameter used to describe the function of surface charge on droplets and represent the electrical characteristics of an emulsion droplets to understand the adsorption of any charged counter ions thus reflecting the electro kinetic potential in the dispersion (Che Marzuki et al., 2019). The value of zeta potential also provide the information of the tendency of particles in the emulsion produced to aggregate or to remain discrete. The particles is considered to be in stable state when the zeta potential value obtained is more than +30mV or below -30mV (Gupta & Trivedi, 2018). The rule

of thumb of zeta potential stated that the value in between -5mV to +5mV indicate rapid coagulation; the range of -20mV to +20mV indicate short term stability; and zeta potential value of more than +60mV or below -60mV results in excellent stabilized nanoemulsion. Figure 2-15 summarized the stability of nanofluids for different zeta potential values (Saxena, Kumar, & Mandal, 2018). In fact, particles with zeta potential between $|-25mV|$ to $|-30mV|$ is having an energy barrier that is strong enough to prevent destabilization of the emulsion by coalescence (De Azevedo Ribeiro et al., 2015).

Zeta potential, [mV]	Stability behavior of the colloid
0 to ±5	Rapid coagulation or flocculation
±10 to ±30	Incipient instability
±30 to ±40	Moderate stability
±40 to ±60	Good stability
more than ±61	Excellent stability

Figure 2-15: Stability of nanofluids for different zeta potential values (Saxena et al., 2018)

CHAPTER 3

METHODOLOGY

3.1 Materials

Passion fruit seed oil were purchased online from the website of iHerb.com on the brand name of Leven Rose from Colorado, USA. Sucrose ester was provided by Dai-Ichi Kogyo Seiyaku Co., Ltd (DKS), Japan. Tween 80 was purchased from Evergreen Chemical, Malaysia. Lecithin was purchased from Sigma-Aldrich Co. (St. Louis, MO) and polyglycerol polyricinoleate was purchased from Shanghai Sunwise Chemical Co., Ltd, China. Distilled water was used for preparation and producing emulsions.

3.2 Research Approach

Figure 3-1 showed the overview of the research approach, methods and analysis. For Part 1 and Part 2, IFT measurement was carried out to accomplish objective 1 which is to identify the concentration of combined surfactants and the ratio of surfactants to surfactants that result in lowest possible interfacial tension. Part 3 and Part 4 aimed to achieve objective 2 whereby the development of passion fruit oil nanoemulsion was carried out by varying the ratio of surfactant to surfactant as well as the surfactant to oil to water ratio (SOW). Particle size, polydispersity index and zeta potential measurement were carried out to evaluate the emulsion produced. Process parameters such as temperature, stirring speed and oil dropping rate were varied to produce most stable nanoemulsion with smallest particle size in accordance to complete objective 3.

The procedures for conducting the study were as followed:

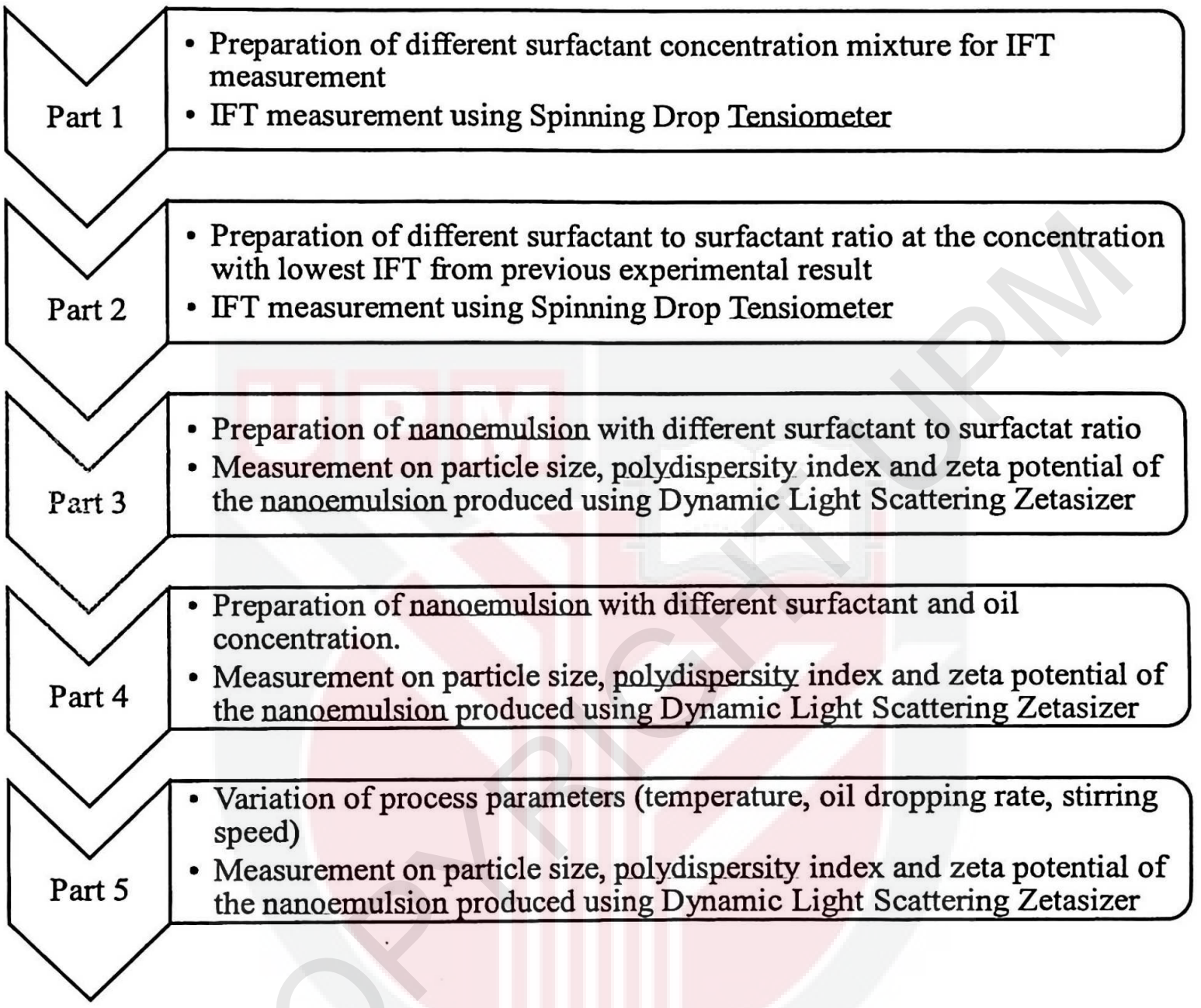


Figure 3-1: Overall methodology flow diagram

3.3 Preparation and Analysis on Interfacial Tension of Samples

3.3.1 Varying the Concentration of Surfactant in Aqueous Solution

There were five types of surfactants or combination of surfactants used in this study namely (1) Sucrose Ester:Lecithin (SE:LCT); (2) Sucrose Ester:Polyglycerol Polyricinoleate (SE:PGPR); (3) Tween 80: Lecithin (T80:LCT); (4) Tween 80:Polyglycerol Polyricinoleate (T80:PGPR); and (5) Tween 80 alone. This experiment

was conducted at constant surfactants to surfactants ratio where (a) SE:LCT at 1.5:1; (b) SE:PGPR at 1:1; (c) T80:LCT at 1.5:1; (d) T80:PGPR at 1:1; and (e) Tween 80 alone. The IFT measurement was done on varying the concentration of surfactant sets in the aqueous solution by 0%, 0.5%, 1.0%, 1.5% and 2.0% w/w.

3.3.2 Varying the Ratio of Surfactant to Surfactant

Based on the results obtained from section 3.3.1, the surfactants concentration that gave lowest IFT were used for the variation on the surfactant to surfactant ratio. The ratio used were (1) 100:0; (2) 95:5; (3) 90:10; (4) 80:20; (5) 50:50; (6) 20:80; (7) 0:100.

3.3.3 Sample Preparation

The samples were prepared by firstly dissolved the surfactants or combination of surfactants in distilled water as continuous phase using magnetic stirrer. The stirring were carried for 5 minutes on hot plate at temperature of 55°C for combination that involved sucrose ester and 35°C for combination involved Tween 80. After 5 minutes of stirring, the heat supply was stopped and the mixtures continued stirred for another 15 minutes. Then, the mixture were let to cool down to room temperature. The density of each set of samples was measured.

3.3.4 Interfacial Tension (IFT) Measurement

The interfacial tension (IFT) measurements of each set of samples were done by using Spinning Drop Tensiometer (SVT 20N, Dataphysics Instruments, Germany). The capillary tube was firstly filled slowly with an aqueous mixture until full by using syringe and needle and air bubbles must not be presented in the tube. After that, a drop of passion

fruit oil was added. The closing of the cover on the tube was on normal direction at 0° to avoid presence of air bubbles. The filled capillary tube was placed into the Spinning Drop Tensiometer. The SVT 20 software was opened and the parameters such as density, temperature and rotational speed was keyed in before locking the capillary and starting the analysis. Each set of mixture was repeated twice and the results were recorded.

3.4 Preparation and Analysis of Passion Fruit Oil Nanoemulsion

3.4.1 Varying the Ratio of Surfactant to Surfactant

Passion fruit oil nanoemulsion was prepared at constant SOW ratio at varying surfactant to surfactant ratio. Each set of combination of surfactants are carried out at different concentration. For SE:LCT, SOW is at 5:5:90 while for SE:PGPR pair was carried out at SOW at 2.5:2.5:95. The ratio of surfactant to surfactants that used to make emulsion were (1) 100:0; (2) 95:5; (3) 90:10; (4) 80:20; (5) 50:50; (6) 20:80; (7) 0:100.

3.4.2 Varying the Ratio of Surfactant to Oil to Water (SOW)

Surfactant to oil to water ratio (SOW) is in comprised to the 3 phase diagram of emulsion where the concentration of each component will affect the emulsion produced. For SE:LCT the ratio of SOW used were changed from 5-5-90 to 2.5-2.5-90 and 7.5-7.5-85. For SE:PGPR the ratio of SOW were changed from 2.5-2.5-95 to 1.5-1.5-97 and 5-5-90. Besides that, SE alone were tested with constant concentration of SE which is at 7.5% and varying the oil concentration at 1%, 3%, 5%, 7%, 7.5%, 8% and 10%. SE alone in the formulation also tested by increasing the surfactant ratio to 10% at constant concentration of PFO (7.5%).

3.4.3 Sample Preparation

First of all, the surfactants or combination of surfactants were added into distilled water and mixed using magnetic stirrer on a hot plate with speed control at 500rpm and temperature at 55°C. After 5 minutes, the mixture was continue stirred at the same speed with reduced temperature to 25°C for another 15 minutes. Then, passion fruit oil was added slowly at dropping rate of 1 drop/20 seconds into the mixture using 5ml transfer pipette with tip diameter of 2mm (refer Figure 3-2). After that, the mixture was continued stir at 25°C on same speed for another 15 minutes to allow the homogenization of the emulsion. Each set of samples was repeated twice.

Figure 3-2: Transfer pipette used for oil dropping

3.4.4 Particle Size, Polydispersity Index (PDI) and Zeta Potential Measurement

The measurement of passion fruit oil nanoemulsion on the particles size, polydispersity index and zeta potential were done by using dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments Ltd, Malvern, UK). The measurement is carried out on the next day after sample preparation. Firstly, the

nanoemulsion prepared were diluted with distilled water at ratio of distilled water: emulsion at 9:1 to minimize multiple scattering effects. Then, the diluted nanoemulsion were tested on the mean droplet diameter and polydispersity index (PDI) in particle size cell probe with cuvette at appropriate setting. The placement of the cuvette must be correct to allow the light penetration for obtaining the results. For measurement of zeta potential, zeta potential cell probe was used with SOP setting for the test. Syringes were used to transfer the diluted emulsion to the cell probe slowly to avoid formation of air bubbles.

3.5 Variation on Process Parameters in Preparation of Passion Fruit Oil Nanoemulsion

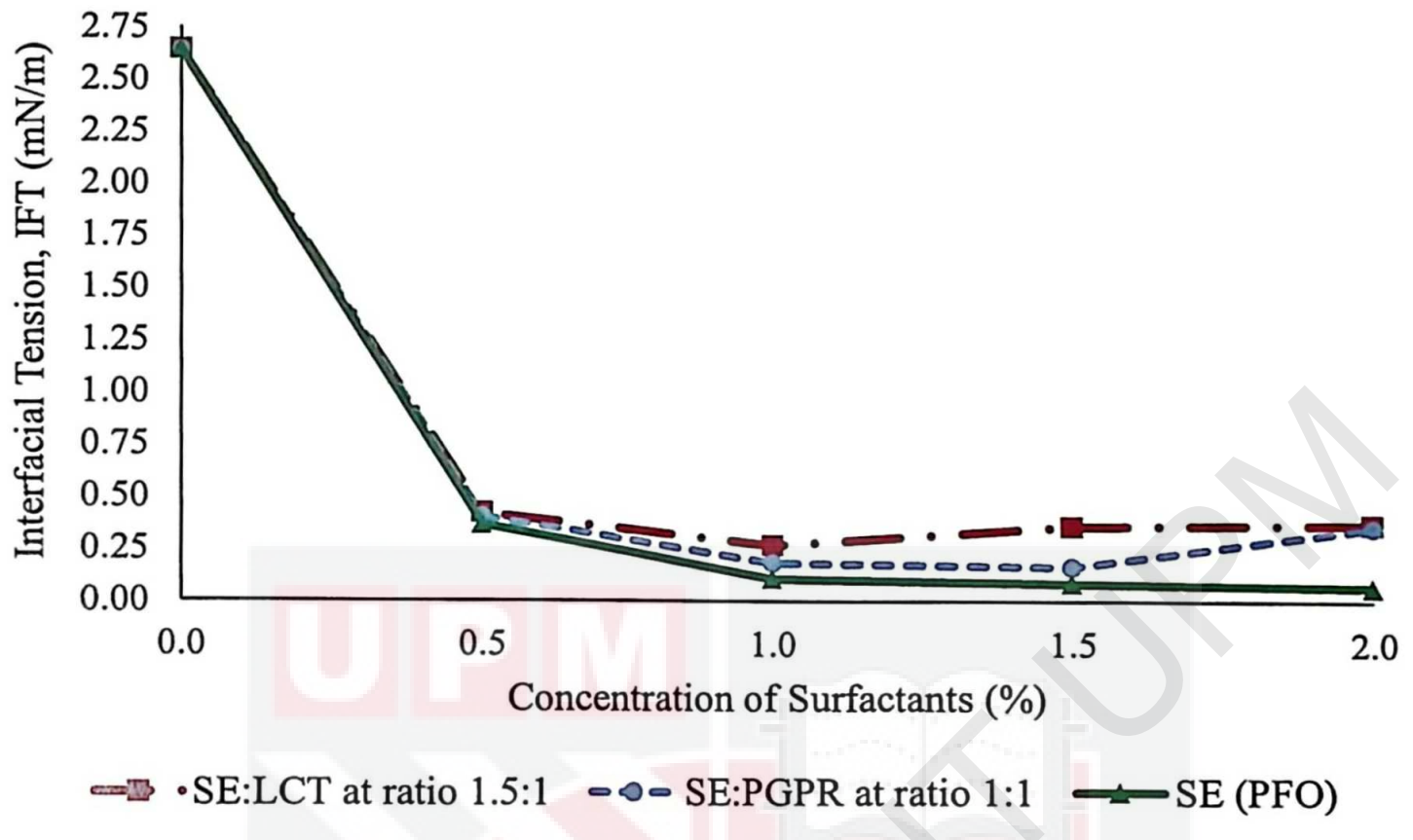
Variation on the process parameters in producing nanoemulsion including mixing temperature, stirring speed and oil dropping rate. In the variation of mixing temperature, the sample preparation is similar as above whereby the final mixing temperature after passion fruit oil added to the continuous phase was varied to identify the effect of increasing mixing temperature on the emulsion produced and hence the optimum mixing temperature can be identified. The mixing temperature was increased by 5°C from 25°C to 55°C. For the parameter of stirring speed, it is being increased and decreased at interval of 100rpm and therefore the initial speed of 500rpm was varied to 300rpm; 400rpm; 600rpm; and 700rpm. This is used to identify the changes in the stirring speed on the emulsion produced and hence the optimum speed can be known. Oil dropping rate is being varied from 1 drop/20 seconds to all oil dropped at once; 1 drop/10 seconds; 1 drop/30 seconds; and 1 drop/40 seconds. The procedures to analyze the particle size and polydispersity index (PDI) are the same as above.

CHAPTER 4

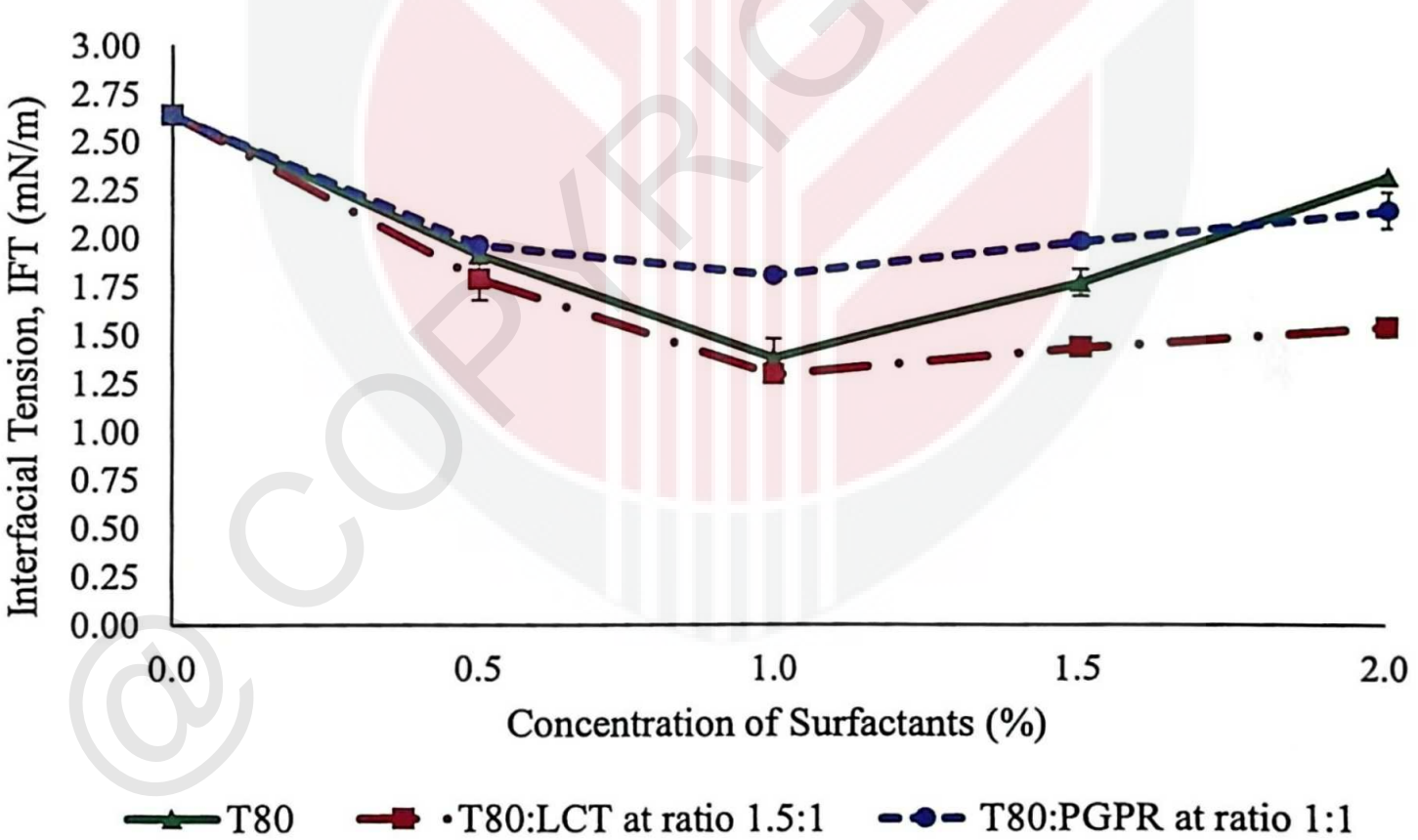
RESULTS AND DISCUSSION

4.1 Effect of Surfactant Concentration on Interfacial Tension for Different Surfactant Combination with Passion Fruit Oil

Based on Figure 4-1(a), the lowest IFT for SE:LCT combination was at the concentration of 1% which result in IFT of 0.27mN/m while for the combination of SE:PGPR, the lowest IFT is 0.17mN/m at 1.5% concentration of surfactants. For SE alone used in the system, the lowest IFT, 0.069mN/m is achieved at concentration of 2%. The lowering of IFT between oil and water is achieved when surfactants are adsorbed on the liquid-liquid interface (Hirasaki, Miller, & Puerto, 2011). With the increase in concentration of surfactants, the reduction in IFT was observed up to a minimum value which is also known as critical micelle concentration (CMC). Similar profile was seen with Tween 80 and its combination as shown in Figure 4-1 (b). The IFT is reduced from 2.64mN/m (at 0% concentration of surfactants) to 1.92mN/m for Tween 80 alone; 1.79mN/m for T80:LCT; and 1.97mN/m for T80:PGPR at 0.5% concentration. Both T80 alone and T80:LCT achieved the lowest IFT at 0.5% while for T80:PGPR the lowest IFT was observed at 1.0% concentration with the value of 1.81mN/m. Change in surfactant concentration will affect the IFT where higher concentration of surfactant in the solution will lead to a higher density of surfactant molecules at the interface, which in turn leads to a lower IFT (Aveyard, Binks, & Mead, 1985). Comparing between SE and T80, it can be seen that SE and its combination are able to reduce IFT to much lower compare to T80. The range of concentration that is expected to produce nanoemulsion is up to 1% and a higher concentration may not be beneficial or may even be contribute to the rise in IFT.



(a)



(b)

Figure 4-1: Graph of interfacial tension (IFT) against different concentration of surfactants for (a) sucrose ester and its combination and (b) tween 80 and its combination

4.2 Effect of Surfactant to Surfactant Ratio on Interfacial Tension for Different Surfactant Combination with Passion Fruit Oil

The concentration of surfactant is kept constant at the CMC (from Part 4.1) and the ratio of surfactants to surfactants is varied. For the combination of SE:LCT and T80:PGPR, 1% of surfactant concentration were used while for SE:PGPR was 1.5% surfactant concentration.

Based on Figure 4-2, when sucrose ester is used alone, the IFT value is lowest. Increasing the amount of lecithin in the combinations tend to increase the IFT value. This is in comprised with the reduction on HLB value. For the combination involving sucrose ester, at the ratio between SE:LCT and SE:PGPR at 95:5 to 80:20, the IFT is similar. After the ratio of surfactant reached 50:50, there is drastic increase in the IFT on further increase the amount of lipophilic surfactants. When T80:PGPR is used in the system, increasing the amount of PGPR tend to increase the IFT. Addition of PGPR for T80:PGPR at 95:5 has showed significant increase in the IFT compared to absence of PGPR in the system.

For the combination between T80 and PGPR, increase the amount of PGPR in the system tend to increase in the IFT value. When T80 was used alone in the system, although the HLB is high (at 15), it is not able to achieve low IFT. Figure 4-3 showed that similar HLB between combination involving SE and T80 do not result in similar IFT. This is related to the molecular weight of the surfactants where T80 is much greater than SE leading to higher viscosity and affect the mobility of surfactant in reaching to the interface of oil and water (Gbadamosi, Junin, Manan, Agi, & Yusuff, 2019). This has indicated that combination between T80 and PGPR might not be suitable for producing nanoemulsion

since the interfacial tension between oil and water interface could not be lower using this combination. Hence, it has found out that packing geometry is the key point when preparing for an emulsion. Based on the IFT, it can be inferred that the ratio of 100:0 to 80:20 give a low IFT which is more possible to produce nanoemulsion while the ratio between 50:50 to 0:100 are not able to achieve small particle size since the IFT at these ratio could not be lowered. On the other hand, T80 and its combinations has a very high IFT and it is unlikely to produce nanoemulsion with the low energy method. Therefore, combination of SE:LCT and SE:PGPR will be used in the preparation of emulsion using low energy approach.

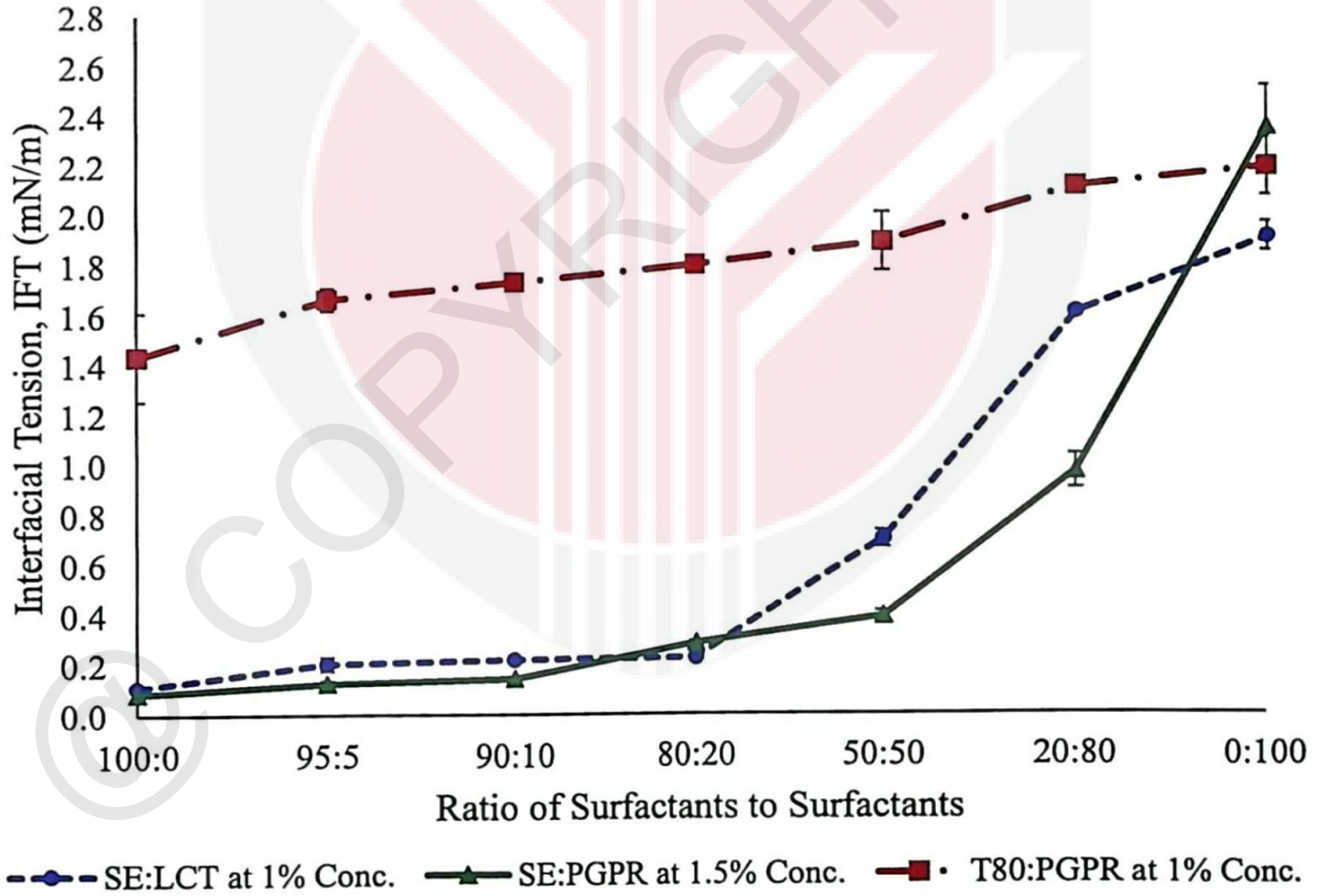


Figure 4-2: Graph of interfacial tension (IFT) against different surfactants to surfactants ratio for Sucrose Ester:Lecithin (SE:LCT); Sucrose Ester:Polyglycerol Polyricinoleate (SE:PGPR); and Tween 80:Polyglycerol Polyricinoleate (T80:PGPR)

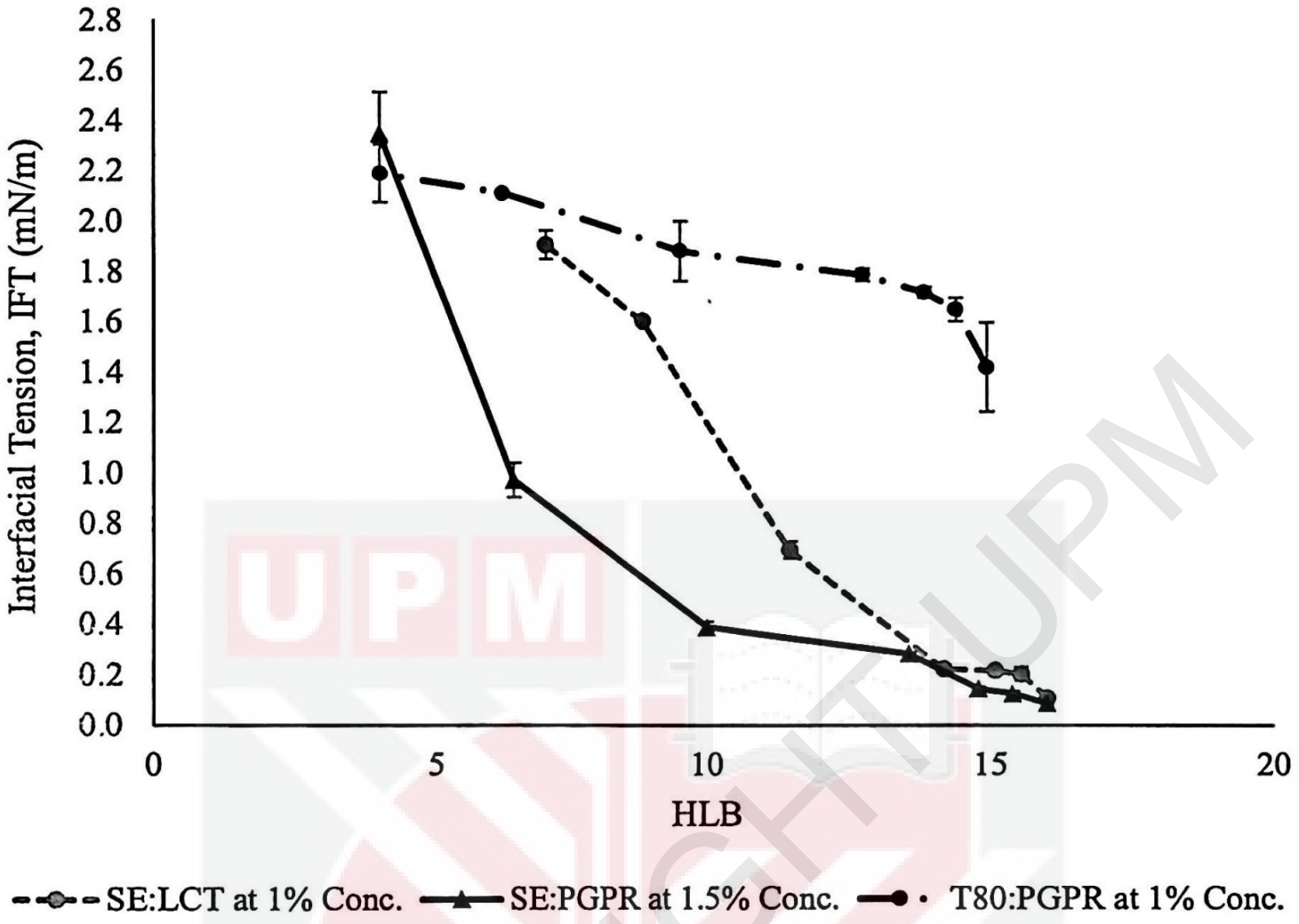


Figure 4-3: Graph of interfacial tension (IFT) against hydrophilic-lipophilic balance (HLB) for Sucrose Ester:Lecithin (SE:LCT); Sucrose Ester:Polyglycerol Polyricinoleate (SE:PGPR); and Tween 80:Polyglycerol Polyricinoleate (T80:PGPR)

4.3 Effect of Varying Surfactant to Surfactant Ratio on Particle Size, Polydispersity Index (PDI) and Zeta Potential of the Emulsion Produced

Based on Figure 4-4 (a), increase the concentration of lecithin to the ratio of SE:LCT at 75:25 showed decreasing trend in the particle size. At the ratio of SE:LCT at 75:25, the particles size obtained is 336nm. Further increase in the concentration of lecithin causing separation of the mixtures. This can be explained in comprise to the IFT obtained where increase the concentration of lipophilic surfactants causing IFT to increase further. Based on the IFT results, even though the SE alone result in lowest IFT, the packing geometry and the balance between the hydrophilic groups and lipophilic groups as well as the concentration of surfactants used will affect the particle size of the emulsion produced in different extent. At the ratio of SE:LCT at 75:25, it can be inferred that the balance between hydrophilic and lipophilic groups are more optimum and the packing geometry is more favorable to result in smaller particles size.

Based on Figure 4-4 (b), for the combination of SE:PGPR at ratio of 100:0 and 95:5, separation has observed. The smallest size obtained from this formulation is at the ratio of SE:PGPR at 90:10 which achieved the particles size of 493.4nm. When increasing the concentration of PGPR, the particles size increase sharply and separation occurred at the ratio of 20:80 and 0:100. Looking on the IFT result for SE:PGPR, the IFT is lower (0.12mN/m) among the other combination. However, at the concentration of 2.5% surfactants and 2.5% PFO, particle size of the emulsion produced is large. On the other hand, it can be seen that even IFT is smaller when there is absence of PGPR, emulsion could not be achieve. Thus, packing geometry between combinations of surfactants is important to provide bilayer structure for the formation of nanoemulsion.

According to Figure 4-5, similar HLB between SE:LCT and SE:PGPR do not result in same particle size. This is because HLB is a universal property as it exclude the molecular weight and chemical nature of surfactants. Therefore, it is again down to the point of packing geometry between combinations of surfactants determine the outcome of the emulsion produced. Based on the formulation that result in smallest particle size, more lecithin is required than PGPR when combined with sucrose ester. This is because PGPR has greater molecular weight than lecithin hence lower concentration is required.

Polydispersity index (PDI) is defined as the degree of non-uniformity of a size distribution of particles. It represent the distribution of size population within a given sample that ranging from 0 to 1 where 0 indicating a perfectly uniform sample with respect to particle size while 1 indicating a highly polydisperse sample with multiple particle size populations. PDI of 0.3 and below is considered to be acceptable and indicates a homogenous population (M. Chen, Liu, & Fahr, 2011). From Table 4-1 and Table 4-2, the decrease in the particle size of the emulsion produced also lead to decrease in the PDI that indicating a more uniform particle distribution is obtained. It is being revealed that a low degree of polydispersity was obtained with the use of high HLB values. This high HLB lead to low coalescence rate and contribute to low polydispersity and more uniform particles are formed (Lindner, Bäumler, & Stäbler, 2018). Reducing the HLB of the surfactants used lead to increase in the particle size which at the same time increase the PDI of the system indicated that the uniformity is not achieved since low HLB is not suitable for the formation of o/w emulsion.

Zeta potential indicates the electrostatic attraction or repulsion between dispersed phase droplets that affect the stability of the formulation. The magnitude of the charge on

droplets influences the droplet coalescence that should be avoided in producing a stable nanoemulsion for enhancing the shelf life. The greater the chance of droplet coalescence and break up due to excessive attraction between the droplets at lower zeta potential value (Kumar, Ali, & Baboota, 2016). Based on Table 4-1 and Table 4-2, it can be noticed that the zeta potential measured for each sample of emulsion produced are at the range of greater than -70mV which means that it is very stable emulsion. This is because the surfactants used for produced the emulsion are nonionic type and it does not significantly affect the zeta potential and aggregation stability of the emulsion produced (Petryshyn, Yaremko, & Soltys, 2010). The emulsions with an HLB value above 11 indicated higher zeta-potential values than the emulsions with an HLB value under 10. This result is probably associated with the presence of the polyoxyethylene group. The zeta potential value can be increased due to the hydrogen bonds with the OH^- groups, hydrogen bonds at the ether-oxygen of the polyoxyethylene chain, with the succeeding oxonium formation. Therefore, increasing of the polyoxyethylene group per chain makes the zeta potential value higher. However, as the surface concentration of the chain increases, the ether-oxygen becomes closer to the surface of the emulsion, resulting in shielding and the zeta-potential value decreases due to the crowding effect. In conclusion, if the HLB value increases beyond the critical point, the zeta-potential value becomes smaller (Hong, Kim, & Lee, 2018).

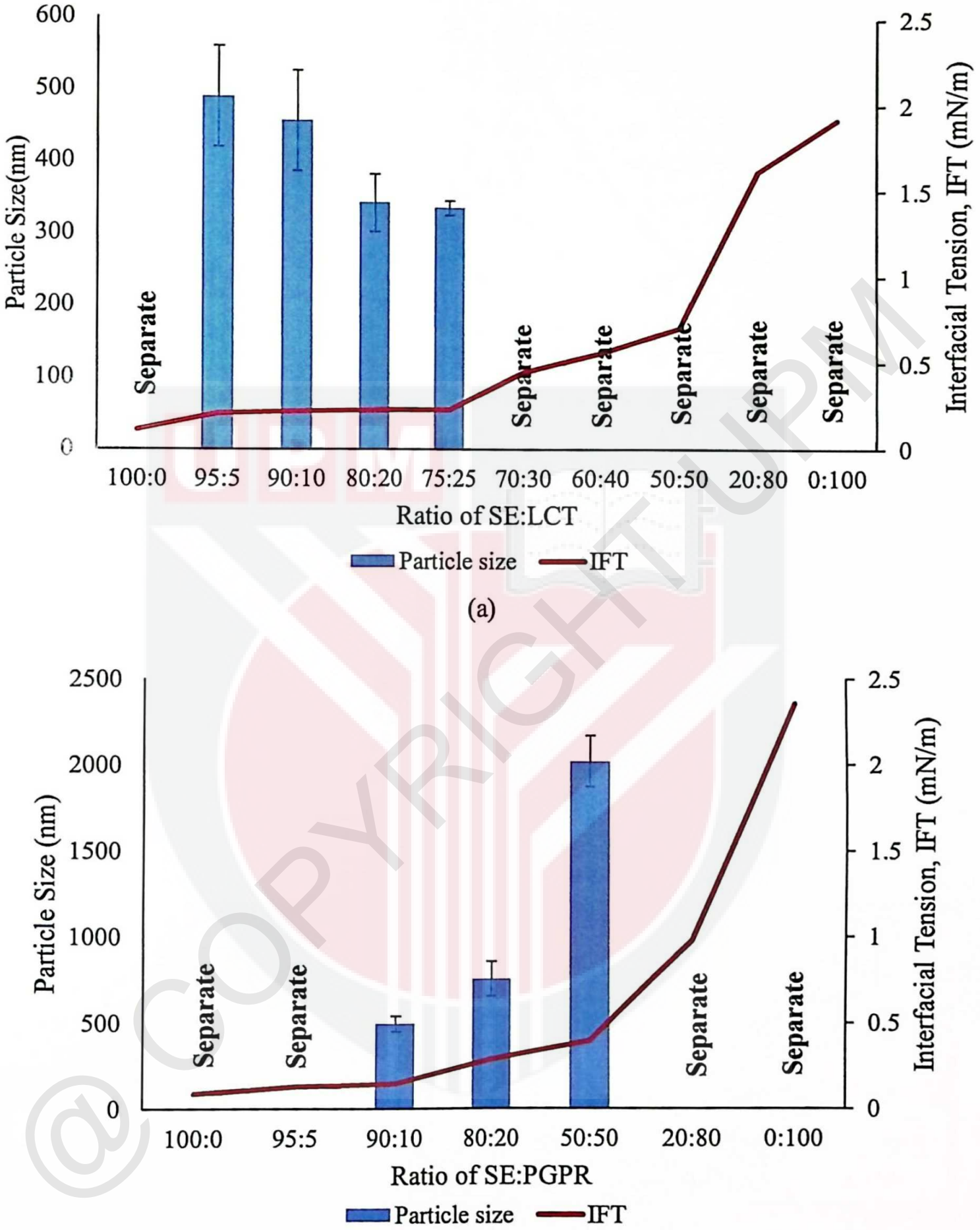


Figure 4-4: Graph of particle size against ratio of surfactants to surfactants at constant process parameters of 25°C mixing temperature, 500rpm stirring speed and 1drop/20secnds oil dropping rate (a) SE:LCT at 5% concentration; and (b) SE:PGPR at 2.5% concentration

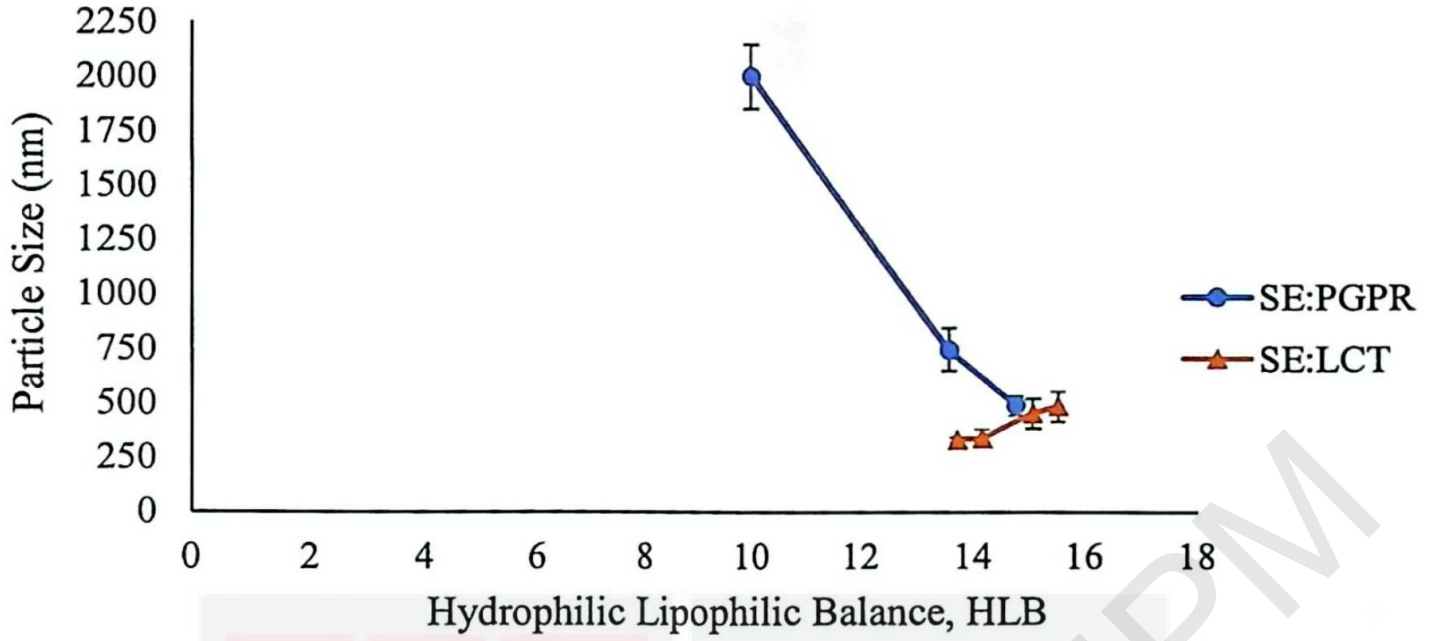


Figure 4-5: Graph of particle size against HLB of surfactants

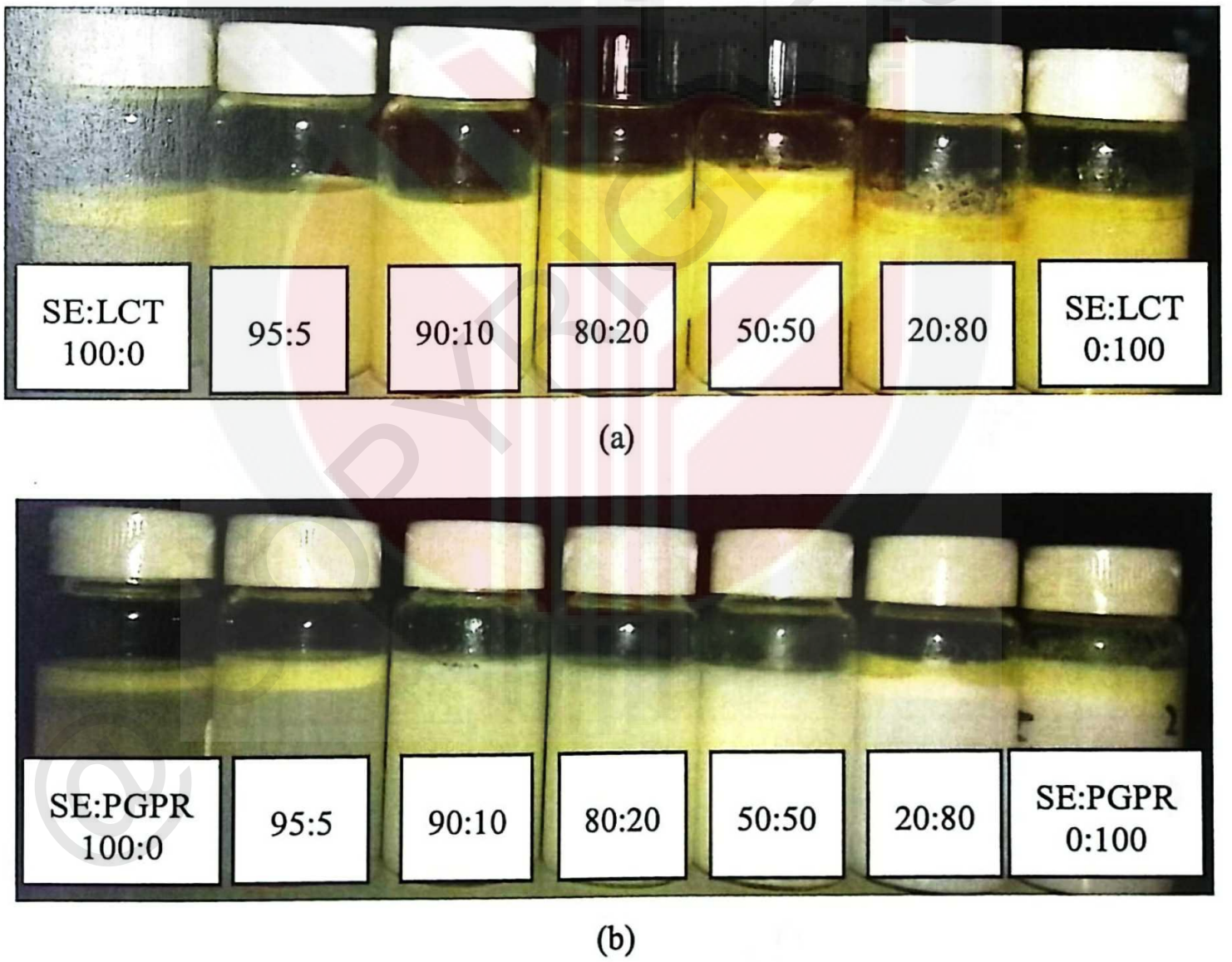


Figure 4-6: Passion fruit oil emulsion with process parameters of 25°C mixing temperature, 500rpm stirring speed and of combination (a) sucrose eater:lecithin (SE:LCT) at 5% PFO and 5% surfactants and (b) sucrose ester:polyglycerol polyricinoleate (SE:PGPR) at 2.5% PFO and 2.5% surfactants.

Table 4-1: Results of combination of sucrose ester:lecithin (SE:LCT) on passion fruit oil emulsion at 5% PFO and 5% surfactants.

HLB	Ratio of SE:LCT	Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
16	100:0	separate		
15.55	95:5	489.8	0.311	-77.92
15.1	90:10	456.7	0.288	-76.76
14.2	80:20	342.6	0.235	-79.67
13.75	75:25	336.0	0.266	-85.56
13.3	70:30	separate		
12.5	60:40	separate		
11.5	50:50	separate		
8.8	20:80	separate		
7	0:100	separate		

Table 4-2: Results of combination of sucrose ester:polyglycerol polyricinoleate (SE:PGPR) on passion fruit oil emulsion at 2.5% PFO and 2.5% surfactants.

HLB	Ratio of SE:PGPR	Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
16	100:0	separate		
15.4	95:5	separate		
14.8	90:10	493.4	0.314	-85.60
13.6	80:20	752.4	0.557	-73.99
10	50:50	2018.6	0.763	-69.99
6.4	20:80	separate		
4	0:100	separate		

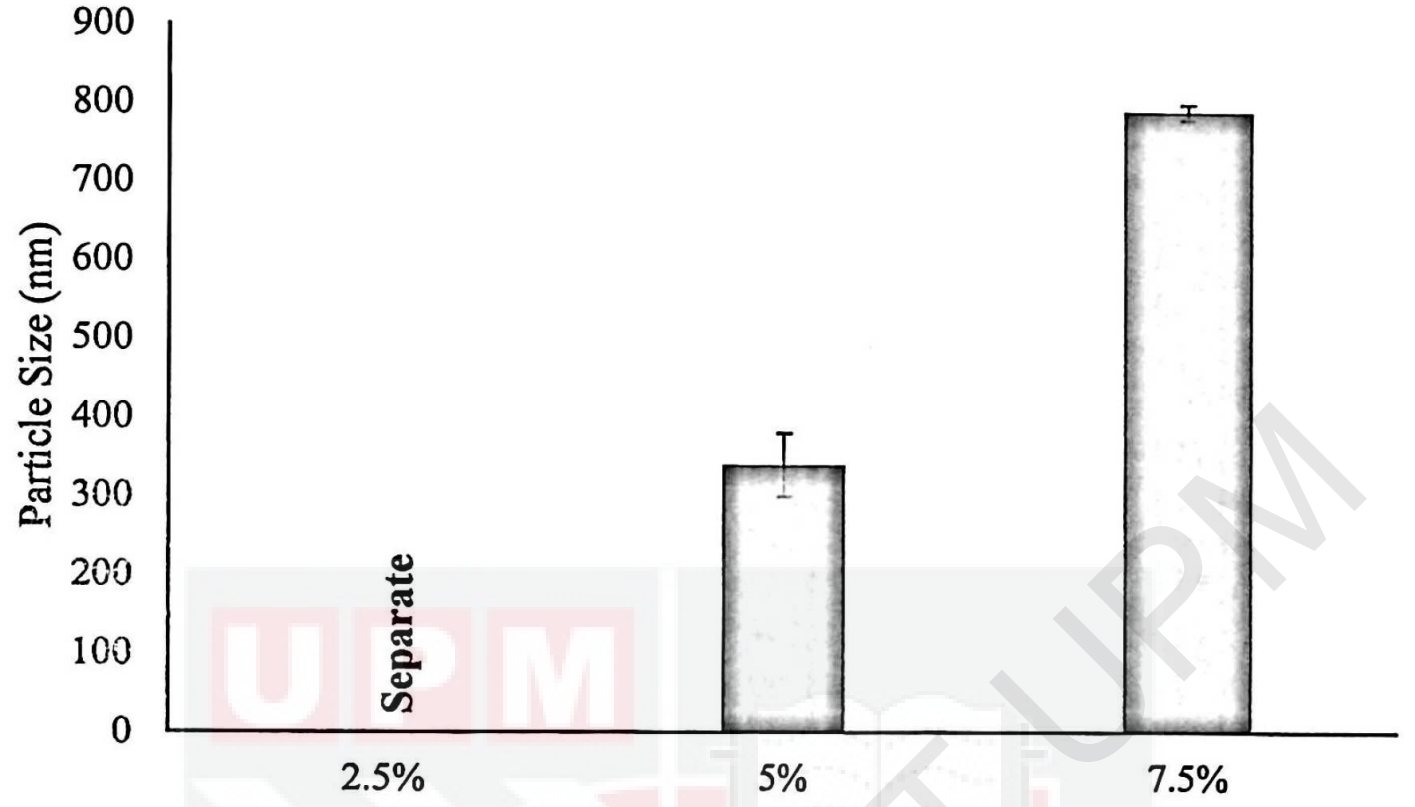
4.4 Effect of Surfactant to Oil to Water Ratio (SOW)/ 3 Phase Diagram on Particle Size and Polydispersity Index (PDI) of the Emulsion Produced for Different Surfactant Combination with Passion Fruit Oil

4.4.1 Effect of Surfactant Concentration on Particle Size for the Emulsion Produced Using Combination of Sucrose Ester: Lecithin (SE:LCT) and Sucrose Ester: Polyglycerol Polyricinoleate (SE:PGPR)

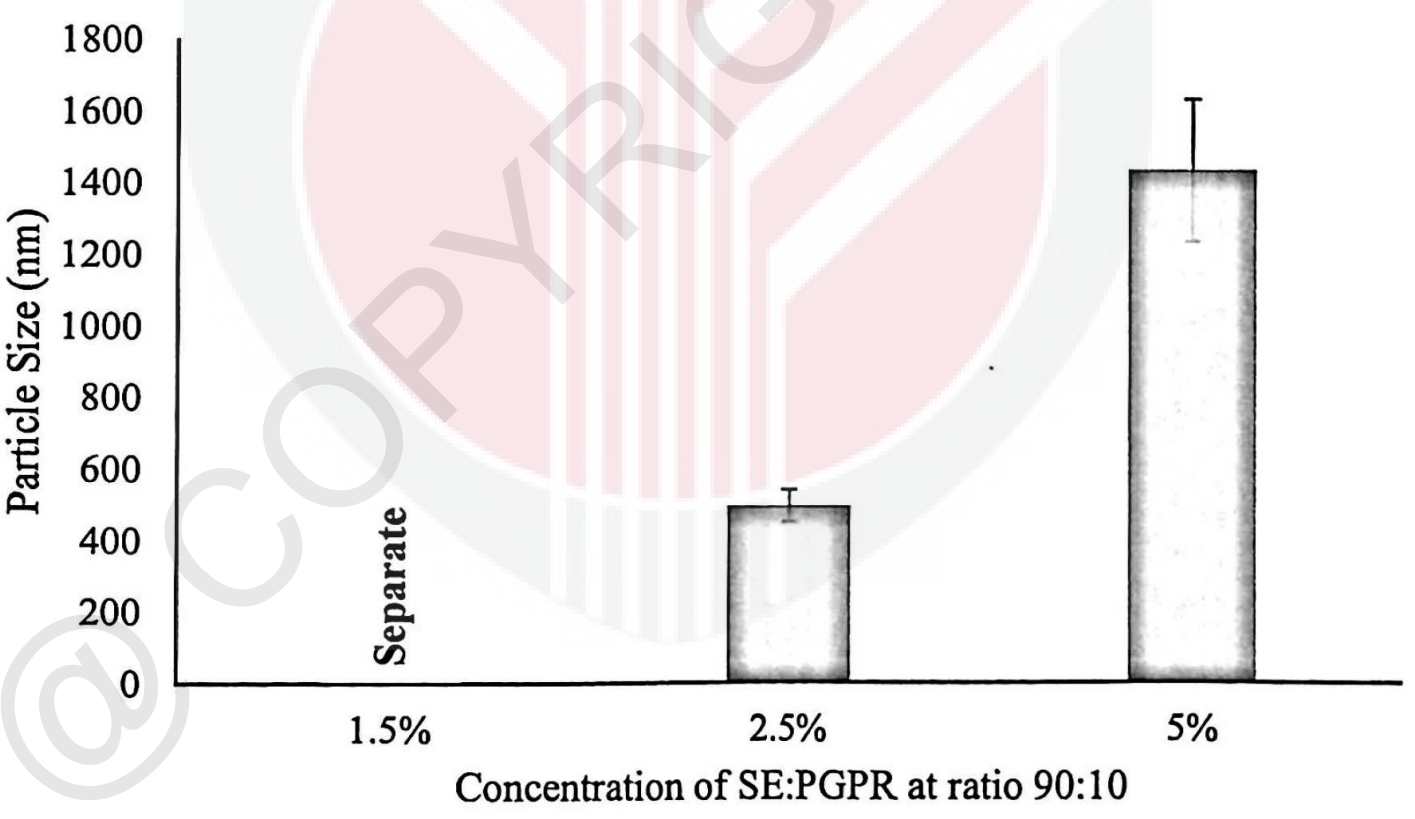
Based on Figure 4-7 (a), when decreasing concentration of combined surfactants for SE:LCT at constant ratio 80:20 to 2.5%, separation between oil and aqueous phase are observed for different surfactants ratio combination. This is because at low surfactants concentration, the system is unstable as there is insufficient surfactants to reduce oil-water interfacial tension. Increasing the concentration of surfactants from 5% to 7.5% lead to increase in particle size from 342.6nm to 798.1nm. The formulation of combined surfactants of SE:LCT at 7.5% is not preferable since it could not achieve nanoemulsion. Increasing the concentration of surfactants further can cause surfactant molecules to aggregate and produce elongated tube and stacked lamellae at CMC lead to increase in particle size (Shih, Lin, Panjaitan, & Sari, 2016). Therefore, the formulation for combination of SE:LCT that is used for process parameters variation section is 5% PFO, 5% surfactants at ratio of SE:LCT as 75:25 with 90% water at which the smallest particle size can be obtained.

Refer to Section of 4.3, combination of SE:PGPR at 2.5% PFO and 2.5% surfactants is being modified to increase surfactants concentration to 5% as well as decrease that to 1.5%. Figure 4-7 (b) has showed that for 90:10 SE:PGPR, at the

formulation of 1.5% surfactants, separation between oil and aqueous phase was observed which indicated that emulsion do not take place. This could be due to the fact that lowering the concentration of surfactants causing there is inadequate surfactants to reduce oil-water interfacial tension and it does not facilitate formation of emulsion (Jusoh & Othman, 2016). Again, increasing the concentration of surfactants to 5% did not enhance the particle size of the emulsion produced to smaller results. This is because increasing the concentration between SE:PGPR causing the solution to be more viscous and milky. Nanoemulsion could not be achieved in higher concentration of surfactants used for the formulation involved SE:PGPR. The smallest particle size that can be achieved by this formulation is 979.5nm at the ratio of 80:20. Therefore, this formulation resulted to be not preferable since it do not show the ability in producing nanoemulsion. At the next section, evaluation on the changes in the concentration of oil is done to identify the optimum oil concentration in producing nanoemulsion at constant 2.5% surfactants concentration. It is being concluded from this section that optimum surfactants concentration is very important to allow formation of appropriate structure that promote formation of nanoemulsion. The recommended used of surfactants in a formulation of skin care products is within the range of 2% to 8%.



(a)



(b)

Figure 4-7: Graph of particle size against concentration of surfactants at 25°C mixing temperature, 500rpm stirring speed and 1drop/20sec oil dropping rate for (a) SE:LCT at 80:20 and (b) SE:PGPR at 90:10

4.4.2 Effect of surfactant to oil ratio on Particle Size and Polydispersity Index (PDI) for the Emulsion Produced Using Combination of Sucrose Ester: Polyglycerol Polyricinoleate (SE:PGPR)

The effect of surfactant to oil ratio is investigated using formulation of SE:PGPR at 90:10 since it did not result in small particle size despite having a low IFT similar to SE:LCT. The new formulation is SE:PGPR at ratio of 90:10 in 2.5% concentration with 1.5% PFO and 3.5% PFO. Figure 4-8 and Table 4-3 showed that both increasing and decreasing the concentration of PFO causing separation between oil and aqueous phase. Increase in the oil concentration led to decrease in specific surface area and increase in polydispersity of emulsion at lower surfactant concentration and less homogenization which may result in separation of oil and water (Dapčević Hadnadev, Dokić, Krstonošić, & Hadnadev, 2013). Reducing oil concentration do not result in smaller particle size too. This inferred that the concentration of oil must be optimum in the formulation in order to produce nanoemulsion. As a result, to proceed with the effect of process parameters, formulation involve SE:PGPR that is being chosen is 2.5% PFO, 2.5% surfactants at ratio of SE:PGPR as 90:10 with 95% water that results the smallest particles size among all formulations of SE:PGPR. In an O/W emulsion type of cosmetic product, the recommended used of seed oil is about 0.5% to 5%. Too high concentration of oil can lead to destabilization on the products produced upon storage.

Table 4-3: Results of varying oil concentration at constant surfactant:surfactant ratio

PFO (%)	Water (%)	Ratio of Surfactant Combination at 2.5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		SE	PGPR			
1.5	95	90	10	separate		
2.5	95	90	10	493.4 ± 45	0.314	-85.6033
3.5	95	90	10	separate		

Figure 4-8: Passion fruit oil emulsion of combination of sucrose ester:polyglycerol polyricinoleate (SE:PGPR) of 2.5% surfactants at (1) 1.5%; (2) 2.5% and (3) 3.5% PFO

4.4.3 Effect of Oil Concentration in Surfactant to Oil to Water Ratio (SOW) on Particle Size and Polydispersity Index (PDI) for the Emulsion Produced Using Sucrose Ester alone (SE)

From Section 4.4.1, SE alone at 7.5% concentration with 7.5% PFO resulted in lowest particle size among all formulation of passion fruit oil emulsion. Therefore, variation on oil concentration is done to identify the optimum concentration between the components in producing nanoemulsion. Figure 4-9 showed that smallest particle size is obtained at 1% PFO and 7.5% SE which is 145.2nm. At constant surfactant concentration

of 7.5%, increasing the oil concentration lead to increase in the particle size. This can be due to that inadequate surfactants concentration required to cover the oil droplets and increased coalescence due to increased oil concentration (McClements, 2015). However, in the formulation of 1% PFO, the PDI value is not the lowest which indicating that there is non-uniformity in the particle distribution.

Figure 4-11 showed that at constant PFO concentration, increased the surfactant concentration to 10%, the particles size increase sharply from 266.8nm to 723.5nm. This indicated that there is excessive surfactants in the mixtures. This can be explained as the CMC has reached where upon increase the surfactant concentration lead to increase in the viscosity of the dispersion medium that correspond to low diffusion rate of the surfactant molecule to the surface of oil droplets result in difficulties to stabilize the oil droplets against coalescence in short time (Rehfeld, 1967). Since low oil concentration is not preferable with high surfactant concentration, therefore, 5% PFO with 7.5% SE and 87.5% water is used for variation on its process parameters.

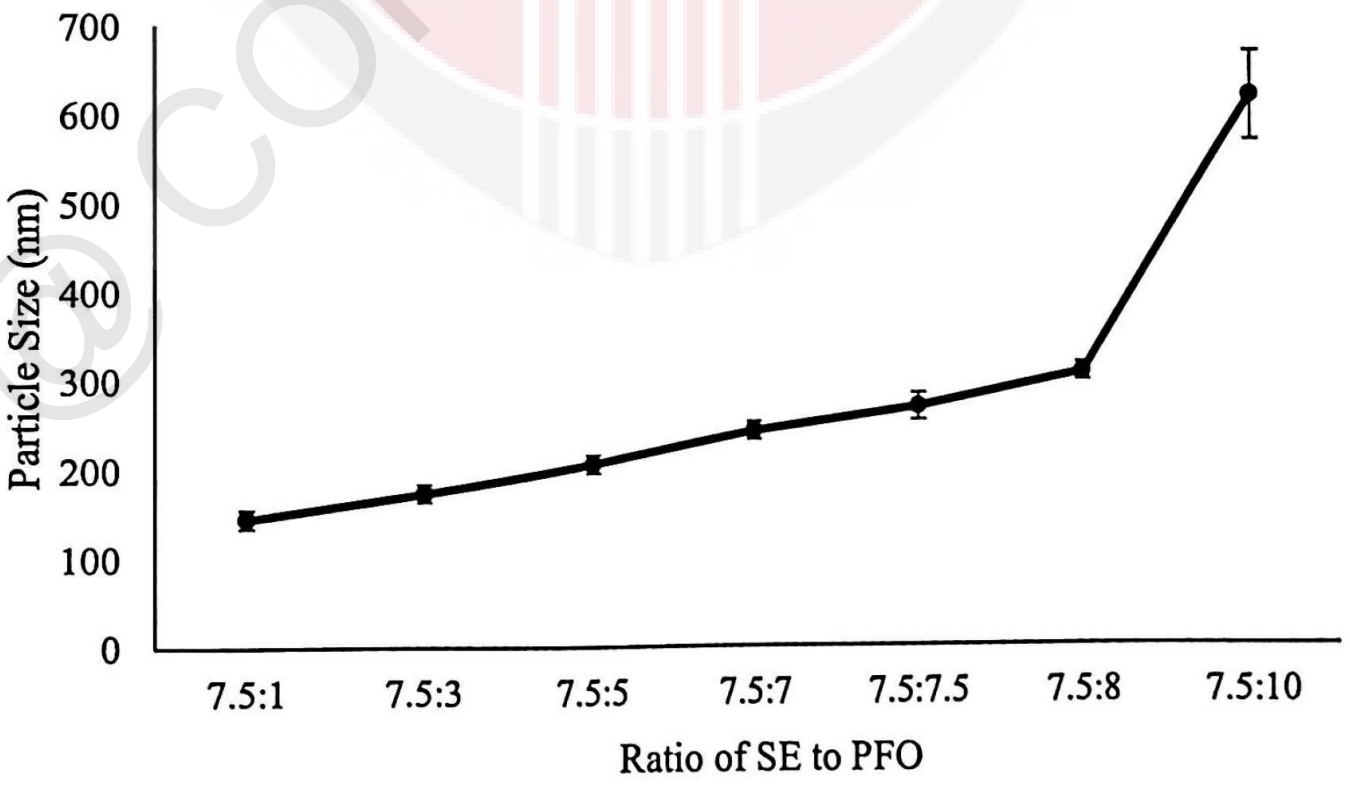


Figure 4-9: Graph of particle size against ratio of sucrose ester (SE) to passion fruit oil (PFO)

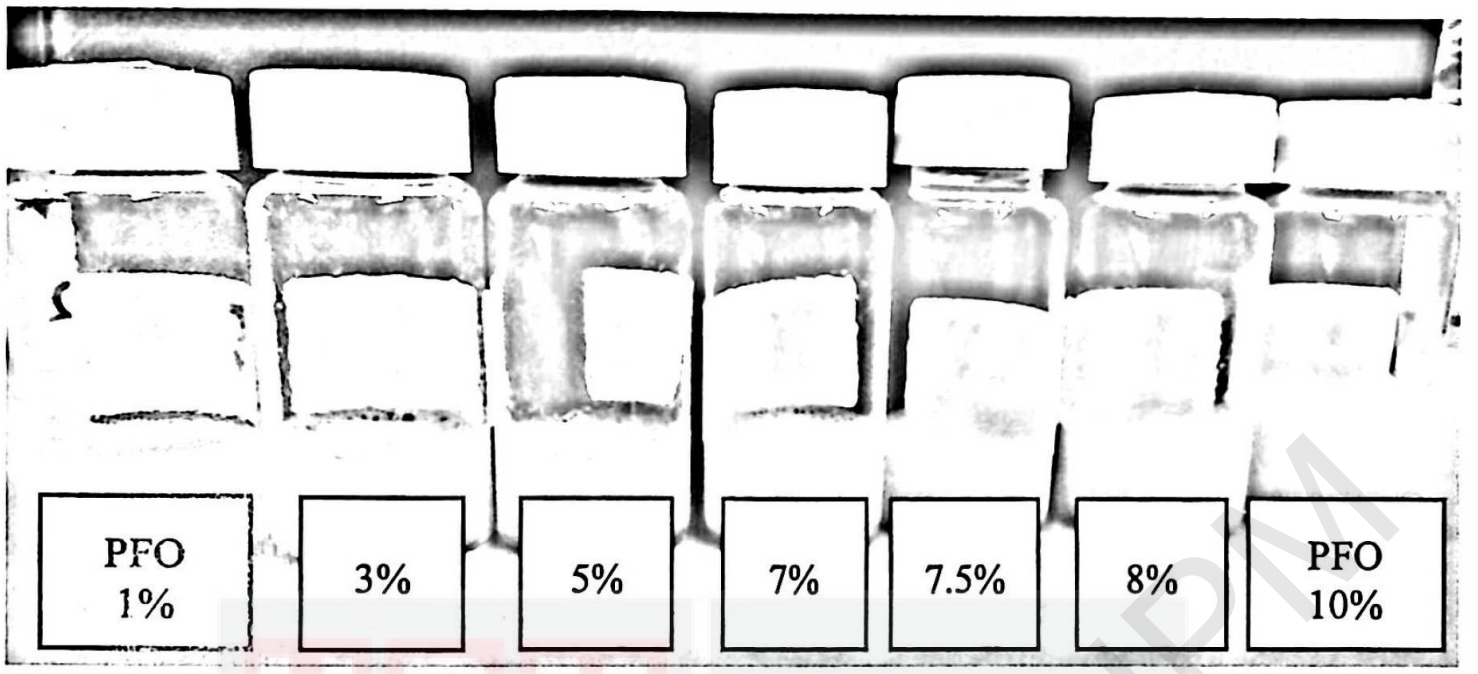


Figure 4-10: Passion fruit oil emulsion of sucrose ester (SE) at 7.5% and PFO at different concentration (1) 1%; (2) 3%; (3) 5%; (4) 7%; (5) 7.5%; (6) 8%; and (7) 10%

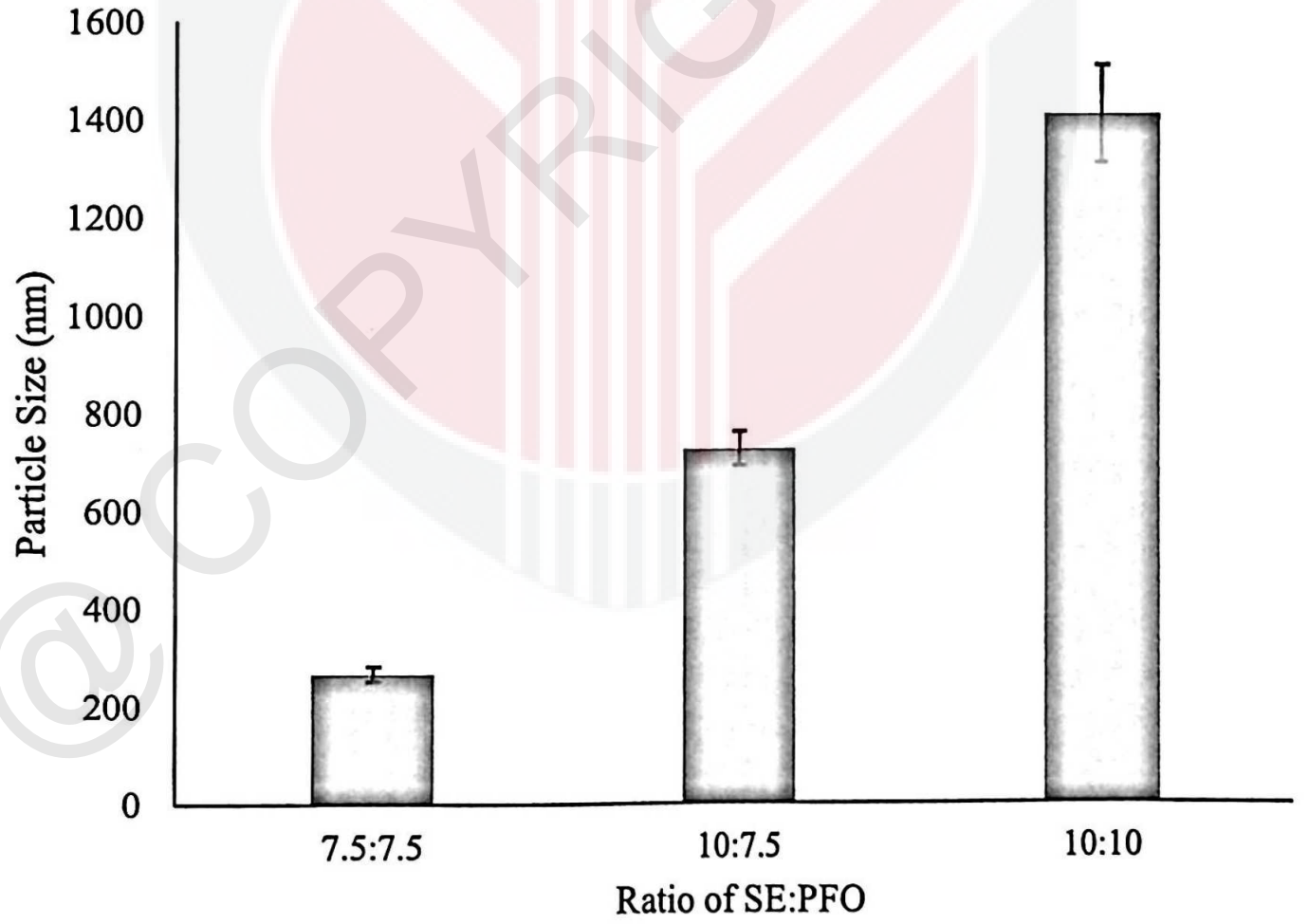


Figure 4-11: Graph of particle size against ratio of sucrose ester (SE) to passion fruit oil (PFO)

4.5 Effect of Process Parameters (Mixing Temperature, Oil Dropping Rate and Stirring Speed) on the Particle Size and Polydispersity Index (PDI) of the Emulsion Produced

4.5.1 Effect of Mixing Temperature on the Particle Size and Polydispersity Index (PDI) for the Emulsion Produced Using Different Formulation. (1) Sucrose Ester: Lecithin (SE:LCT); (2) Sucrose Ester: Polyglycerol Polyricinoleate (SE:PGPR); and (3) Sucrose Ester alone (SE).

Temperature parameter has a strong effect in the physical properties of a system. For instance, the interfacial characteristics of oil and water, and emulsifying agent solubility are depending on the thermodynamic conditions. Temperature plays an important role in emulsification especially when nonionic surfactants system were used. Temperature factor cause effect in the viscosity and also interfacial tension of nonionic surfactants as well as the interaction forces within the system (Zakaria et al., 2012). Based on the selection of formulation in section 4.4, the mixing temperature are varied from 25°C to 55°C with increment of 5°C.

Based on section 4.4.1, the selected formulation is 5% PFO, 5% surfactants at ratio of SE:LCT as 75:25 with 90% water. Figure 4-12 showed that increasing the mixing temperature from 25°C to 50°C having decreasing trend in the particle size of the emulsion produced. The particle size decreased from 336nm to 215.6nm which is close to the desired region on producing nano-size. This can be explained as the utilization of energy has been absorbed or utilized by emulsion droplets to be more kinetically energetic and thus induced less occurrence of aggregation. Besides that, for nonionic surfactant with

higher HLB value, to maintain the actual HLB value of the system, the emulsion temperature is preferred to be increased (Liu et al., 2006). The head group of surfactant become progressively dehydrated with increasing temperature which indicated that the packing parameters of surfactant molecules tend to be more unity lead to lower interfacial tension which promote the formation of O/W nanoemulsion (McClements & Rao, 2011). High temperature that induced more kinetic energy allow dispersed liquid to overcome the net attractive force of the continuous phase that result in smaller particle size (Chen & Tao, 2005). However, further increase the mixing temperature to 55°C result in increasing in the particles size to 305.4nm. Increasing the temperature to certain extent lead to impaired in the interfacial adsorption of the emulsifier causing coalescence to occur and destabilizing the emulsion (Chen & Tao, 2005). Therefore, the optimum temperature for this formulation of emulsion is at 50°C.

Based on Figure 4-12, for formulation of SE:PGPR, the increase in the mixing temperature from 25°C to 30°C causing a great increase in the particle size which is from 483.4nm to 644.8nm. Further increase in the mixing temperature of the system lead to separation between oil and water. The increment in temperature is said to be unusual for this formulation that tends to coagulate the particles indirectly causing damage to the emulsion quality. The increase in the temperature that lead to separation can be due to the imbalance between the head and tail groups of the surfactants in the system. Other than that, increasing temperature causing dehydration of the head group in the surfactant making the interaction between head and water to be weaken. This in turn will change the packing geometry of the surfactant. As a result, for the formulation of SE:PGPR, the optimum temperature is 25°C.

In section 4.4.3, the formulation chosen for undergoing process parameters variation is 5% PFO with 7.5% SE and 87.5% water. The particle size at 25°C is 205nm and increase to 692nm at 55°C. As the temperature increased, the degree of hydration of the polyoxythylene chains in nonionic surfactants decreased causing the surfactant to become more hydrophobic. High temperature decrease the solubility of hydrophilic surfactants by dehydration of the polar head group of the nonionic surfactant molecules lead to leakage of oil water interface thereby allowing aggregation of droplets (Yilmaz & Borchert, 2005). Excess emulsification energy in the system lead to increase in the probability of collision and coalescence of newly formed colloid particle that resulting in bigger particle coalescence (Garcia et al., 2011). The optimum temperature for SE formulation is at 25°C.

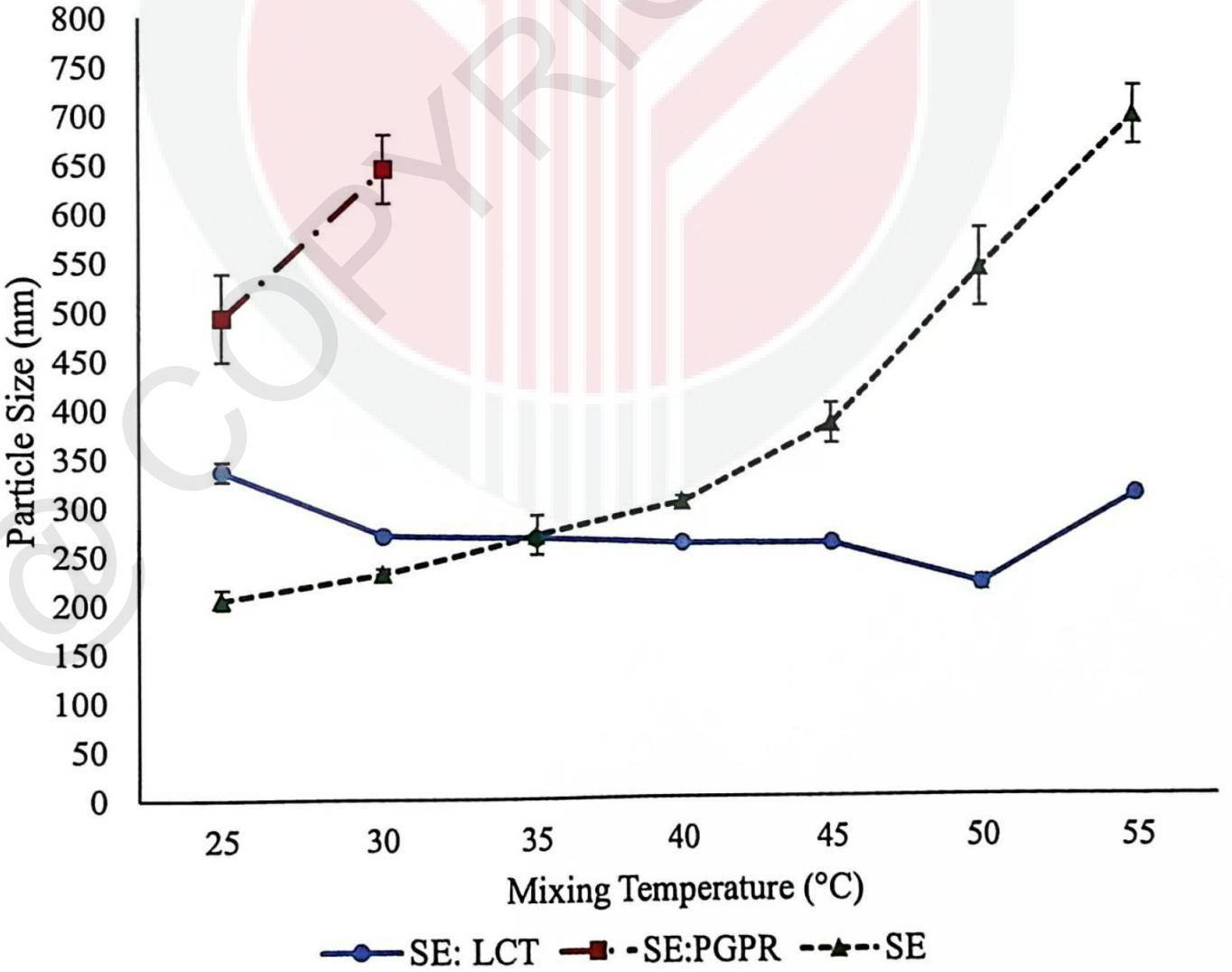
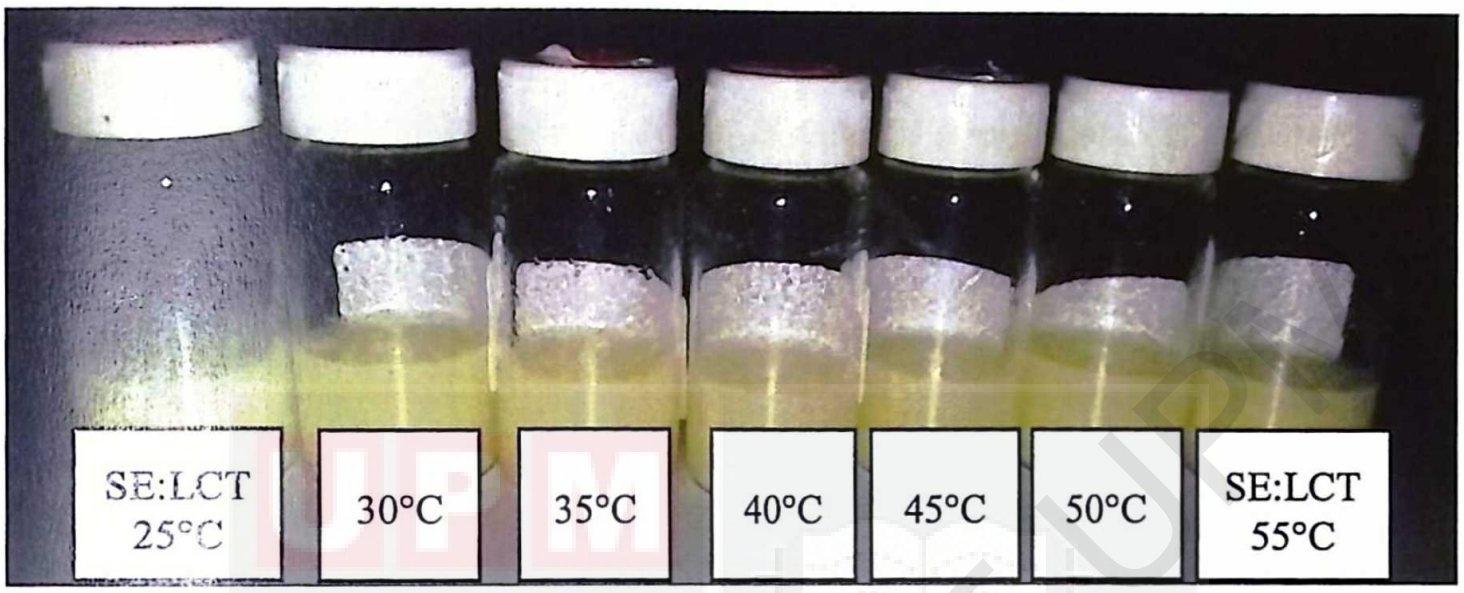


Figure 4-12: Graph of temperature variation for (i) sucrose ester:lecithin (SE:LCT); (ii) sucrose ester:polyglycerol polyricinoleate (SE:PGPR); and (iii) sucrose ester (SE)



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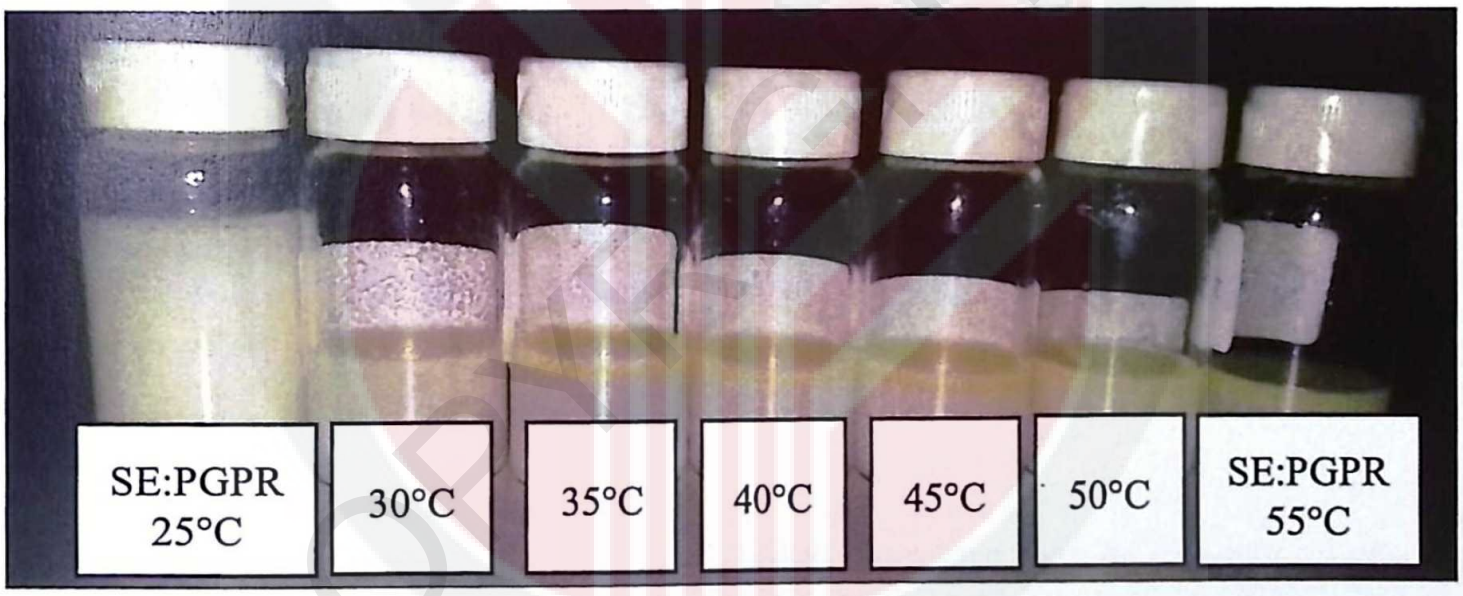

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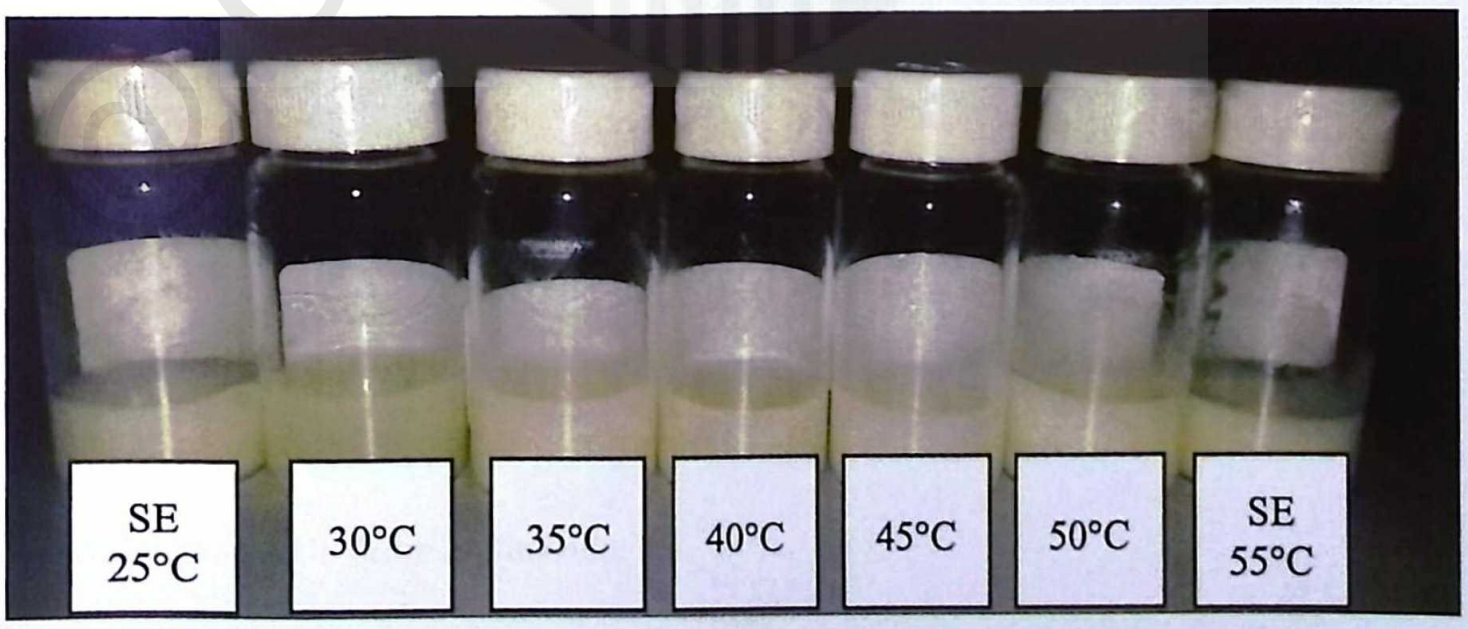


(c)

Figure 4-13: Passion fruit oil emulsion at different mixing temperature for (a) SE:LCT at 75:25; (b) SE:PGPR at 90:10; and (c) SE alone

4.5.2 Effect of Oil Dropping Rate on the Particle Size and Polydispersity Index (PDI) for the Emulsion Produced Using Different Formulation. (1) Sucrose Ester: Lecithin (SE:LCT); (2) Sucrose Ester: Polyglycerol Polyricinoleate (SE:PGPR); and (3) Sucrose Ester alone (SE).

Addition rate of organic phase (oil) into aqueous phase in spontaneous emulsification method was reported to be one of the controlling factors. In general, it is being expected that each surfactant, oil, water combination would have different optimum rate of addition depending on factors such as the SOW phase diagram and phase properties such as rheology and microstructure (Santana, Perrechil, & Cunha, 2013). The variation of the oil dropping rate (all in once, 1drop/10sec, 1drop/30sec, 1drop/40sec) were carried out after obtaining the optimum mixing temperature for each set of nanoemulsion formulation.

Based on Figure 4-14, the smallest particle size for SE:LCT formulation was found mixing temperature of 50°C. It showed that increasing the oil dropping rate from all poured at once to 1 drop/20 seconds reduce the particle size from 323.7nm to 246.4nm. However, further increase the dropping rate cause slight increase in the particle size. The particle size at dropping rate of 1 drop/40 seconds was 278.1nm. This is probably due to that long addition time causing the relatively unstable samples start to destabilize lead to increase in droplet size (Su et al., 2017). Therefore, the optimum oil dropping rate for this formulation is at 1 drop/20 seconds.

According to Figure 4-14, the optimum mixing temperature for formulation of SE:PGPR was 25°C. By using the optimum mixing temperature, variation in oil dropping rate result in different conditions to the particle size. It can be seen that increasing the oil dropping rate lead to decrease in the particle size from 1432.5nm to 1193.1nm. It is being found out that if the addition rate of organic phase (oil) into the aqueous phase is carried out too quickly, large viscous SOW clumps may form caused difficulties in breaking and dispersion. In a model system, it was found that if the organic phase is added too quickly significantly larger droplets may be formed (Yang, Marshall-Breton, Leser, Sher, & McClements, 2012). Other than that, long times favor the formation of small droplets due to the fact that long times help the system to reach equilibrium in the viscous zone incorporating more oil into this phases which is required for a proper nanoemulsion formation through low energy method (Solè et al., 2010). The particle size formed were larger than previous result indicated that there is possibility of contamination occurred in the materials lead to increase in the particle size as compared to previous experimental results. Thus, the optimum oil dropping rate for SE:PGPR formulation is 1 drop/40 seconds.

Formulation of SE alone is optimum at mixing temperature of 25°C. Figure 4-14 indicated that increasing the dropping rate from all poured at once to 1 drop/20 seconds lead to decrease in the particle size from 619.2nm to 217.8nm. Addition rate is an important factor and should be adjusted to ensure that it is slow enough for the o/w phase to be formed. Low addition rate or longer time used for dropping oil allow the system time to equilibrate (Pey et al., 2006). Nonetheless, further increasing the oil dropping rate from 1 drop/20 seconds to 1 drop/30 and 40 seconds respectively causing the particle size to

increase to 278.1nm. Each SOW system would have different maximum addition rate in achieving small particle size on a reasonable timescale. In general, most researches are using addition times between 5 to 15 minutes which is likely to be appreciably longer than actually required to form small droplets (J. Komaiko, 2016). As a result, the optimum oil dropping rate suggested for SE alone used in the nanoemulsion formulation is 1 drop/20 seconds.

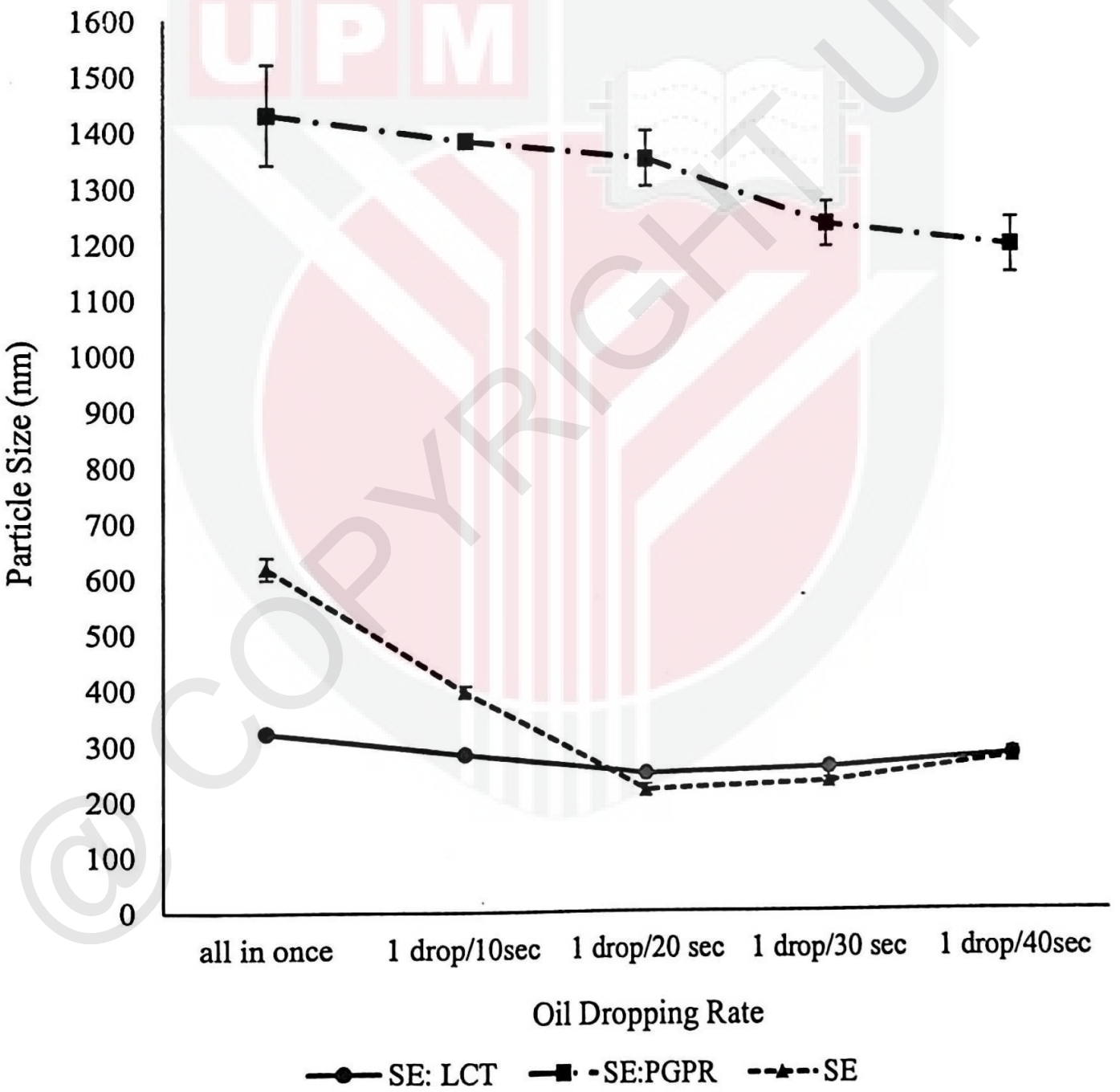
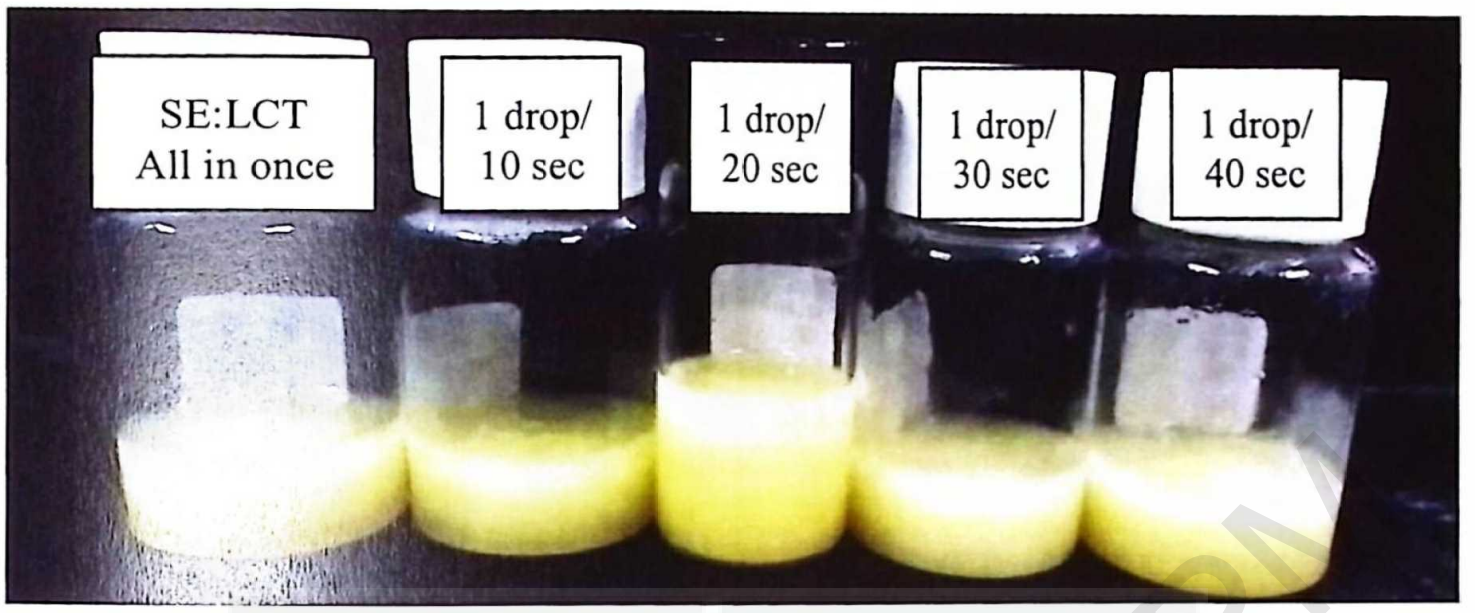


Figure 4-14: Oil dropping rate variation for (i) sucrose ester:lecithin (SE:LCT); (ii) sucrose ester:polyglycerol polyricinoleate (SE:PGPR); and (iii) sucrose ester (SE)



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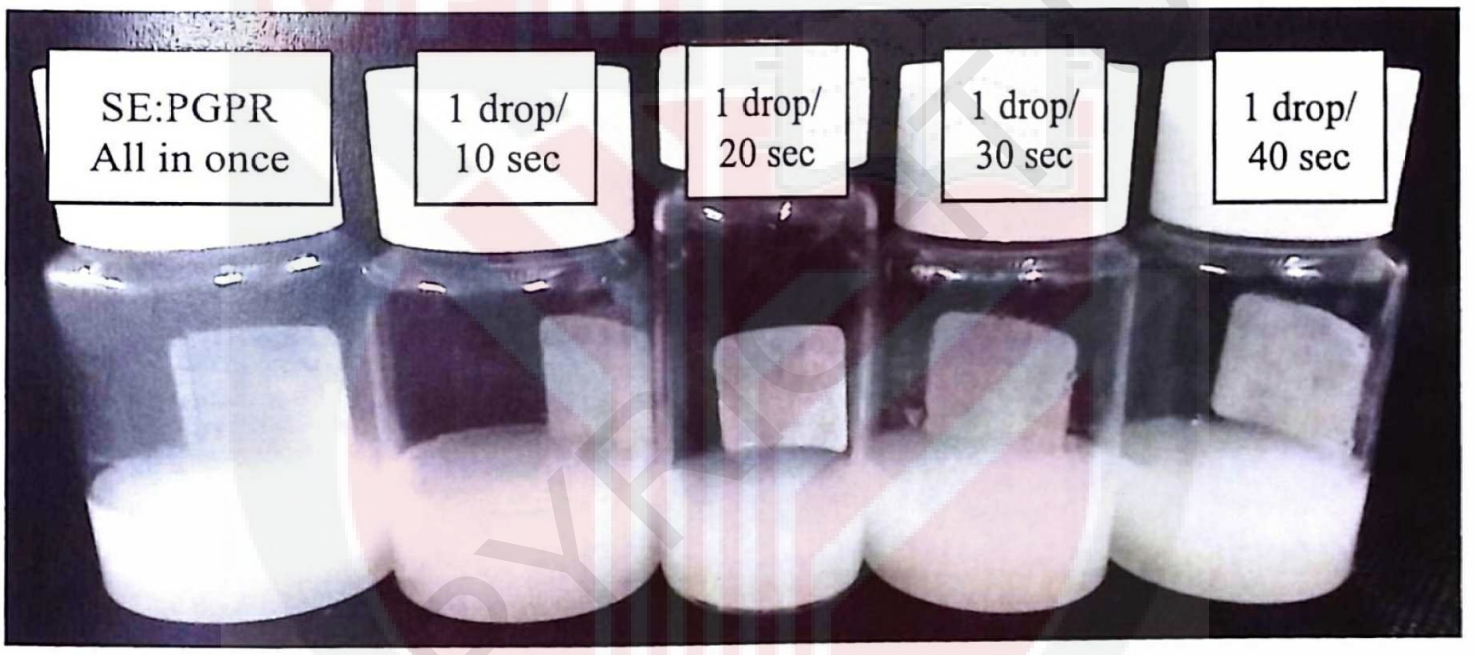

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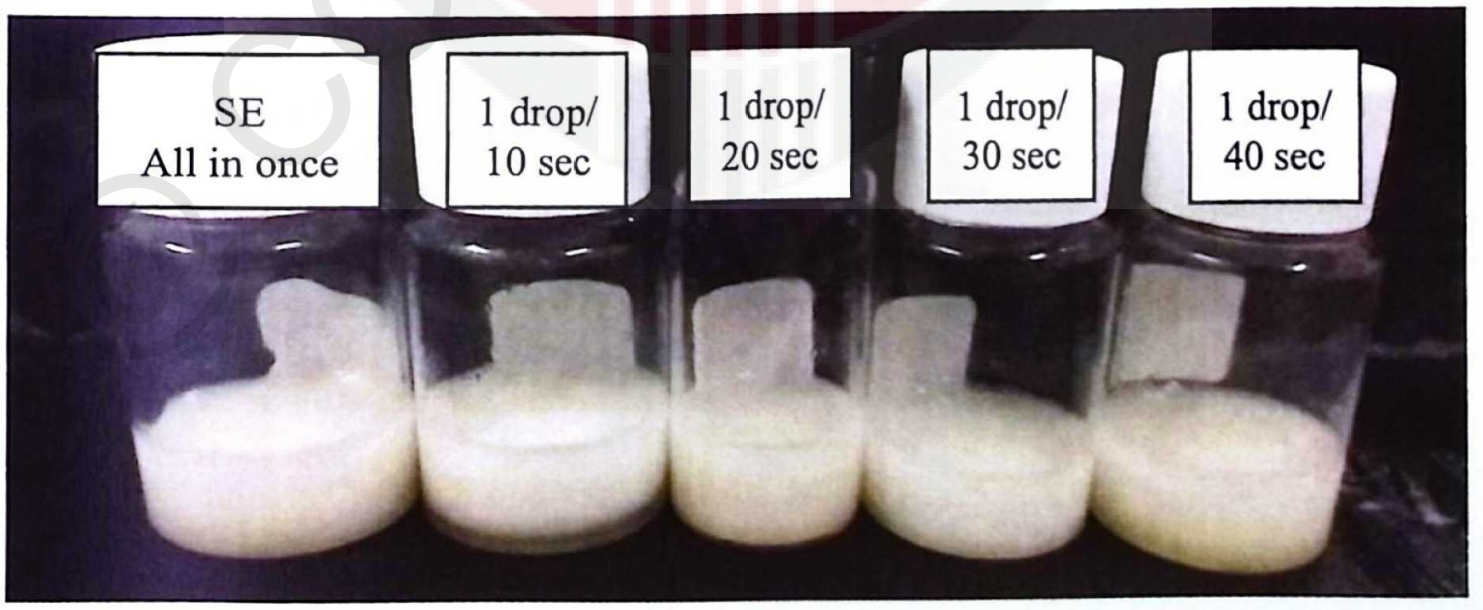


(c)

Figure 4-15: Passion fruit oil emulsion at different oil dropping rate for (a) SE:LCT at 75:25; (b) SE:PGPR at 90:10; and (c) SE alone

4.5.3 Effect of Stirring Speed on the Particle Size and Polydispersity Index (PDI) for the Emulsion Produced Using Different Formulation. (1) Sucrose Ester: Lecithin (SE:LCT); (2) Sucrose Ester: Polyglycerol Polyricinoleate (SE:PGPR); and (3) Sucrose Ester alone (SE).

Agitation conditions during emulsion formation by spontaneous emulsification influence the particle size with particle diameter typically decreasing with increasing stirring speed. Stirring is necessary to facilitate the transport of surfactant, oil and water molecules as well as facilitate the disruption of the bicontinuous microemulsion formed at the boundary between the organic and aqueous phases (Saberi, Fang, & McClements, 2013). The variation of the stirring speed (300rpm, 400rpm, 600rpm, 700rpm) were carried out after obtaining the optimum mixing temperature for each set of nanoemulsion formulation.

Stirring has an effect on both breakup and coalescence since it provides the shear to elongate the drop before breaking as well as provide the inertia to gather drops and help them coalesce (Tolosa, Forgiarini, Moreno, & Salager, 2006). At the optimum mixing temperature of 50°C for SE:LCT formulation, increasing stirring speed from 300rpm to 500rpm lead to reduction in the particle size from 265.5nm to 247.4nm while further increase in the stirring speed up to 700rpm causing the particle size to increase to 334.1nm (refer Figure 4-16). The reduction in the particle size with increasing stirring speed may due to the applied mechanical energy broke up and distributed the organic and aqueous phases so that concentration gradients could be maintained at the oil water interface. However, mild mixing is preferable during spontaneous emulsification method to produce

very fine droplets (Saberi et al., 2013). Hence, 500rpm of stirring is the optimum speed for the formulation with SE:LCT.

Studies have shown that the effect of stir speed may be dependent on the surfactant concentration. As instance, at lower surfactant concentration, increasing the stir speed decrease the particle size (An, Yan, Li, & Li, 2014). For formulation of SE:PGPR, at lower stirring speed, the emulsion system could not achieve and separation between oil and water phases were observed. This is probably due to the energy supplied by the stirring force to the system is insufficient to break up the oil droplet to be emulsified with the aqueous phase. Increasing the stirring speed to 500rpm bring up the particle size of 1352.0nm and further increase the speed increase slightly on the particle size to 1693.7nm at 700rpm. It can be seen that the higher the mechanical shear involved, the easier the drop being broke up and the smaller the droplet size (Aravand & Semsarzadeh, 2008). Thus, based on the result, the optimum stirring speed for this formulation is at 500rpm.

When a system is allowed to stir to accumulate sufficient kinetic energy during the formation of emulsion, then a smaller size distribution of emulsion droplets would be produced. Subsequently, a system would generate better stability as smaller droplets are less attracted to each other due to weaker Van der Waals forces between them (Zakaria et al., 2012). The emulsion formulation of SE alone used in this studied showed that increasing the stirring speed from 300rpm to 500rpm reduced the particle size from 491.6nm to 217.8nm. Generally, higher mixing speed would produce smaller droplet size which have higher interfacial area and droplet-to-droplet interaction resulting in more stable emulsion (Lim, Wong, Law, Samyudia, & Dol, 2015). However, further increasing the stirring speed lead to increase in the particle size up to 428.6nm at 700rpm. This is due

to that when the stirring speed was too high, it promotes destabilization mechanisms like coalescence and sedimentation resulting in larger final droplets (Pey et al., 2006). Thus, the optimum stirring speed is 500rpm.

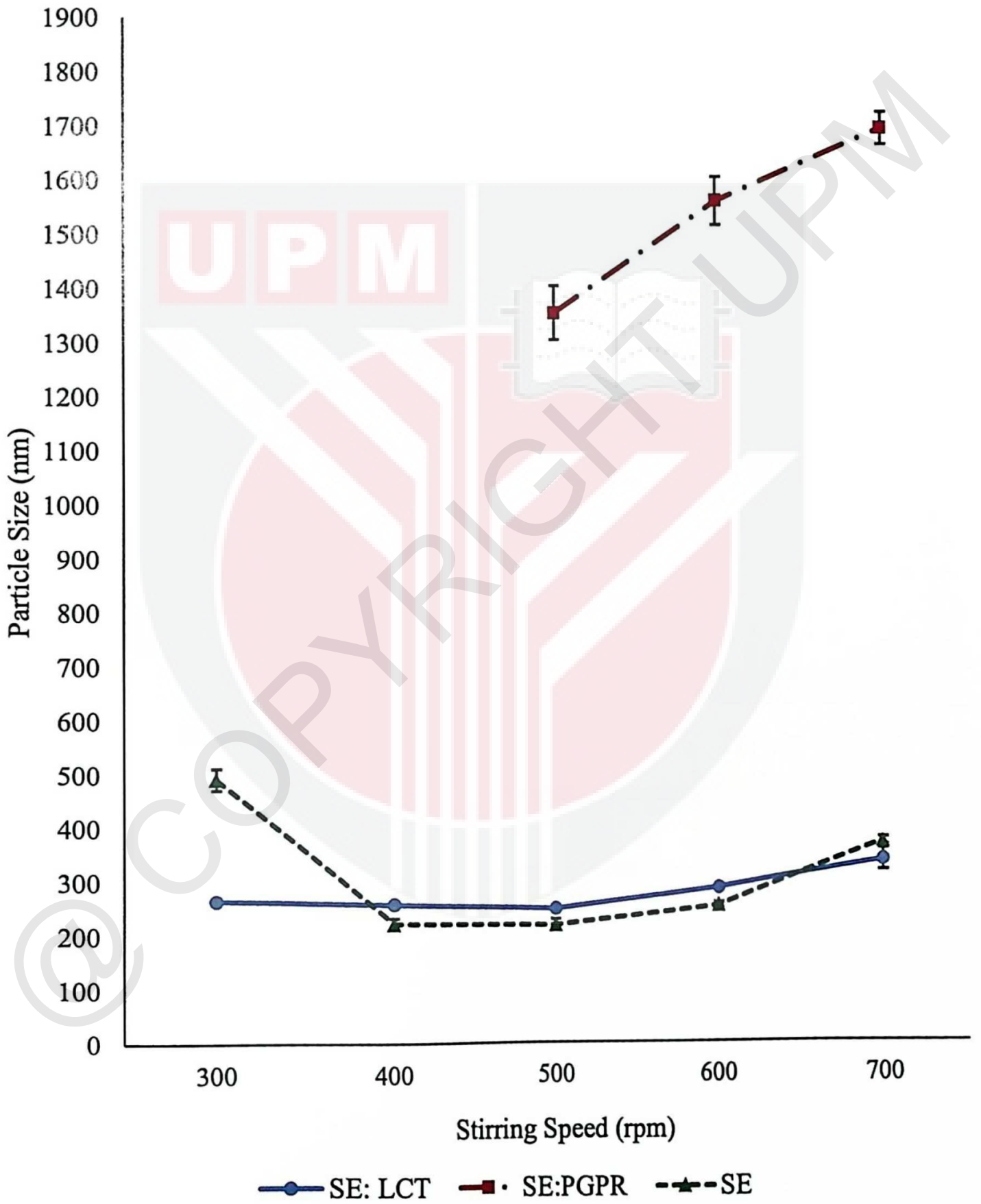
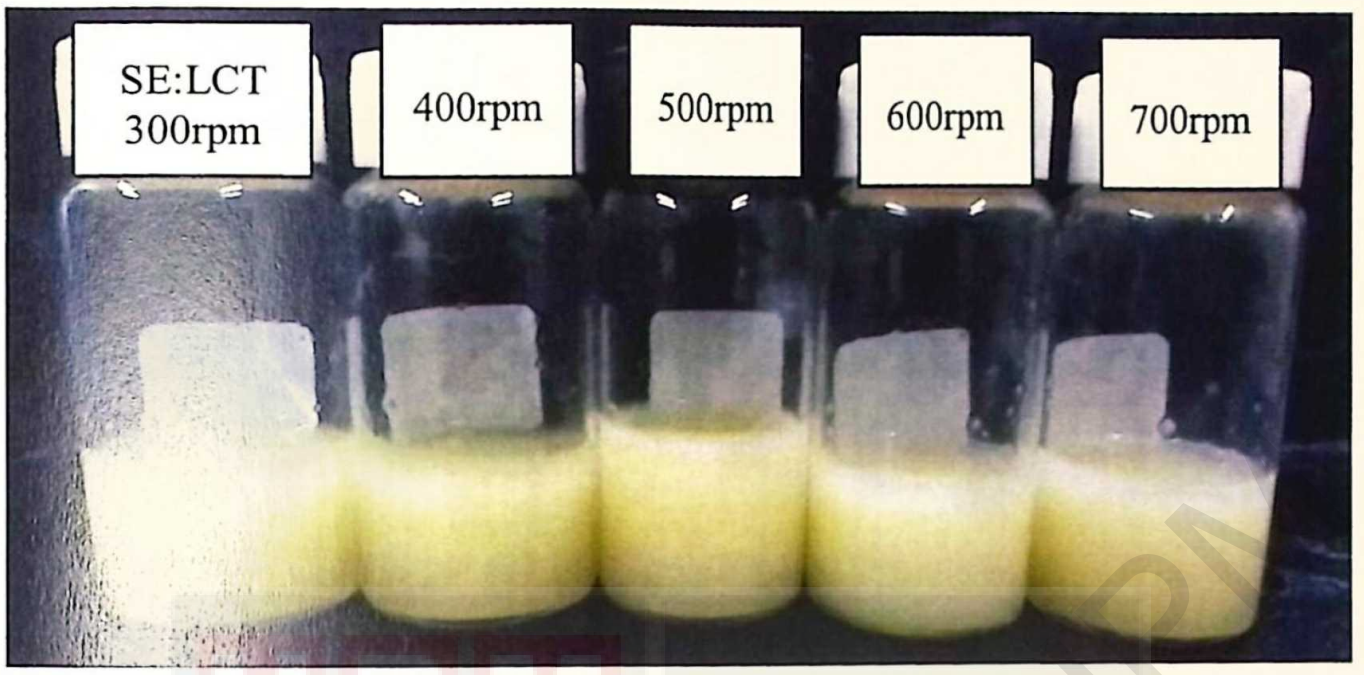
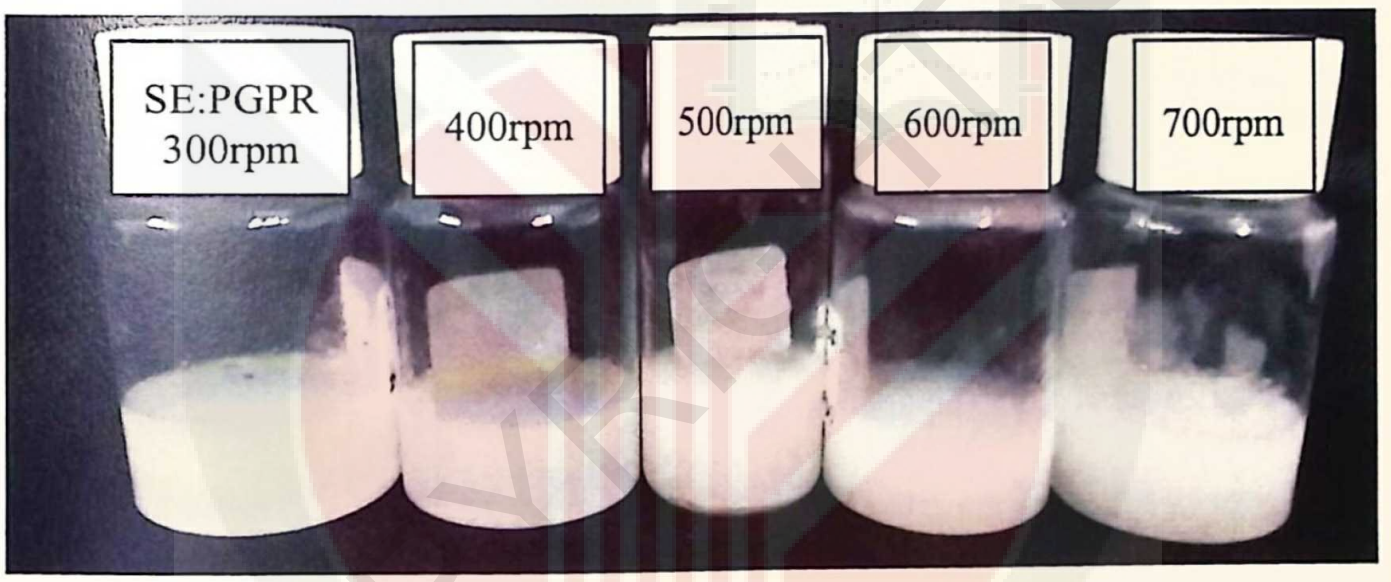


Figure 4-16: Stirring speed variation for (i) sucrose ester:lecithin (SE:LCT); (ii) sucrose ester:polyglycerol polyricinoleate (SE:PGPR); and (iii) sucrose ester (SE)



(a)



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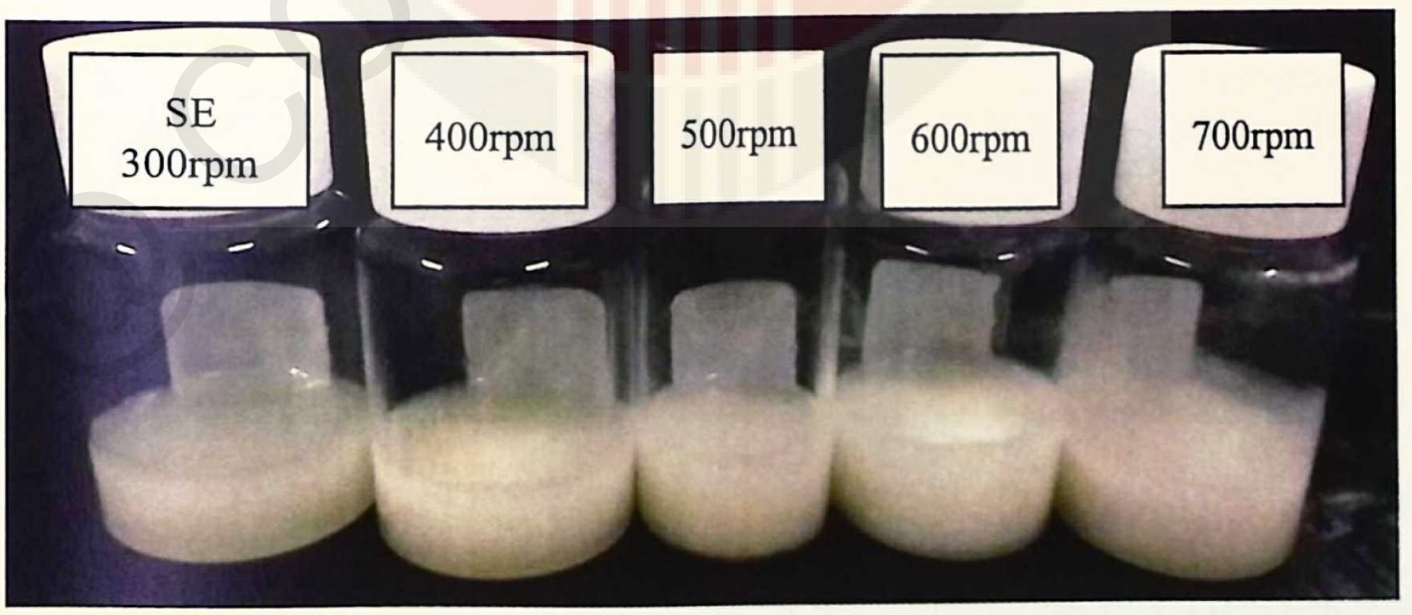

(c)

Figure 4-17: Passion fruit oil emulsion at different stirring speed for (a) SE:LCT at 75:25; (b) SE:PGPR at 90:10; and (c) SE alone

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

In this study, a combined approach on the formulation and parametric study of low energy process to produce nanoemulsion was investigated. The effect of using different combination of food grade surfactants including sucrose ester (SE) alone; sucrose ester:lecithin (SE:LCT); sucrose ester: polyglycerol polyricinoleate (SE:PGPR); tween 80:lecithin (T80:LCT); and tween 80: polyglycerol polyricinoleate (T80:PGPR) to produce passion fruit oil nanoemulsion was studied.

Based on the findings, SE and its combination were able to reduce IFT lower than Tween 80 and its combination. It has been found out that ratio of combined surfactants from 100:0 to 80:20 is more likely to produce nanoemulsion with low IFT obtained. Then, the ratio between combinations of surfactants is studied where different formulations result in different particle size. As a result, the smallest particle size of 336 nm was found to be at ratio of 75:25 at the formulation of 5% SE:LCT while for the combination between SE:PGPR, 90:10 found to be ratio that result in smaller particle size of 493.3 nm. According to the results, it can be seen that surfactants combination that result in the same HLB did not produce the same particle size. Combination between SE:PGPR tend to result in a larger particle size. Hence, it inferred that packing geometry is the key point in determining the size of emulsion produced. On the stability test, the zeta potential resulted in stable range which is below -60 mV. Therefore, focus is given on the result of particle size produced instead of zeta potential in this study.

It is being concluded that, the concentration of surfactants used in the formulation for producing nanoemulsion is important. For the combination of SE:LCT, 5% concentration result the smallest particle size while SE:PGPR required only 2.5% concentration. This is because PGPR is bigger molecule as compared to lecithin and hence the concentration needed will be lower. On the other hand, concentration of oil is critical too as too less or too much oil in the system can lead to separation between oil and water phases. Therefore, the optimum concentration of surfactants as well as oil must be determined in order to successfully produce nanoemulsion.

Variation has been carried out on the process parameters such as mixing temperature, stirring speed and oil dropping rate to determine the effect on the final particle size of the emulsion produced. Based on the findings, different formulation resulted in different optimum parameters. In conclusion, SE:PGPR formulation required 25°C mixing temperature incorporated with 500rpm and 1drop/40second dropping rate to produce smaller particle size. The smallest particle size of 246.4nm is obtained in the formulation of 5% concentration SE:LCT at ratio of 75:25, 5% PFO and 90% water at the process parameters of 50°C mixing temperature, 500rpm stirring speed and 1drop/20sec oil dropping rate. This has shown that different parameters affect the emulsion produced in different extend. However, the controlling factor in producing an emulsion is on the chemical formulation instead of the physical parameters.

With the result from this study, it provide the information on which passion fruit seed oil has the potential to be used as nanoemulsion for cosmetics products. This help to reduce the wastage of juice industry that dispose the seed after extraction. Besides that, this study has explored the formulation of different food grade surfactants used for

formation of nanoemulsion that is advantageous for further investigation since it is potential to use low energy approach in triglycerides for producing nanoemulsion. With the successful of the emulsion produced using low energy approach, it showed that the method can be implemented in industry as it is easy to be performed and cost saving. On the impact of health and safety context, the recommended concentration of surfactants used in cosmetic products is 2% to 8% and the recommended dose of oil is 0.5% to 5%. Other than that, using food grade surfactants instead of extended surfactants is important in cosmetic products which safeguard the products produced from toxicity and safe to be applied.

Future work can be carried out by determining the effect of the size of oil droplets when added into the continuous phase on the particle size of the nanoemulsion produced. Besides that, it is being recommended that parameter of emulsification time can be determine to identify the optimum time for emulsification to occur. Furthermore, stability test can be carried out by checking the emulsion produced at 2, 4, 8 and 12 weeks intervals as well as in different storage conditions such as at temperature of 4°C, 20°C and 40°C.

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APPENDICES

Figure A-1: Spinning drop tensiometer

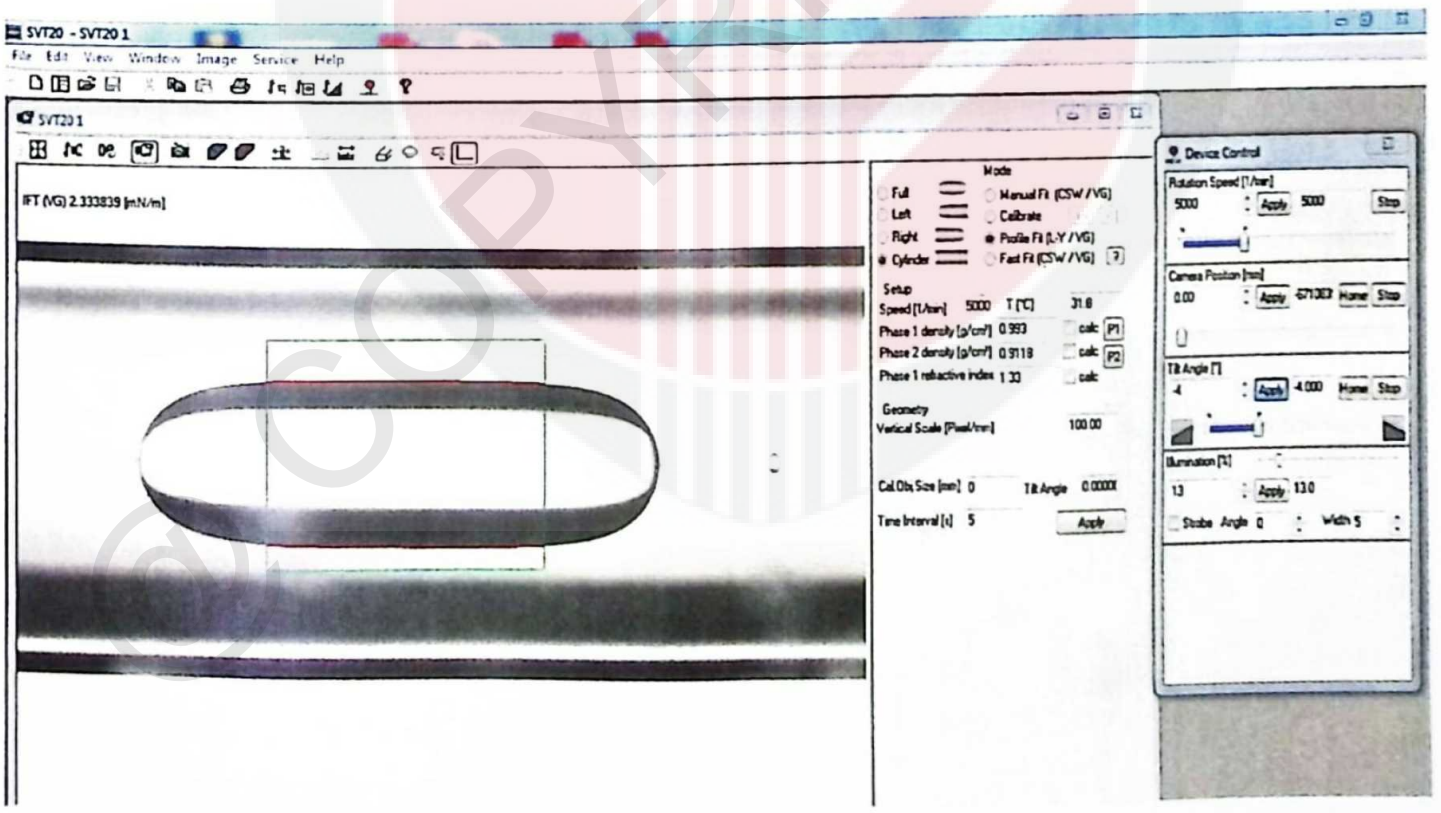

Figure A-2: IFT measurement using spinning drop tensiometer

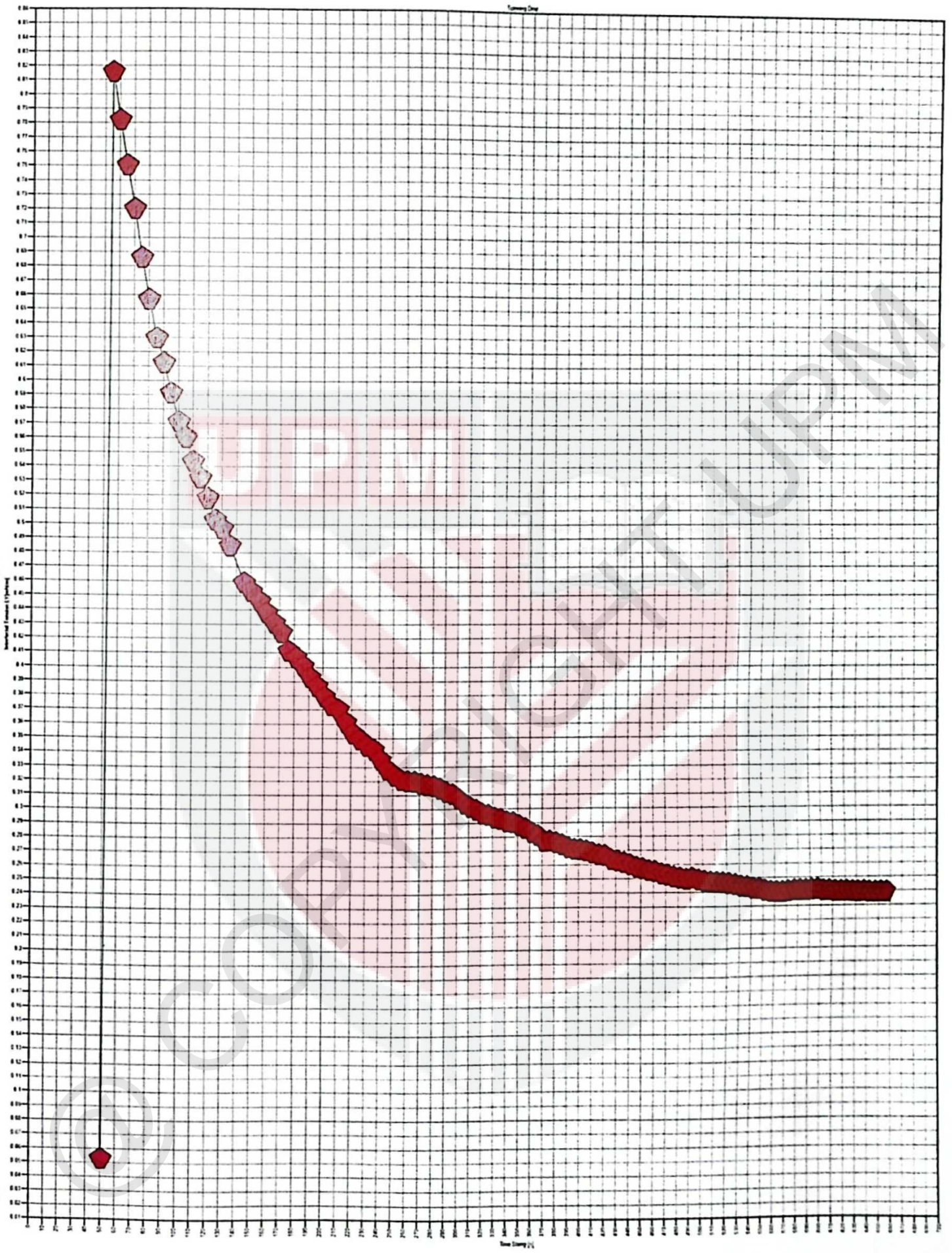

Figure A-3: Graph of interfacial tension against time

Table A-1: Raw data of interfacial tension at different concentration

No	Surfactant Combination	Surfactant Concentration	Ratio	Density (g/cm^3)	RPM	IFT Value (mN/m)		
						1st	2nd	Mean
1	-	-	-	0.946	7000	2.659	2.625	2.642
1A	SE:LCT	0.5%	1.5:1	0.993	7000	0.403	0.441	0.422
1B	SE:LCT	1.0%	1.5:1	0.995	7000	0.263	0.279	0.271
1C	SE:LCT	1.5%	1.5:1	0.995	7000	0.355	0.371	0.363
1D	SE:LCT	2.0%	1.5:1	0.996	7000	0.363	0.382	0.372
2A	SE:PGPR	0.5%	1:1	0.992	5000	0.394	0.419	0.406
2B	SE:PGPR	1.0%	1:1	0.992	5000	0.190	0.183	0.187
2C	SE:PGPR	1.5%	1:1	0.993	5000	0.165	0.170	0.168
2D	SE:PGPR	2.0%	1:1	0.993	6000	0.374	0.343	0.358
3A	T80:LCT	0.5%	1.5:1	0.993	7000	1.871	1.716	1.794
3B	T80:LCT	1.0%	1.5:1	0.995	7000	1.291	1.306	1.298
3C	T80:LCT	1.5%	1.5:1	0.996	7000	1.475	1.413	1.444
3D	T80:LCT	2.0%	1.5:1	0.996	7000	1.584	1.515	1.549
4A	T80:PGPR	0.5%	1:1	0.991	6000	1.987	1.951	1.969
4B	T80:PGPR	1.0%	1:1	0.991	6000	1.833	1.791	1.812
4C	T80:PGPR	1.5%	1:1	0.991	6000	1.969	2.019	1.994
4D	T80:PGPR	2.0%	1:1	0.992	6000	2.224	2.090	2.157
5A	T80	0.5%	-	0.989	7000	1.886	1.953	1.920
5B	T80	1.0%	-	0.990	7000	1.210	1.560	1.385
5C	T80	1.5%	-	0.991	7000	1.829	1.733	1.781
5D	T80	2.0%	-	0.991	7000	2.338	2.329	2.333

Table A-2: Raw data of interfacial tension at different ratio

No	Surfactant Combination	Surfactant Concentration	Ratio	Density (g/cm^3)	RPM	IFT Value (mN/m)		
						1st	2nd	Mean
1A	SE:LCT	1.0%	1.0:0	0.993	4000	0.109	0.110	0.110
1B	SE:LCT	1.0%	0.95:0.5	0.991	3000	0.224	0.185	0.205
1C	SE:LCT	1.0%	0.9:0.1	0.991	3000	0.227	0.212	0.219
1D	SE:LCT	1.0%	0.8:0.2	0.995	3000	0.226	0.225	0.226
1E	SE:LCT	1.0%	0.5:0.5	0.995	5000	0.678	0.725	0.702
1F	SE:LCT	1.0%	0.2:0.8	0.996	5000	1.612	1.617	1.615
IG	SE:LCT	1.0%	0:1.0	0.992	6000	1.874	1.956	1.915
2A	SE:PGPR	1.5%	1.0:0	0.992	4000	0.092	0.082	0.087
2B	SE:PGPR	1.5%	0.95:0.5	0.993	5000	0.135	0.120	0.128
2C	SE:PGPR	1.5%	0.9:0.1	0.993	5000	0.140	0.150	0.145
2D	SE:PGPR	1.5%	0.8:0.2	0.993	5000	0.281	0.290	0.286
2E	SE:PGPR	1.5%	0.5:0.5	0.994	5000	0.379	0.407	0.393
2F	SE:PGPR	1.5%	0.2:0.8	0.994	5000	0.928	1.025	0.977
2G	SE:PGPR	1.5%	1.0:0	0.994	6000	2.237	2.475	2.356
3A	T80:PGPR	1.0%	1.0:0	0.990	5000	1.310	1.560	1.435
3B	T80:PGPR	1.0%	0.95:0.5	0.990	5000	1.633	1.699	1.666
3C	T80:PGPR	1.0%	0.9:0.1	0.992	5000	1.749	1.719	1.734
3D	T80:PGPR	1.0%	0.8:0.2	0.992	5000	1.823	1.786	1.804
3E	T80:PGPR	1.0%	0.5:0.5	0.992	5000	1.12	1.980	1.896
3F	T80:PGPR	1.0%	0.2:0.8	0.992	5000	2.125	2.115	2.120
3G	T80:PGPR	1.0%	1.0:0	0.993	5000	2.115	2.278	2.197

Table A-3: Raw data on formulation of 5% sucrose ester:lecithin (SE:LCT) and 5% PFO in emulsion

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Conc.		Avg. Particle Size (nm)			Avg. PDI			Avg. Zeta Potential (mV)		
		SE	LCT	1st	2nd	Avg.	1st	2nd	Avg.	1st	2nd	Avg.
5	90	100	0	separate								
5	90	95	5	541.1	438.8	489.9 ± 70	0.365	0.257	0.311	-81.26	-74.58	-77.92
5	90	90	10	406.2	507.1	456.7 ± 70	0.244	0.333	0.288	-82.67	-70.85	-76.76
5	90	80	20	376.9	315.0	342.6 ± 40	0.264	0.206	0.235	-87.71	-71.67	-79.67
5	90	75	25	339.5	332.5	336.0 ± 10	0.227	0.225	0.226			
5	90	70	30	separate								
5	90	60	40	separate								
5	90	50	50	separate								
5	90	20	80	separate								
5	90	0	100	separate								

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-4: Raw data on formulation of 2.5% sucrose ester:polyglycerol polyricinoleate (SE:PGPR) and 2.5% PFO in emulsion

PFO (%)	Water (%)	Ratio of Surfactant Combination at 2.5% Conc.		Avg. Particle Size (nm)			Avg. PDI			Avg. Zeta Potential (mV)		
		SE	PGPR	1st	2nd	Avg.	1st	2nd	Avg.	1st	2nd	Avg.
2.5	95	100	0	separate								
2.5	95	95	5	separate								
2.5	95	90	10	453.7	533.0	493.4 ± 45	0.312	0.352	0.314	-72.97	-98.24	-85.60
2.5	95	80	20	745.5	759.3	752.4 ± 100	0.631	0.489	0.557	-65.31	-74.67	-69.99
2.5	95	50	50	2159.8	1877.4	2018.6 ± 150	0.853	0.694	0.763	-80.74	-66.54	-73.99
2.5	95	20	80	separate								
2.5	95	0	100	separate								

* Note: The reading of each repetition are the average of 3 cycles run.

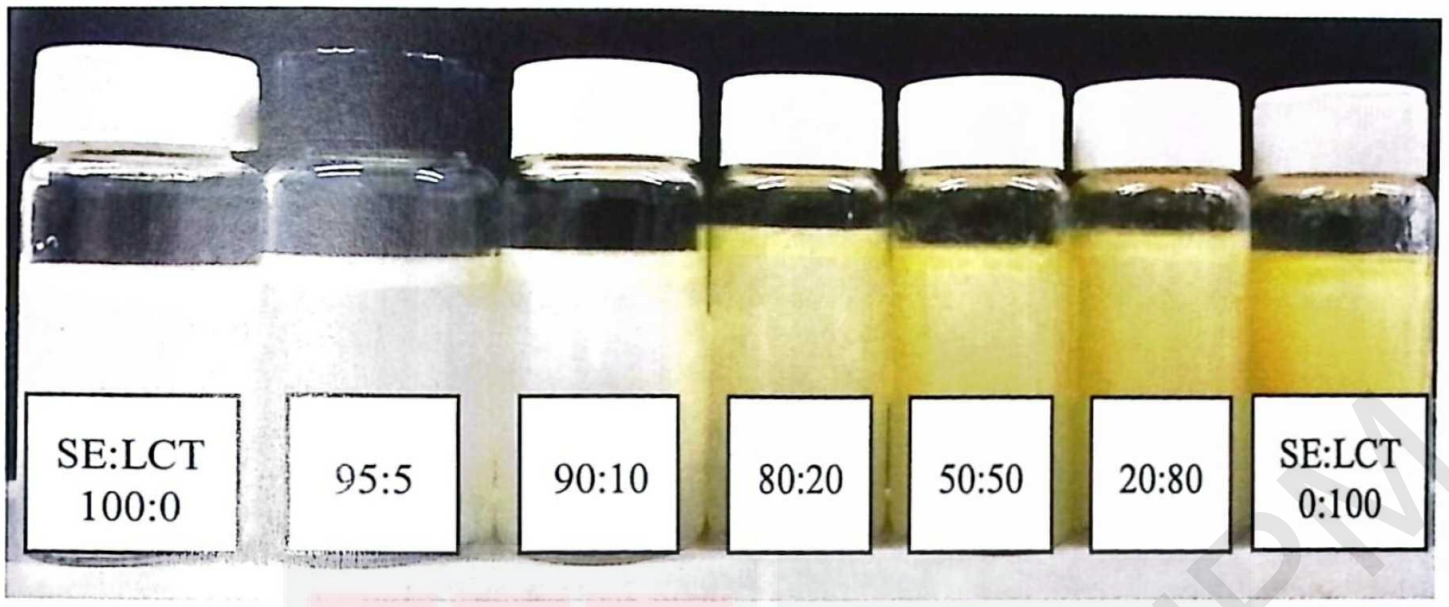

Figure A-4: Passion fruit oil emulsion of combination of sucrose ester:lecithin (SE:LCT) at 2.5% PFO and 2.5% surfactants

Figure A-5: Passion fruit oil emulsion of combination of sucrose ester:lecithin (SE:LCT) at 7.5% PFO and 7.5% surfactants

Table A-5: Raw data on formulation of 7.5% sucrose ester:lecithin (SE:LCT) and 7.5% PFO in emulsion

PFO (%)	Water (%)	Ratio of Surfactant Combination at 7.5% Conc.		Avg. Particle Size (nm)			Avg. PDI			Avg. Zeta Potential (mV)		
		SE	LCT	1st	2nd	Avg.	1st	2nd	Avg.	1st	2nd	Avg.
7.5	85	100	0	252.8	253.8	253.3 ± 10	0.172	0.229	0.201	-77.07	-74.78	-75.93
7.5	85	95	5	4138.8	3967.7	4053.2 ± 200	2.011	1.811	1.906	-71.22	-78.32	-74.77
7.5	85	90	10	2101.5	2626.6	2364.0 ± 300	0.976	1.572	1.274	-78.44	-79.26	-78.85
7.5	85	80	20	793.7	802.5	798.1 ± 10	0.475	0.480	0.477	-75.44	-74.19	-74.81
7.5	85	50	50	separate								
7.5	85	20	80	separate								
7.5	85	0	100	separate								

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-6: Raw data on formulation of sucrose 5% ester:polyglycerol polyricinoleate (SE:PGPR) and 5% PFO in emulsion

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Conc.		Avg. Particle Size (nm)			Avg. PDI			Avg. Zeta Potential (mV)		
		SE	PGPR	1st	2nd	Avg.	1st	2nd	Avg.	1st	2nd	Avg.
5	90	100	0	separate								
5	90	95	5	1618.9	1342.0	1480.5 ± 200	0.885	0.773	0.829	-74.51	-71.16	-72.84
5	90	90	10	1609.0	1259.5	1434.3 ± 200	0.837	0.751	0.794	-68.80	-73.38	-71.09
5	90	80	20	941.2	1017.7	979.5 ± 50	0.602	0.581	0.592	-76.95	-71.38	-74.17
5	90	50	50	1931.2	1793.3	1862.3 ± 100	0.692	0.631	0.621	-76.56	-74.95	-75.76
5	90	20	80	separate								
5	90	0	100	separate								

* Note: The reading of each repetition are the average of 3 cycles run.

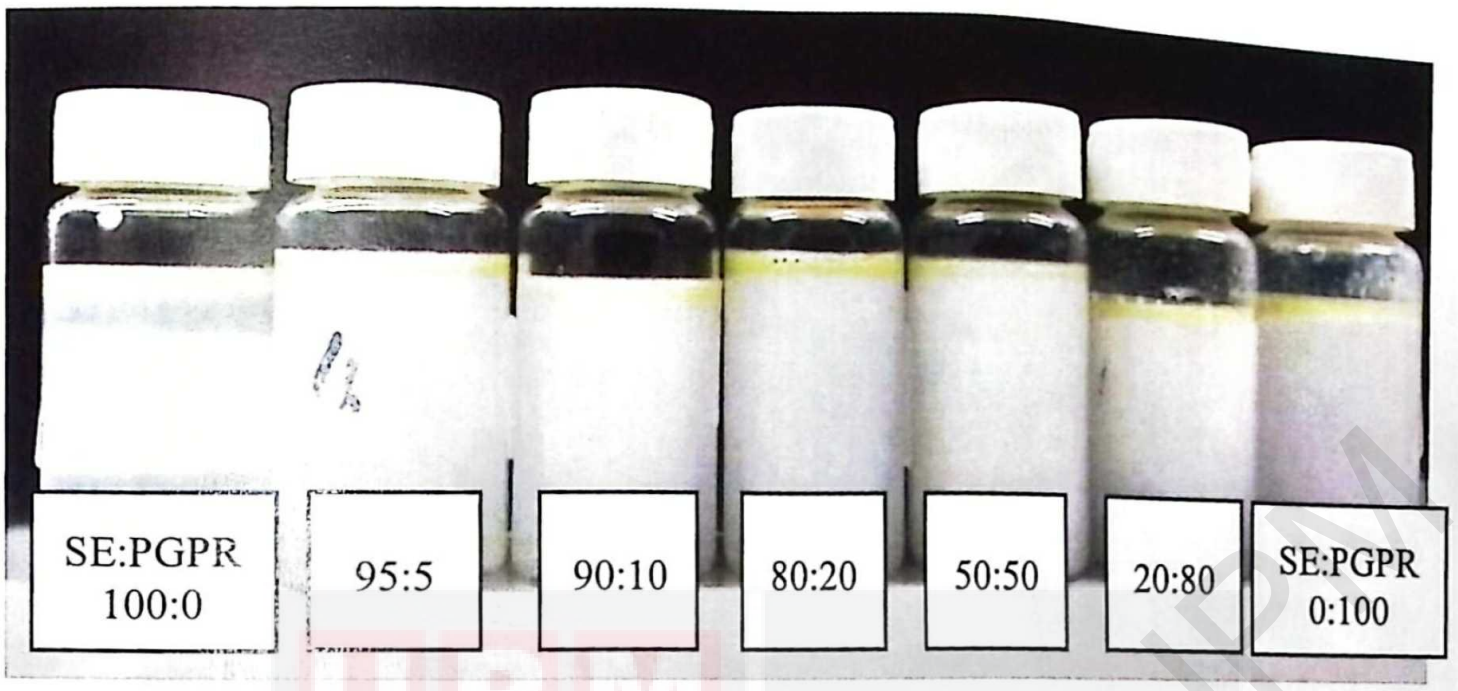

Figure A-6: Passion fruit oil emulsion of combination of sucrose ester:polyglycerol polyricinoleate (SE:PGPR) at 1.5% PFO and 1.5% surfactants

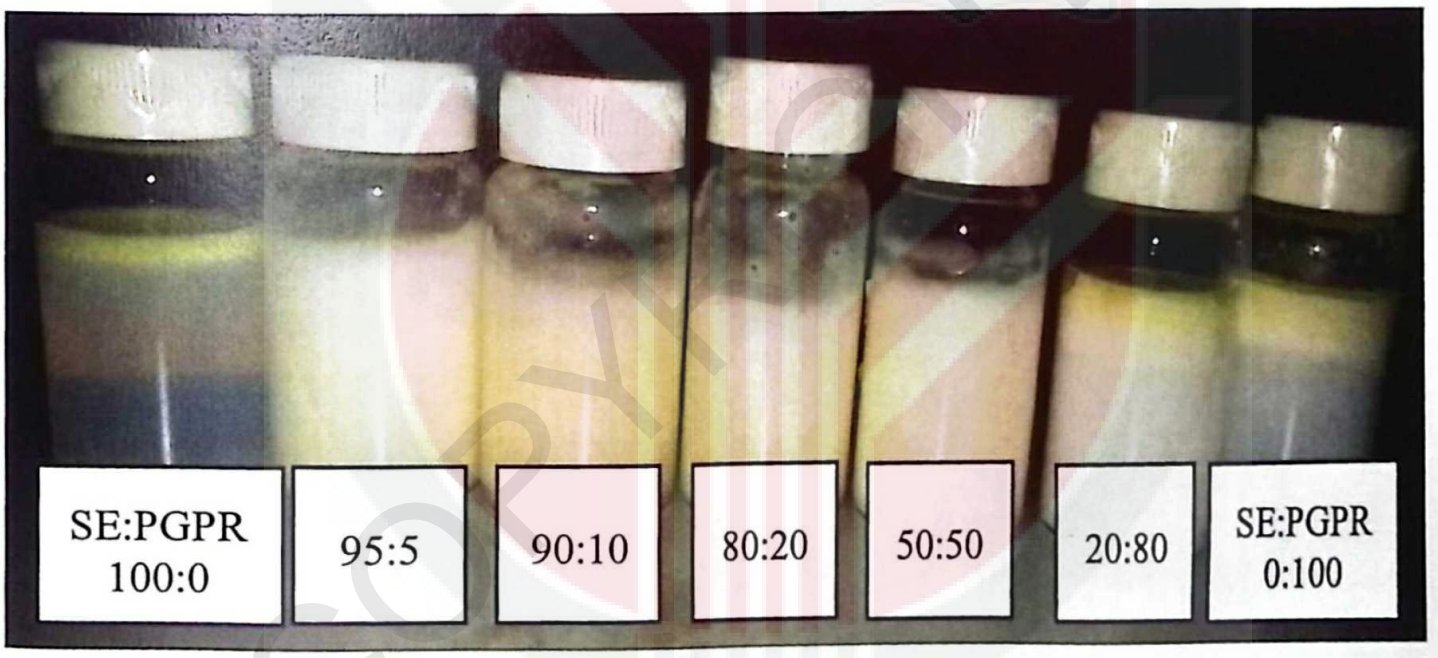

Figure A-7: Passion fruit oil emulsion of combination of sucrose ester:polyglycerol polyricinoleate (SE:PGPR) at 5% PFO and 5% surfactants

Table A-7: Raw data on formulation of 7.5% sucrose ester (SE) at different concentration of PFO in emulsion

PFO (%)	Water (%)	Conc. of Sucrose Ester (%)	Avg. Particle Size (nm)			Avg. PDI		
			1st	2nd	Avg.	1st	2nd	Avg.
1	91.5	7.5	156.1	134.2	145.2 ± 10	0.267	0.281	0.274
3	89.5	7.5	170.3	177.0	173.7 ± 10	0.190	0.130	0.160
5	87.5	7.5	206.4	203.6	205.0 ± 10	0.149	0.165	0.157
7	85.5	7.5	232.8	247.7	240.2 ± 10	0.166	0.180	0.173
7.5	85	7.5	277.7	255.9	266.8 ± 15	0.178	0.201	0.189
8	84.5	7.5	302.9	306.0	304.4 ± 10	0.210	0.212	0.211
10	82.5	7.5	653.5	569.6	611.6 ± 50	0.405	0.343	0.374

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-8: Raw data on formulation of 7.5% PFO with different concentration of sucrose ester (SE) in emulsion

PFO (%)	Water (%)	Conc. of Sucrose Ester (%)	Avg. Particle Size (nm)			Avg. PDI		
			1st	2nd	Avg.	1st	2nd	Avg.
7.5	85	7.5	277.7	255.9	266.8 ± 15	0.178	0.201	0.189
7.5	82.5	10	727.6	719.5	723.5 ± 35	0.419	0.457	0.438
10	80	10	1308.2	1555.6	1405.5 ± 100	0.702	0.851	0.777

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-9: Raw data on variation of mixing temperature for sucrose ester: lecithin (SE:LCT) in emulsion

Mixing Temperature (°C)	PFO (%)	Water (%)	Ratio of Surfactants at 5% Conc.		Avg. Particle Size (nm)			Avg. PDI		
			SE	LCT	1st	2nd	Avg.	1st	2nd	Avg.
25	5	90	75	25	339.5	332.5	336.0 ± 10	0.227	0.225	0.226
30	5	90	75	25	268.2	271.4	269.8 ± 5	0.192	0.203	0.198
35	5	90	75	25	263.2	268.7	266.0 ± 5	0.194	0.198	0.196
40	5	90	75	25	262.2	258.8	258.9 ± 5	0.192	0.185	0.189
45	5	90	75	25	261.3	254.1	257.7 ± 5	0.192	0.206	0.199
50	5	90	75	25	219.6	211.6	215.6 ± 7	0.216	0.250	0.233
55	5	90	75	25	305.7	305.1	305.4 ± 4	0.208	0.209	0.209

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-10: Raw data on variation of mixing temperature for sucrose ester:polyglycerol polyricinoleate (SE:PGPR) in emulsion

Mixing Temperature (°C)	PFO (%)	Water (%)	Ratio of Surfactants at 2.5% Conc.		Avg. Particle Size (nm)			Avg. PDI		
			SE	PGPR	1st	2nd	Avg.	1st	2nd	Avg.
25	2.5	95	90	10	453.7	533.0	493.4 ± 45	0.312	0.352	0.314
30	2.5	95	90	10	673.0	616.5	644.8 ± 35	0.432	0.384	0.408
35	2.5	95	90	10	separate					
40	2.5	95	90	10	separate					
45	2.5	95	90	10	separate					
50	2.5	95	90	10	separate					
55	2.5	95	90	10	separate					

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-11: Raw data on variation of mixing temperature for sucrose ester alone (SE) in emulsion

Mixing Temperature (°C)	PFO (%)	Water (%)	Conc. Of Sucrose Ester (%)	Avg. Particle Size (nm)			Avg. PDI		
				1st	2nd	Avg.	1st	2nd	Avg.
25	5	87.5	87.5	206.4	203.6	205.0 ± 10	0.149	0.165	0.157
30	5	87.5	87.5	233.7	227.7	230.7 ± 5	0.164	0.166	0.165
35	5	87.5	87.5	256.7	281.4	269.1 ± 20	0.166	0.218	0.192
40	5	87.5	87.5	303.9	297.5	300.7 ± 5	0.230	0.222	0.226
45	5	87.5	87.5	363.1	395.7	379.4 ± 20	0.239	0.271	0.255
50	5	87.5	87.5	503.1	571.2	537.2 ± 40	0.327	0.367	0.347
55	5	87.5	87.5	716.2	667.8	692.0 ± 30	0.453	0.411	0.432

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-12: Raw data on variation of oil dropping rate for sucrose ester: lecithin (SE:LCT) in emulsion

Oil Dropping Rate	PFO (%)	Water (%)	Ratio of Surfactants at 5% Conc.		Avg. Particle Size (nm)			Avg. PDI		
			SE	LCT	1st	2nd	Avg.	1st	2nd	Avg.
All in once	5	90	75	25	325.6	321.7	323.7 ± 5	0.215	0.215	0.215
1 drop/10 sec	5	90	75	25	280.4	288.3	284.4 ± 7	0.188	0.207	0.198
1 drop/20 sec	5	90	75	25	245.0	249.8	247.4 ± 3	0.190	0.183	0.187
1 drop/30 sec	5	90	75	25	252.8	261.4	257.1 ± 8	0.200	0.188	0.190
1 drop/40 sec	5	90	75	25	287.4	268.8	278.1 ± 10	0.206	0.180	0.196

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-13: Raw data on variation of oil dropping rate for sucrose ester:polyglycerol polyricinoleate (SE:PGPR) in emulsion

Oil Dropping Rate	PFO (%)	Water (%)	Ratio of Surfactants at 2.5% Conc.		Avg. Particle Size (nm)			Avg. PDI		
			SE	PGPR	1st	2nd	Avg.	1st	2nd	Avg.
All in once	2.5	95	90	10	1392.5	1485.8	1432.5 ± 90	0.703	0.802	0.752
1 drop/10 sec	2.5	95	90	10	1382.7	1385.0	1383.8 ± 10	0.626	0.747	0.687
1 drop/20 sec	2.5	95	90	10	1393.6	1310.3	1352.0 ± 50	0.703	0.645	0.674
1 drop/30 sec	2.5	95	90	10	1265.1	1199.2	1232.1 ± 40	0.662	0.670	0.666
1 drop/40 sec	2.5	95	90	10	1230.4	1155.9	1193.1 ± 50	0.618	0.670	0.644

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-14: Raw data on variation of oil dropping rate for sucrose ester alone (SE) in emulsion

Oil Dropping Rate	PFO (%)	Water (%)	Conc. Of Sucrose Ester (%)	Avg. Particle Size (nm)			Avg. PDI		
				1st	2nd	Avg.	1st	2nd	Avg.
All in once	5	87.5	7.5	621.5	616.9	619.2 ± 20	0.388	0.375	0.382
1 drop/10 sec	5	87.5	7.5	396.3	396.0	396.1 ± 10	0.264	0.260	0.262
1 drop/20 sec	5	87.5	7.5	226.2	209.4	217.8 ± 10	0.167	0.155	0.161
1 drop/30 sec	5	87.5	7.5	231.2	233.6	232.4 ± 5	0.160	0.161	0.165
1 drop/ 40 sec	5	87.5	7.5	273.5	272.4	272.9 ± 3	0.212	0.204	0.200

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-15: Raw data on variation of stirring speed for sucrose ester: lecithin (SE:LCT) in emulsion

Stirring Speed (rpm)	PFO (%)	Water (%)	Ratio of Surfactants at 5% Conc.		Avg. Particle Size (nm)			Avg. PDI		
			SE	LCT	1st	2nd	Avg.	1st	2nd	Avg.
300	5	90	75	25	267.4	263.7	265.5 ± 5	0.188	0.186	0.187
400	5	90	75	25	261.1	256.0	258.5 ± 5	0.182	0.181	0.182
500	5	90	75	25	245.0	249.8	247.4 ± 3	0.190	0.183	0.187
600	5	90	75	25	245.0	249.8	285.1 ± 3	0.200	0.203	0.203
700	5	90	75	25	316.6	351.6	334.1 ± 20	0.210	0.243	0.277

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-16: Raw data on variation of stirring speed for sucrose ester:polyglycerol polyricinoleate (SE:PGPR) in emulsion

Stirring Speed (rpm)	PFO (%)	Water (%)	Ratio of Surfactants at 2.5% Conc.		Avg. Particle Size (nm)			Avg. PDI		
			SE	PGPR	1st	2nd	Avg.	1st	2nd	Avg.
300	2.5	95	90	10	separate					
400	2.5	95	90	10	separate					
500	2.5	95	90	10	1393.6	1310.3	1352.0 ± 50	0.703	0.645	0.674
600	2.5	95	90	10	1551.1	1565.5	1558.3 ± 45	0.759	0.730	0.745
700	2.5	95	90	10	1672.4	1716.3	1693.7 ± 30	0.826	0.861	0.844

* Note: The reading of each repetition are the average of 3 cycles run.

Table A-17: Raw data on variation of stirring speed for sucrose ester alone (SE) in emulsion

Stirring Speed (rpm)	PFO (%)	Water (%)	Conc. Of Sucrose Ester (%)	Avg. Particle Size (nm)			Avg. PDI		
				1st	2nd	Avg.	1st	2nd	Avg.
300	5	87.5	7.5	482.4	507.4	491.6 ± 20	0.313	0.330	0.321
400	5	87.5	7.5	215.6	229.1	222.4 ± 10	0.163	0.159	0.161
500	5	87.5	7.5	226.2	209.4	217.8 ± 10	0.167	0.155	0.161
600	5	87.5	7.5	253.4	251.3	252.4 ± 5	0.187	0.172	0.180
700	5	87.5	7.5	322.9	413.2	428.6 ± 70	0.225	0.295	0.279

* Note: The reading of each repetition are the average of 3 cycles run.