UNIVERSITI PUTRA MALAYSIA

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ABSTRACT

Nanoemulsions are generally defined as biphasic dispersion of two immiscible liquids either water in oil (W/O) or oil in water (O/W) droplets with the mean droplet diameter size less than 200 nm which can be produced using high or low energy method. Low energy method depends highly on the type of oil and surfactant. Previous research has shown that long-chain triglycerides was not able to produce nanoemulsion using low energy method. The ability to produce nanoemulsion using low energy method required a balance interaction between surfactant with water and surfactant with oil, and this was not found to be achieved using single surfactant nor with combination of surfactant at different hydrophilic-lipophilic balance (HLB) that have similar molecular geometry of the tail and head part of the surfactant. However, a balance interaction might be achieved if the packing geometry of the surfactants is optimized.

In this study, combination of food grade surfactants that produced different packing geometries was investigated. The main hydrophilic surfactants used are sucrose ester (HLB: 16) and Tween 80 (HLB 15) which are crucial to produce oil-in-water emulsion. These two surfactants were combined with distilled monoglycerides (contain one hydrophobic tail), lecithin (contain two hydrophobic tail) and polyglycerol polyricinoleate (contain three or more hydrophobic tail). Passion fruit oil which is long-chain triglyceride oil was used in this study as it may add value to agro-industrial waste and contains active ingredients that are beneficial for the skin.

From the result of the study, the combination of surfactant that produces the lowest interfacial tension (IFT) is sucrose ester with polyglycerol polyricinoleate, followed by sucrose ester with lecithin and finally sucrose ester with distilled monoglycerides. Similar trend was found with the combination using Tween 80. Based

on the combination of surfactant used, the lowest IFT achieved is 0.034 mN/m using Tween 80: polyglycerol polyricinoleate which indicated the possibility to produce nanoemulsion. This also indicated that the surfactant which contains more tail groups produced the lowest IFT when combined with a single short tail of high HLB surfactant.

It was found that the lowest IFT was obtained from sucrose ester alone which is 0.014 mN/m at 1.5% of concentration, followed with the combination of sucrose ester with polyglycerol polyricinoleate and combination of sucrose ester with lecithin at third place. The combination of sucrose ester and lecithin at the only 5% concentration with 5% concentration of passion fruit oil can produce passion fruit oil nanoemulsion at low interfacial tension of oil-water phase using low energy method. The smallest particle size which is 187.9 nm was obtained at a ratio of 80:20 (sucrose ester:lecithin) at 5% concentration to emulsify 5% of passion fruit oil. However, based on 5% concentration of surfactant used, nanoemulsion cannot be produced with the combination of sucrose ester:polyglycerol polyricinoleate and Tween 80:polyglycerol polyricinoleate although the obtained IFT is low. This is due to improper concentration of surfactant used. In this study, it was found that combinations with sucrose ester have an excellent stability behaviour of the particle size in the emulsion compared to the combinations with Tween 80 although the particle size obtained is at the macromolecule range.

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LIST OF ABBREVIATION

HLB	Hydrophilic-Lipophilic Balance
IFT	Interfacial Tension
SE	Sucrose Ester
T80	Tween 80
MDG	Distilled Monoglyceride
LCT	Lecithin
PGPR	Polyglycerol Polyricinoleate
PFO	Passion Fruit Oil
Conc.	Concentration
Avg.	Average

CHAPTER 1: INTRODUCTION

1.1 Background

Passion flower plant originally comes from South America and Africa, produce the passion fruit (Passiflora edulis Sims f. flavicarpa Degener) which also called as 'maracuja'. In Malaysia, the passion fruit called as 'buah markisa' is well known in Malaysia and can be easily obtained at Johor, Melaka and Kelantan. The seeds that come from the passion fruit have a high nutritional value which is a good source of fat, especially high in unsaturated fatty acids that are essential to the human diet (Lopes et al., 2010). The extracted oil from the passion fruit seeds contains polyphenolic compounds carotenoid families and vitamin C that is very beneficial for the skin. The seeds of the passion fruit can be easily obtained as a thousand tons of seeds produces from the passion fruit as an agricultural by-product during juice extraction in the juice industry (Lopes et al., 2010). So in this study, the passion fruit oil that beneficial for human health was extracted from the seeds to produce nanoemulsion and at the same time, this can add the value on this agricultural by-product.

Because this passion fruit oil (PFO) contains a lot of active ingredients that beneficial for skins, most people used this PFO as the ingredients of their cosmetics. The typical cosmetic usually is not in nano-particle size and this will affect the effectiveness of the diffusion of the active ingredients through the skin because the penetration of this cosmetic will be very low compared to the nanoparticle size. As the particle size of the cosmetic emulsion is small, this will enhance the penetration of the active ingredients in the cosmetic into the skin layers due to the large surface area of droplets. Thus, in this study, the passion fruit oil nanoemulsion was produced to obtain rapid penetration of this formulation through the skins.

There are two methods to produce nanoemulsion which are high and low energy methods. High energy methods can produce nanoemulsion by using ultrasonicators, microfluidizer and high-pressure homogenizer (Singh et al., 2017). While low energy methods can produce nanoemulsion by spontaneous emulsification (SE), emulsion phase inversion (EPI), phase inversion composition (PIC) and phase inversion temperature (PIT). When it comes to the high energy, of course, the high cost is the issue, but when the low energy method was used to produce nanoemulsions, the long chain triglycerides (LCT) oil type in nanoemulsion is the most difficult to be produced and high surfactants amount was used compared to the high energy and also the preparation conditions is more complex. In this study, the modification on the spontaneous emulsification was used to produce nanoemulsion.

When low energy method used in the preparation of nanoemulsions, the most important parameter is the surfactant. This surfactant is a substance used for decreasing the surface tension or lowering the interfacial tension (Salaguer, 2002). So it is clear that the surfactant will be used to lower the interfacial tension (IFT) between the water and oil phase so that it can be emulsified. These surfactants usually classified based on their hydrophilic-lipophilic balance (HLB) and their molecular geometry (Komaiko & Mcclements, 2016). This HLB is actually the balance of the size and strength of the hydrophilic (water-loving or polar) and the lipophilic (oil-loving or non-polar) groups of the emulsifier (ICI Americas Inc, 1980). Next, each surfactant has different molecular geometry that can be characterized by a packing parameter or geometry (p), which this p = aT/aH or in words, this packing geometry is equal to the ratio of the hydrophobic tail group to hydrophilic head group cross-sectional areas (Komaiko & Mcclements, 2016).

1.2 Problem Statement

Usually, when only a single surfactant used in the oil-water system such as Tween 80, it will result in high of interfacial tension between the oil-water interface due to the poorest packing geometry of the surfactant. An understanding of the factors that influence the packing geometry of a surfactant is often extremely useful for optimizing the formation of nanoemulsions by low energy methods (Komaiko & Mcclements, 2016). Therefore, in this study, the combination of surfactants was used to develop synergistic effect on the packing geometry of the surfactants to favours the formation of nanoemulsion using low energy method by lowering the interfacial tension of oil-water interphase.

The production of nanoemulsion using low energy method was successful for the medium-chain triglyceride (MCT) oil such as lemon and thyme oil but will be much complex and difficult when the long-triglyceride (LCT) oil introduce to nanoemulsion using low energy method. So, another problem faced in this study is the passion fruit oil is a vegetable oil or LCT oil type which was difficult to produce the nanoemulsion by using the low energy method. This is one of the reasons why the combination of food grade surfactants was used in producing this nanoemulsion to obtain the bicontinuous microemulsion in order to obtain small oil droplets.

1.3 Objectives

The research is an experimental study of the development passion fruit oil nanoemulsion using the combination of food grade surfactants and the main aim is to produce nanoemulsion using low energy methods. The objectives of the study are:

1. To identify the interfacial tension of surfactants combinations at different packing geometry by varying the concentration and surfactant ratio.
2. To determine the effect of using combination of surfactants at different packing geometry on the nanoemulsion by low energy method.

CHAPTER 2: LITERATURE REVIEW

2.1 Vegetable Oil (VO)

Vegetable oil (VO) is oil that is extracted from various types of fruits, seeds, grains, and nuts. VO are excellent renewable sources for chemical and oleo-chemistry industries because VO can be used in various applications (Lopes et al., 2010). Results from oleo-chemistry show that the use of VO allows the development of competitive, powerful products, which are both consumer-friendly and environment-friendly. Other than that, VO presents low toxicity, good lubricity, low volatility, high viscosity index, inherent biodegradability, solvent for lubricant additives, and easy miscibility with other fluids (Lopes et al., 2010). Moreover, VO are one of the cheapest and most abundant biological sources available in large quantities and having a huge benefit. In industry, the most popular VO are usually made from canola, coconut, corn, cottonseed, olive, palm, palm-kernel, peanut, safflower, soybean, and sunflower which this type of VO is used to add flavour, assist with texture, and to cook food. In this study, the VO that will be used is passion fruit oil that comes from the passion fruit seeds.

2.2 Passion Fruit Oil (PFO)

Passion flower plant originally comes from the South America and Africa, produce the passion fruit (Passiflora edulis Sims f. flavicarpa Degener) which also called as 'maracuja'. In Malaysia, the passion fruit is well known and called as 'buah markisa' and also popular in Johor, Melaka and Kelantan. Passion fruit is a great source of fibre and considered as beneficial for digestive health. Other than that, its contains polyphenolic compounds carotenoid families and vitamin C that is very beneficial for the skin. Thousand tons of seeds produces from the passion fruit as agricultural by-product during juice extraction in the juice industry (Lopes et al.,

2010). Generally, in juice industry, these seeds are just a waste after being crushed for the juice industry. The seeds itself have about 6 to 12% of the total weight of the fruit and they can be as oil source carbohydrates, proteins, and minerals. So, there is large amount of fibre and oil contained in the seed (Chau & Huang, 2004). The passion fruit oil (PFO) comes from the seeds of the passion fruit which is rich in vitamin C, calcium and phosphor (Chau & Huang, 2004). The oil showed high levels of unsaturated fatty acids (87.59%), including mainly linoleic (73.14%) and oleic (13.83%) acids, tocopherol (499.30 mg/kg) and phenolic compounds (1,314.13 mg GAE/kg). The physicochemical characteristics were similar to those of other edible oils and the oil showed significant antioxidant activity (Malacrida & Jorge, 2012). This PFO having pleasant flavour and there is presence of ascorbic acid, flavonoids, b-carotene and potassium also contained fatty acids mainly linoleic acid with about 60% (Lopes et al., 2010). The oil seed extraction can be performed by Soxhlet method. In this study, by using low energy method, the passion fruit oil will be used to produce passion fruit oil nanoemulsion as it has many benefits to our skins, can be easily obtained in our country and added value to agro-waste.

2.3 Nanoemulsions

Nanoemulsions are generally defined as biphasic dispersion of two immiscible liquids either water in oil (W/O) or oil in water (O/W) droplets with the mean droplet diameter size less than 500nm that stabilized by the surfactant (Singh et al., 2017). Because the droplet diameter size of nanoemulsion is small, it will provide a wide surface area that will lead active components in nanoemulsion to be penetrated quickly through the skins (Singh et al., 2017). Moreover, nanoemulsion is metastable system which it has a tendency to break down over time due to various destabilization mechanisms such as gravitational separation, coalescence, flocculation and Ostwald

ripening (Komaiko & Mcclements, 2016). In order to obtain better stability to gravitational separation and droplet aggregation, the nanoemulsions should have smaller size of droplets diameter (Komaiko & Mcclements, 2016). Generally, a clear or hazy appearance will be obtained when having a small droplet size compared to milky white colour that usually obtained from the coarse emulsion (Singh et al., 2017). Other than that, there are two types of emulsion which are water-in-oil and oil-in-water. Water in oil emulsion (W/O) occurred when the water phase is dispersed in oil phase while oil in water emulsion (O/W) occurred when the oil phase is dispersed in water phase (Komaiko & Mcclements, 2016). In order to prepare nanoemulsions, generally there are two methods which are high and low energy methods.

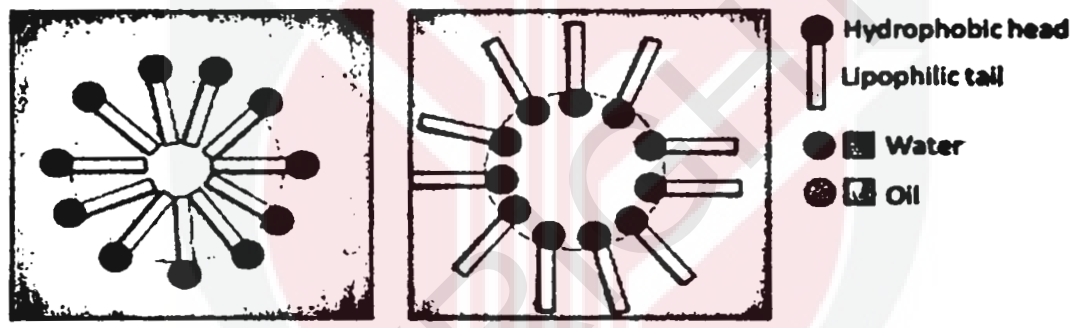

Figure 2-1:Schematic picture of A (oil in water) and B (water in oil) emulsions

2.3.1 High Energy Methods

In high energy methods, the free energy comes from mechanical forces applied to the systems in order to create powerful disruptive forces for size reductions (shear, turbulence or cavitation) that achieved from ultrasonicators, microfluidizer and high pressure homogenizer (Singh et al., 2017). The reason why most of the industries used high energy methods when preparing nanoemulsion is because most of any oil can be subjected to nanoemulsifications (Singh et al., 2017). Other than that, the high energy methods only required a little amount of an amphiphilic surfactants compared to low energy methods. The major disadvantages of this high energy methods are high

instrumental cost and generation of high operational temperature which usually rules out the thermolabile drugs (Singh et al., 2017).

2.3.2 Low Energy Methods

Nanoemulsions prepared by low energy methods were developed after studying the cumulative behaviour of oil, surfactants, co-surfactants, aqueous component, hydrophilic-lipophilic balance of utilized oil surfactant blend and operative temperature (Singh et al., 2017). Because the low energy methods do not require any expensive equipment that used in high energy methods, the optimal conditions for low energy production of nanoemulsions are compulsory to understand. To be specific, it is a must to have a better understanding of the types and amounts of ingredients required to form nanoemulsions by low-energy methods, and to establish the most appropriate preparation methods to use for particular applications (Komaiko & Mcclements, 2016). The advantage of using low energy methods to prepare nanoemulsions is low in terms of cost while the disadvantages of this low energy methods are it is often difficult to produce very small droplets using long chain triglycerides types of oil (LCT) using low-energy methods, which limits this method for many applications and next, the amount of surfactants used is higher compared to the high energy methods. The low energy methods were divided into two which are isothermal methods and thermal methods. In the preparation of nanoemulsions, the isothermal methods are which do not require a change in temperature to produce fine droplets such as spontaneous emulsification, emulsion phase inversion, phase inversion composition and emulsion inversion point (Komaiko & Mcclements, 2016). While the thermal methods require a change in temperature to encourage the formation of a nanoemulsion such as phase inversion temperature.

2.3.3 Spontaneous Emulsification

Spontaneous emulsification (SE) is an isothermal low energy method to produce nanoemulsions. SE can take place through numerous physicochemical mechanisms which generally, the SE happens when two immiscible liquids are place in contact and then emulsify without any external assistance whether thermal or mechanical (Komaiko & Mcclements, 2016). Usually the procedure of this SE is by titrating an organic phase which is the oil with hydrophilic surfactants into a container containing an aqueous phase which is water where the fine oil droplets in the water can be obtained if both phases composition and preparation conditions are optimized (Komaiko & Mcclements, 2016). The proposed mechanism of SE methods is the formation of a bicontinuous microemulsion at the boundary where the titrated organic and aqueous phases was in contact actually leads to the spontaneous generation of fine oil droplets where then the bicontinuous microemulsion boundary phase breaks up by applying some mild stirring in order to facilitate the breakdown of the bicontinuous microemulsion as in Figure 2-2 (Komaiko & Mcclements, 2016). However, to obtain nanoemulsion by SE is not easy because there are a lot of factors that have to be considered and will affect the emulsions such as preparation conditions, oil composition, surfactant type, surfactant concentration, cosolvents, cosurfactants, and system composition (Komaiko & Mcclements, 2016).

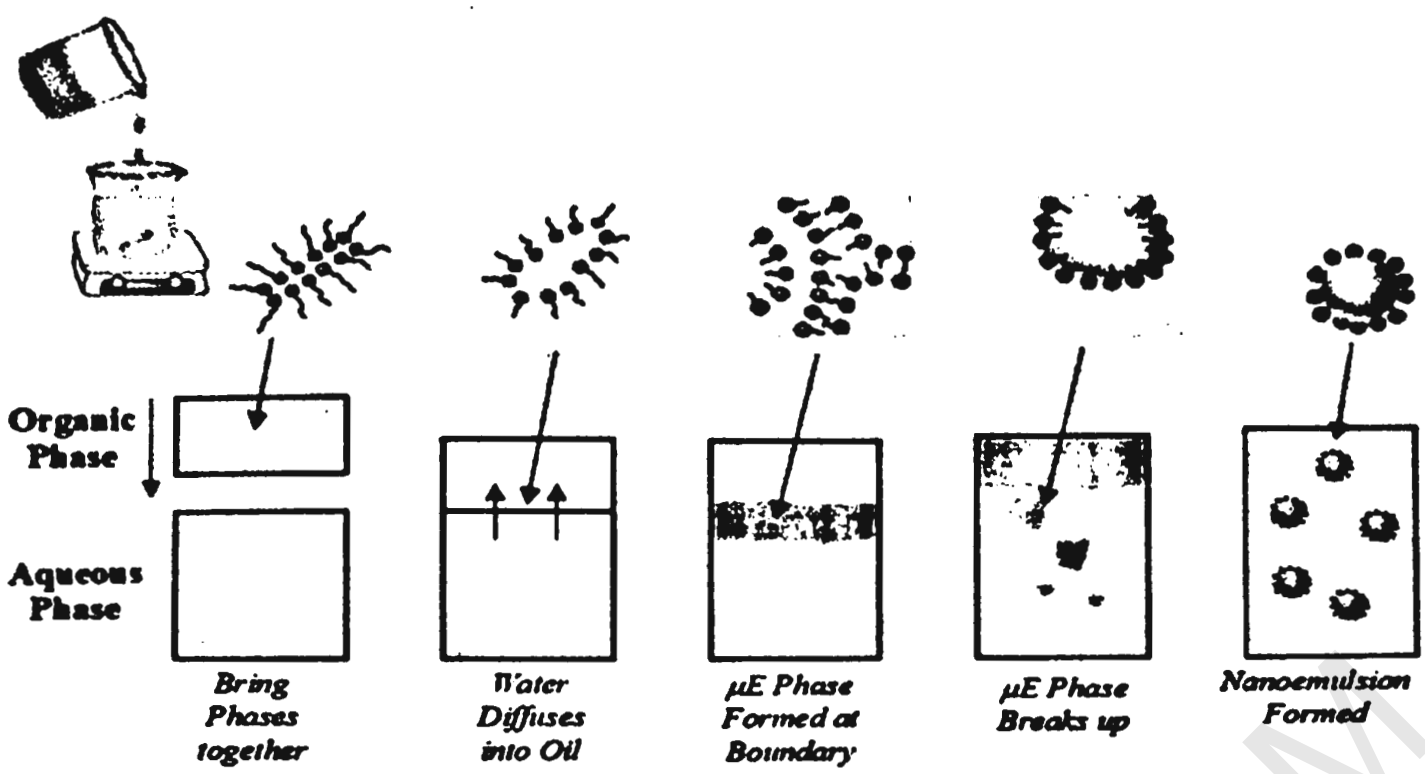

Figure 2-2: Schematic diagram of potential mechanism for formation of nanoemulsion by the spontaneous emulsification (Komaiko & Mcclements, 2016)

2.3.4 Emulsion Phase Inversion

Emulsion phase inversion (EPI) is an isothermal low energy method to produce nanoemulsions. Generally, the EPI is just the opposite method of SE which the addition of an aqueous phase into a stirring organic phase (Komaiko & Mcclements, 2016). When the aqueous phase is initially titrated into the organic phase, a water-in-oil emulsion is formed and as more water is added, a liquid crystalline phase may be formed that can become so viscous that it prevents the stir bar from continuing to rotate (Komaiko & Mcclements, 2016). The proposed mechanism of the EPI method is the formation of this liquid crystalline phase may be an important intermediate step in nanoemulsion production, as it may be related to the generation of the bicontinuous microemulsion that eventually breaks down and forms small droplets as in Figure 2-3 (Komaiko & Mcclements, 2016). Then, the inner oil droplets are spontaneously formed at the boundary between the organic and aqueous phase which may exist as a bicontinuous microemulsion at a certain surfactant-oil-water ratio that breaks down.

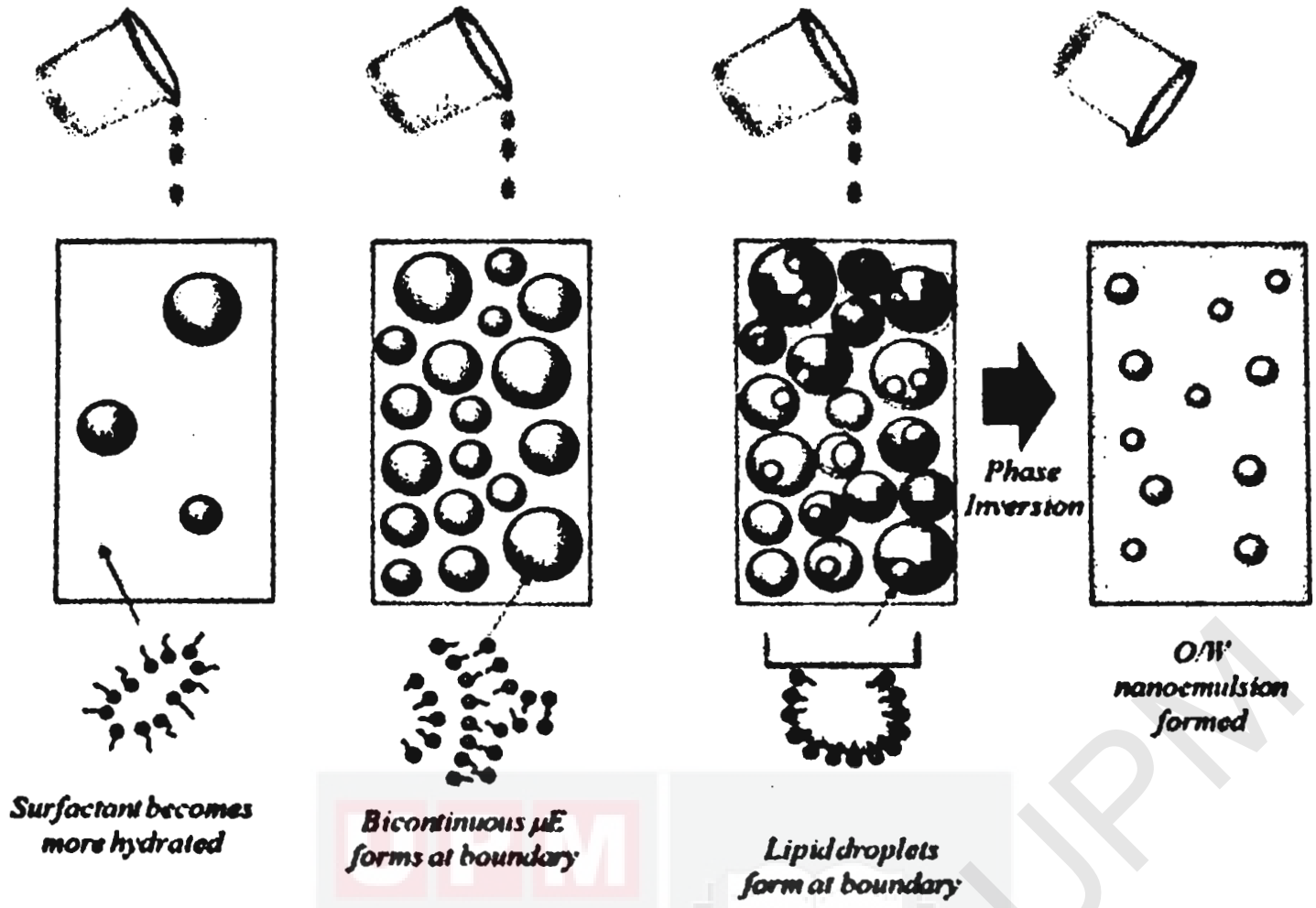

Figure 2-3: Schematic diagram of potential mechanism for formation of nanoemulsion by the emulsion phase inversion (Komaiko & Mcclements, 2016)

2.3.5 Phase Inversion Temperature

Phase inversion temperature (PIT) is a thermal low energy method to produce nanoemulsions. Usually, the PIT method used to produce nanoemulsion from a mixture of the hydrophilic non-ionic surfactant, oil and water using three main steps which firstly the mixture of surfactant, oil and water are stirred at room temperature to form a coarse emulsion, then the mixture is slowly heated up to around or above the PIT and lastly the mixture is either rapidly cooled or diluted into cold water with continuous stirring, which results in the formation of an O/W nanoemulsion as in Figure 2-4 (Komaiko & Mcclements, 2016). The mechanism of the formation of small oil droplets using the PIT methods is related to the changes in the structural and physicochemical characteristics of the surfactants during heating, which the surfactant head groups are highly hydrated or predominantly hydrophilic and tends to be located in the aqueous phase at low temperature while the surfactants is predominantly

lipophilic as the head groups are largely dehydrated and tends to be located in the organic phase when at high temperature (Komaiko & Mcclements, 2016).

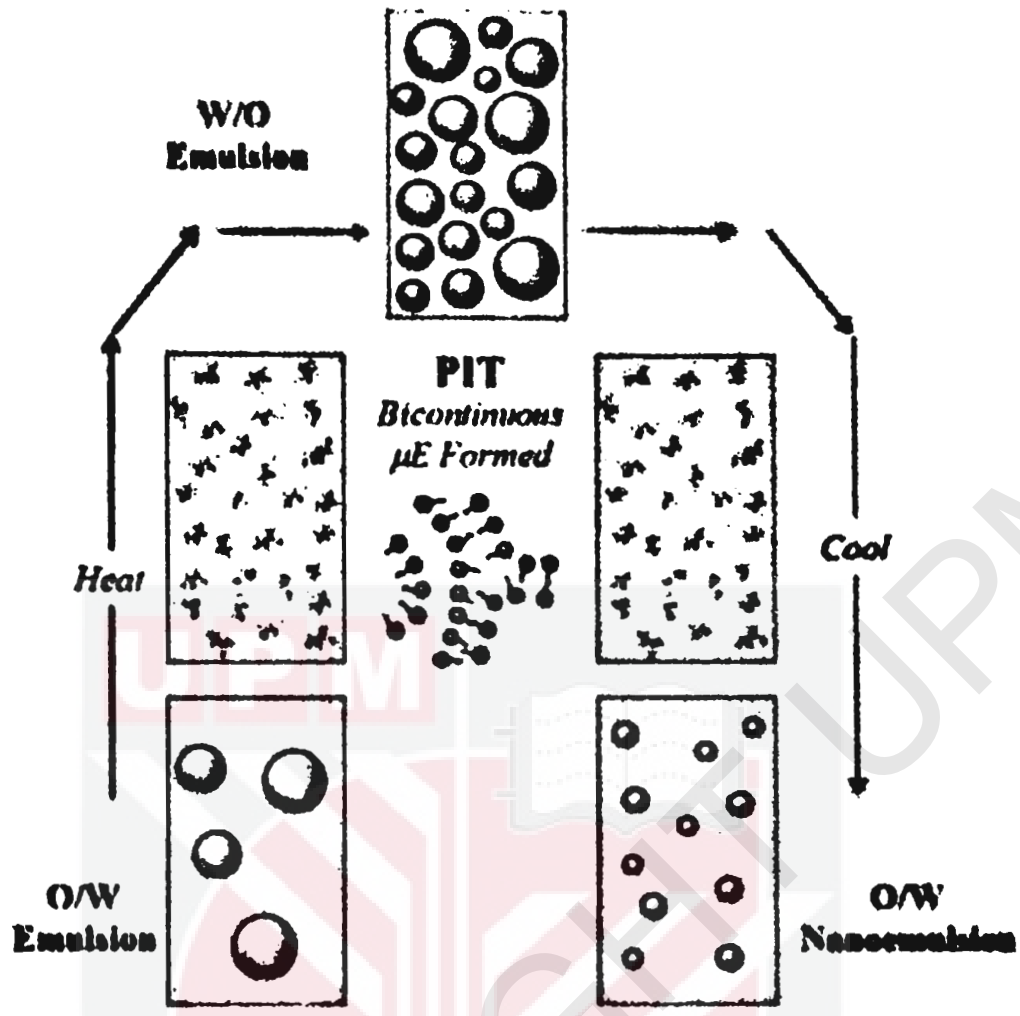

Figure 2-4: Example of formation of nanoemulsion by the phase inversion temperature (Komaiko & Mcclements, 2016)

Another explanation of the mechanism for nanoemulsion formation by the PIT method is based on changes of optimum curvature of the surfactant molecules with temperature. The surfactant head groups are hydrated and have a molecular geometry with p as the packaging geometry, $p < 1$ at low temperature that indicates the formation of oil-in-water emulsion, while at intermediate temperature which is near the PIT, the surfactant head groups are partially dehydrated and have a molecular geometry of $p = 1$ that initiate the formation of planar monolayers as the interfacial tension in extremely low and will result the formation of bicontinuous microemulsion, while at high temperature, the surfactants will have molecular geometry of $p > 1$ as the head groups are dehydrated and initiate the formation of water-in-oil emulsions (Komaiko &

Mcclements, 2016). Thus, to obtain small droplets, the cooling process must typically be carried out rapidly with continuous stirring.

2.3.6 Previous Study Review on Methodology of Producing Nanoemulsion of Long-Chain Triglyceride Oil Using Low Energy Method

The title of this study review is development of avocado oil nanoemulsion hydrogel using sucrose ester stearate (Eid, Elmarzugi, & El-enshasy, 2013). Avocado oil is a type of long-chain triglyceride oil and this oil has been used as an antioxidant due to the high amount of oleic monosaturated fatty acids in the oil. In their study, the objective was to characterize nanoemulsion hydrogel prepared using avocado oil and sucrose ester stearate as surfactant using low energy method. The emulsion produced were examined using zetasizer to determine the particle size of dispersed oil droplets, polydispersibility index (PDI) and zeta potential (Eid et al., 2013). To produce nanoemulsion by low energy method, sucrose ester stearate was mixed with glycerol at 75 °C until it was dissolved then the oil was added to the surfactant mixture at the same temperature and mixed until the mixture cool down to the room temperature (Eid et al., 2013). The emulsions were tested for their particle size, polydispersity index and zeta potential using zetasizer. Then, the optimum nanoemulsion formulation was converted to hydrogels using two different types of Carbopol 934 and 940 (Eid et al., 2013). Therefore, the surfactant was mixed into aqueous phase which in their study is glycerol at certain temperature to dissolved the surfactant, and lastly the oil was added to the surfactant mixture to produce emulsion.

2.4 Surfactants

Surfactant or surface active agent is defined as a substance which demonstrates some superficial of interfacial activity (Salaguer, 2002). In other words, surfactant is a substance used for decreasing the surface tension or lowering the interfacial tension. Surfactant can be manufactured from natural sources like natural oil and fats and also synthetized from a petroleum cut (Salaguer, 2002). In the food industries, the surfactants have been used for many centuries for example, naturally occurring surfactants such a lecithin from egg yolk and various proteins from milk are used for the preparation of many food products such as mayonnaise, salad creams, dressings, deserts and so on (Kralova & Sjöblom, 2017). Other than that, the surfactants also were used in various types of industries.

Surfactant usually referred as an amphiphilic substance which consists of two parts which is polar and apolar group (Salaguer, 2002). The polar group is hydrophilic or water-loving which shows an strong attraction to the polar solvent, usually water, and the apolar group is hydrophobic or water-hating which is an hydrocarbon chain of the alkyl or alkylbenzene type, sometimes with halogen atoms and even a few nonionized oxygen atoms (Salaguer, 2002). Because the surfactant is an amphiphilic molecule, there will be always one of the group (polar or apolar) tends to not like the solvent environment since the molecules demonstrate a very strong tendency to migrate to the interfaces and to place itself on the position where the polar group is in water while the apolar group is in oil (Salaguer, 2002). The basic structure of amphiphilic surfactant is as in Figure 2-5 (Novakovish, 2018).

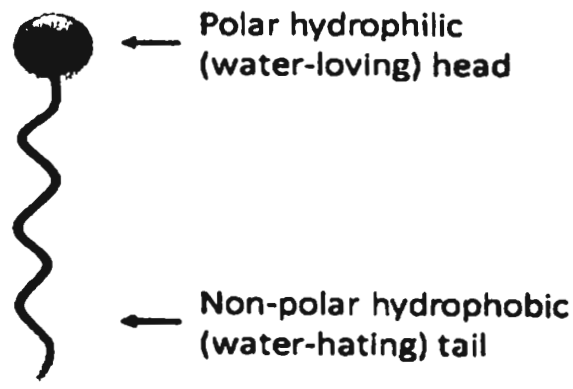

Figure 2-5: The basic structure of surfactant (Novakovish, 2018)

Amphiphilic substance usually called according to their use or commercial point such as soap, detergent, wetting agent, dispersant, emulsifier, foaming agent, bactericide, corrosion inhibitor and antistatic agent (Salaguer, 2002). Surfactants have been classified based on their dissociation in water as this is the most accepted ways to differentiate the surfactants because there are many confusions about the surfactant as they have several uses (Salaguer, 2002). Based on Figure 2-6, surfactant was classified into four different categories which is anionic, nonionic, cationic and zwitterionic surfactants. Surfactant that dissociated in water with an amphiphilic anion and cation is the anionic type, while cationic type is dissociated in water with an amphiphilic cation an anion, but then there is also nonionic surfactant that do not ionize in aqueous solution as its hydrophilic group is a non-dissociable type and lastly there is also zwitterionic surfactant which its molecule exhibit both anionic and cationic dissociation (Salaguer, 2002).

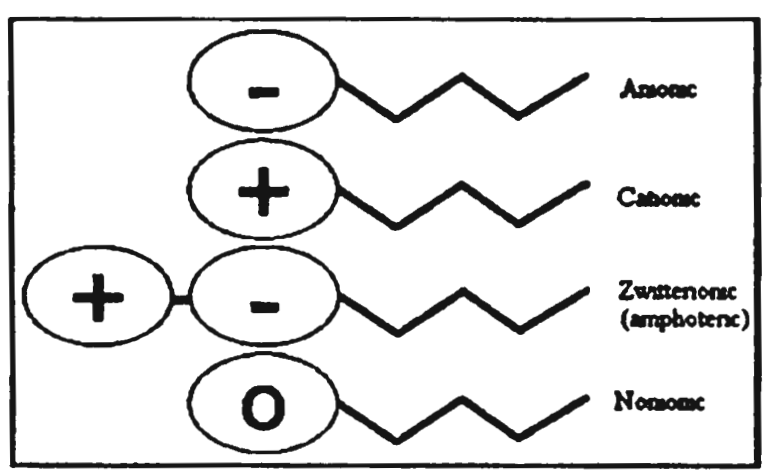

Figure 2-6: Type of surfactant (Novakovish, 2018)

Surfactants were acted as protective coating that can prevent droplet aggregation during and after emulsion formation. In this study, the food grade emulsifier or surfactants were used. The examples of food-grade emulsifiers include small-molecule surfactants, phospholipids, amphiphilic proteins, and amphiphilic polysaccharides. These surfactants usually classified based on their hydrophilic-lipophilic balance (HLB) and their molecular geometry (Komaiko & Mcclements, 2016).

2.4.1 Hydrophilic-Lipophilic Balance (HLB)

There are a lot of emulsifier or surfactants to produce emulsion, but usually to identify the appropriate surfactant required to formulate our emulsion, most of people will choose their surfactant based on its hydrophilic-lipophilic balance (HLB). In the HLB System, each emulsifier is assigned a numerical value which called as HLB. This HLB is actually the balance of the size and strength of the hydrophilic (water-loving or polar) and the lipophilic (oil loving or non-polar) groups of the emulsifier (ICI Americas Inc, 1980). As mentioned before, a surfactant consists of a molecule that combines both hydrophilic and lipophilic groups. Generally, as in Figure 2-7, the lipophilic surfactant is assigned a low HLB number which is below 9.0 that suitable for water-in-oil emulsion while the hydrophilic surfactant is assigned a high HLB number which is above 11.0 that suitable with oil-in-water emulsion, and those in the range of 9-11 are intermediate (ICI Americas Inc, 1980). When two or more emulsifiers are blended, the resulting HLB of the blend is easily calculated by multiply the value of HLB with used amount in percentage, then total up the values together (ICI Americas Inc, 1980). The function of the surfactants that based on the HLB value usually listed as in Figure 2-8.

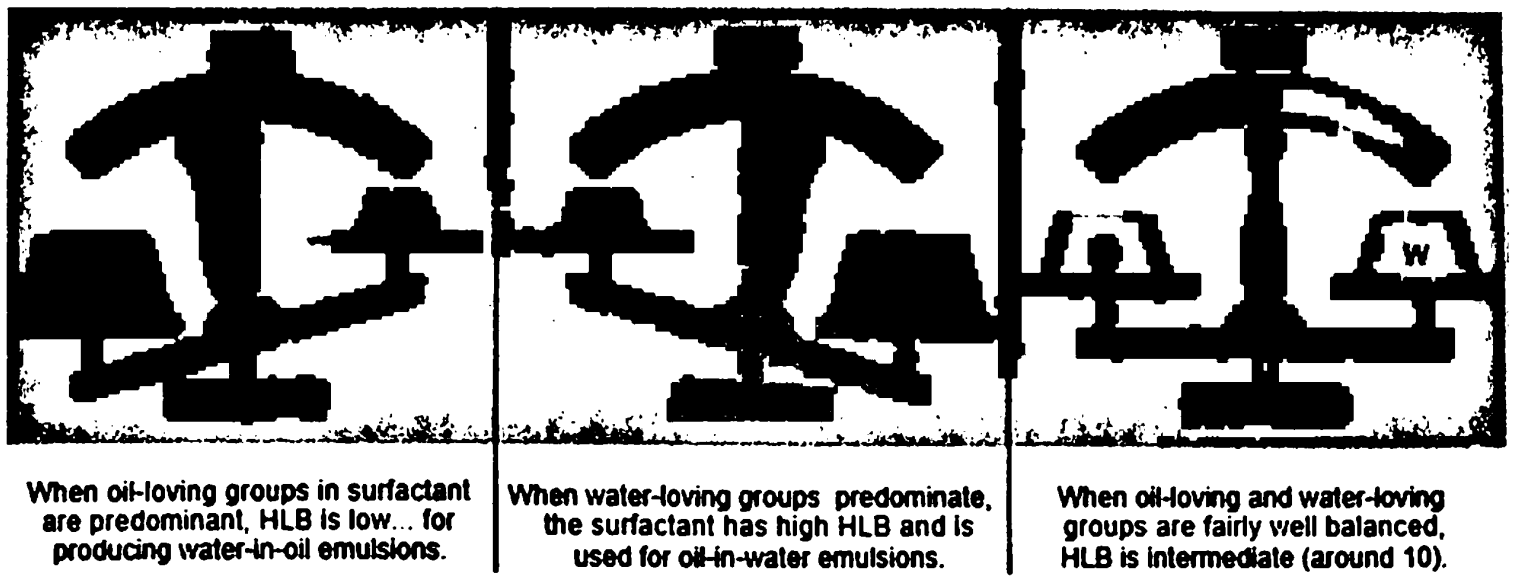

Figure 2-7: The hydrophilic-lipophilic balance of surfactants (ICI Americas Inc, 1980)

HLB Range	Use
4-6	W/O emulsifiers
7-9	Wetting agents
8-18	O/W emulsifiers
13-15	Detergents
10-18	Solubilizers

Figure 2-8: The function of surfactants according to HLB value (ICI Americas Inc, 1980)

In this study, to identify the appropriate surfactants required to formulate passion fruit oil nanoemulsion, it is not just depending on the HLB, but also depends on the molecular geometry of the surfactants or the packing geometry obtained during nanoemulsion.

2.4.2 Packing Parameter/Geometry (p)

Every surfactant has different molecular geometry that can be characterized by a packing parameter (p), which this $p = a_T/a_H$ or in words, this packing geometry is equal to the ration of the tail group to head group cross-sectional areas (Komaiko & Mcclements, 2016). This packing geometry actually determines the optimum packing of surfactants when they assemble into monolayers in production of nanoemulsion, which in turn determines the optimum curvature that tends to be adopted by a given

surfactant (Komaiko & Mcclements, 2016). In order to obtain smaller size of droplets in emulsion using low energy method, the understanding of this packing geometry is compulsory to have the optimize packing geometry of surfactants. As in Figure 2-9, when the packing geometry, p is more than one ($p > 1$), the tail group is significantly larger that the head group which then the monolayer adopts a curvature, where the tail groups point outwards, which it will favours the formation of reverse micelles and water-in-oil emulsions (Komaiko & Mcclements, 2016). On the other hand, the packing geometry, p is less than one ($p < 1$) when the head group is significantly larger that the tail group, which then the monolayer adopts a curvature where the head groups point outward, which favours the formation of micelles and oil-in-water emulsions (Komaiko & Mcclements, 2016). Other than that, if the ratio of head to tail group cross-sectional areas are similar, the packing geometry, p is one ($p = 1$) which then the monolayer tends to be planar which favours the formation of bilayers and vesicles (Komaiko & Mcclements, 2016).

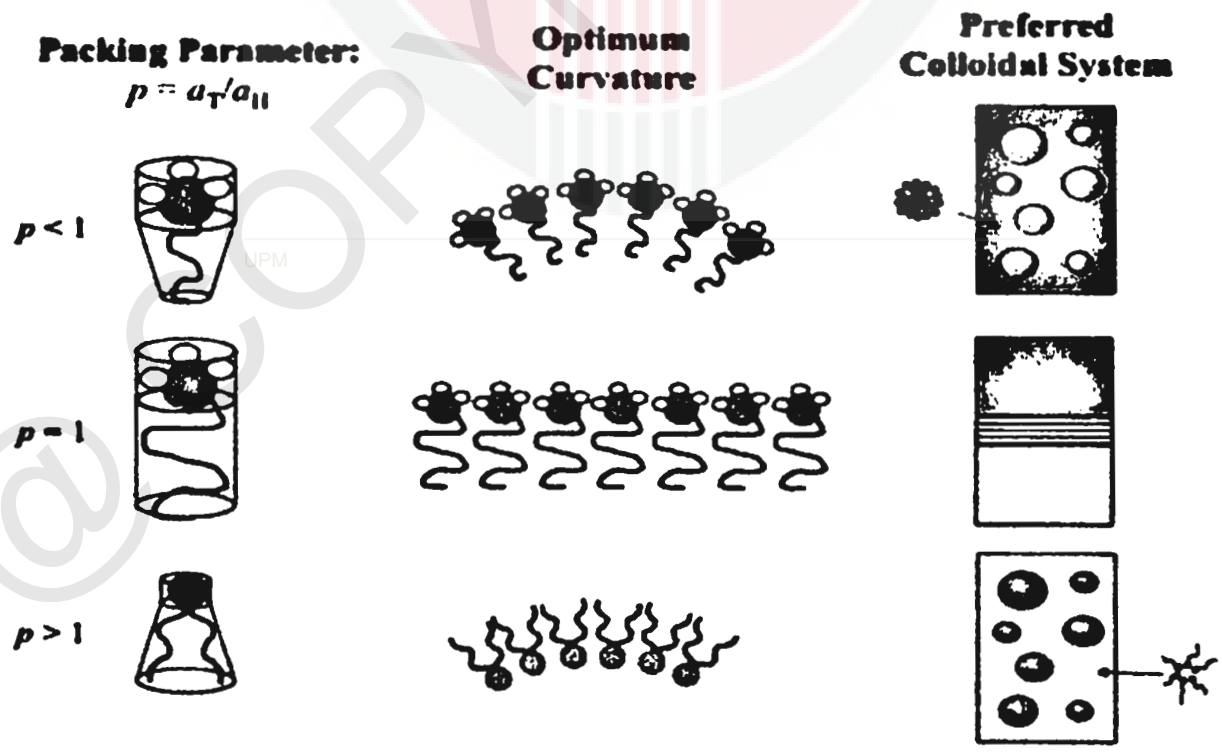

Figure 2-9: Schematic diagram of packing geometry that depends on their molecular geometry (Komaiko & Mcclements, 2016)

Generally, the packing geometry that equal to one will favour the formation of nanoemulsion using low energy methods. Thus, an understanding of the factors that influence the packing geometry of a surfactant is often extremely useful for optimizing the formation of nanoemulsions by low energy methods (Komaiko & Mcclements, 2016). In this study, the combination of surfactants was used which is Tween 80, Lecithin, Sucrose Ester, Distilled Monoglycerides and Polyglycerol Polyricinoleate to obtain optimum packing geometry and helps the formation of nanoemulsion by using low energy methods.

2.5 Interfacial Tension (IFT)

Interfacial tension (IFT) can be defined as the tension between two different types of liquid phases such as organic and aqueous phase. Usually, when two different phases (gas/liquid, liquid/liquid, gas/solid or liquid/solid) are in contact with each other the molecules at the interface experience an imbalance of forces (Biolin Scientific, n.d.). If the measurement of IFT value obtained is low, then it indicates that the two immiscible liquids are same in polarity while if the IFT value obtained is high, it shows that they are in different polarity. The efficiency of a surfactant shows how easy is to reduce the tension using this surfactant and effectiveness corresponds to the minimum value obtained of interfacial tension (Mestre, C., Prieto, C., Ribeiro, n.d.). The combination of surfactants was important because we want to obtain one as the packing geometry. Other than that, based on the packing geometry of the combination of surfactants, the main objectives of using that mixture is to obtain the lowest IFT value because it will initiate the formation of nanoemulsion by producing the microemulsion bilayer. Usually, the lowest of the IFT of oil-water interface was obtained at the point of critical micelle concentration.

2.6 Critical Micelle Concentration (CMC)

The critical micelle concentration is defined as the concentration of surfactants above which micelles form and almost all additional surfactants added to the system turn into micelles (Mestre, C., Prieto, C., Ribeiro, n.d.). When micelles form, the aqueous surfactant solution behaves as a micro heterogeneous medium. The value of the CMC can be determined by the change in the physicochemical properties of the surfactant solution as the surfactant concentration increases (Domínguez, Fernández, González, Iglesias, & Montenegro, 1997). The CMC can be affected by many variables such as temperature and pressure being of relatively minor importance. It decreases with increasing hydrocarbon chain-length of the apolar groups, and for ionic surfactants it also depends on the nature and concentration of counter ions in solution (Domínguez et al., 1997).

2.7 Combination of Surfactants

2.7.1 Sucrose Ester (SE)

Sucrose esters (SEs) are non-ionic surface-active surfactant consisting of sucrose as the hydrophilic group and fatty acids as lipophilic groups (Dona, Sintang, Danthine, Patel, & Rimaux, 2017). Sucrose esters which is hydrophilic surfactant was used in many applications including bakery, confectionery, cereals, dairy, ice cream and sauces. By varying the degree of esterification of the sucrose molecule it is possible to obtain emulsifiers with HLB values ranging from 1 up to 16 (Sisterna, n.d.). In this study, the highest HLB of sucrose ester was used which is 16 that will favours the oil-in-water emulsion. According to the supplier, the Sucrose Ester usually called as SE for short, and the hydrophilic part is sugar and the lipophilic part is fatty acid as in Figure 2-12. Other than that, the emulsions with sucrose ester will produce smaller oil droplets, improved the stability of emulsion and better protection against the

rancidity (Bax, 2015). Moreover, sucrose esters are used as solubilizing, foaming, anti-bacterial and releasing agents, for enhancement or inhibition of crystal growth in fats, and lubrication (Dona et al., 2017). This sucrose ester self-organize into micelles structures when dispersed in a solvent at particular concentration and composition (Dona et al., 2017).

In this experiment, DK Ester F-160 was used, and based on the chemical formula ($C_{33}H_{62}O_{12}$) provided by supplier, the calculated molecular weight is 650.84 g/mol. Based on Figure 2-10, the mono-ester is a sucrose ester that is combined one molecule of fatty acid with one mol. of sucrose. Di-ester is two mol. of fatty acid and Tri-ester is three mol. of fatty acid. The more mono-ester is contained in sucrose ester, the higher hydrophilic property it shows and the more di-, tri-ester and poly-ester, the higher lipophilic property. In this sucrose ester, the percentage for mono-ester is 70% and 30% from Di-ester, Tri-ester and Poly-ester (Mutiara Jesindo Cemerlang, n.d.). Furthermore, it is claimed that by using together with other surface active agents, this DK ESTER is expected to show excellent synergetic effects (Mutiara Jesindo Cemerlang, n.d.).

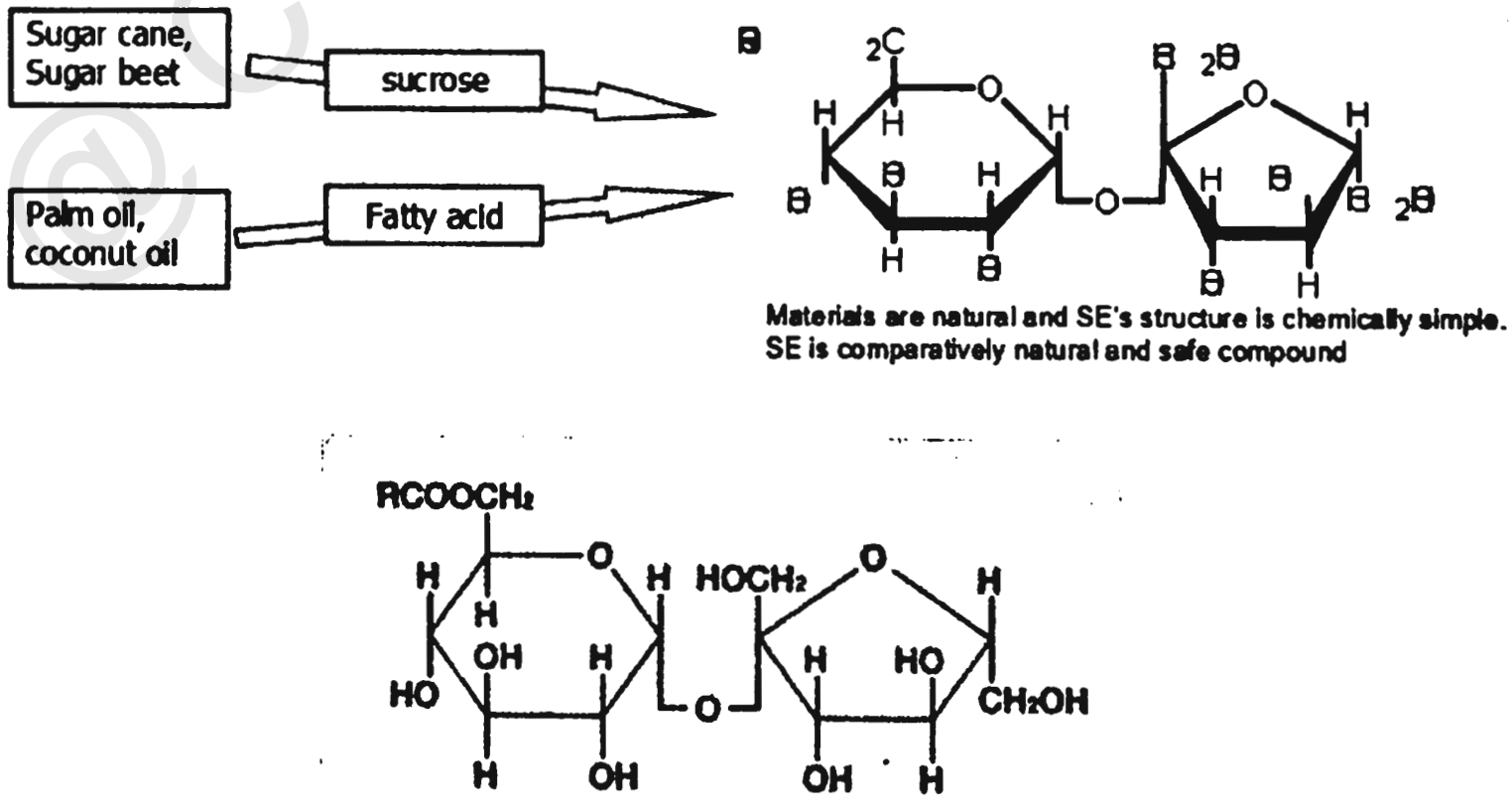

Figure 2-10: The chemical structure of sucrose ester from supplier

2.7.2 Tween 80 (T80)

Tween 80 or Polysorbate 80 or Polyoxyethylene sorbitan (20) monooleate is a hydrophilic non-ionic surfactant which is not present any charge. This Tween 80 have high HLB value which used in this study is 15 and the chemical structure is as in Figure 2-11 which can be presented by having the larger head group (hydrophilic) compared to the tail group (hydrophobic). This Tween 80 is a food emulsifier and in a category of small molecule surfactant. It is used as an emulsifier and dispersing agent when there is high fat content or hydrophobic content to emulsify and disperse the hydrophobic medium components (Acumedia Manufacturer, 2015). Other than that, Tween 80 is the most used synthetic surfactants in development of nanoemulsion due to good emulsifying properties especially in oil-in-water nanoemulsion using low energy method (Wu, Yan, Chen, & He, 2016). This Tween 80 has a single unsaturated tail, so it will have a smaller packing geometry that required to promote the spontaneous formation of very small droplets at the oil-water boundary using low energy methods (Guttoff, Saberi, & Mcclements, 2015). The molecular weight based on the molecular formula ($C_{64}H_{124}O_{26}$) of this Tween 80 and the CAS number (9005-65-6) is 1310 g/mol (ChemicalBook, n.d.-b).

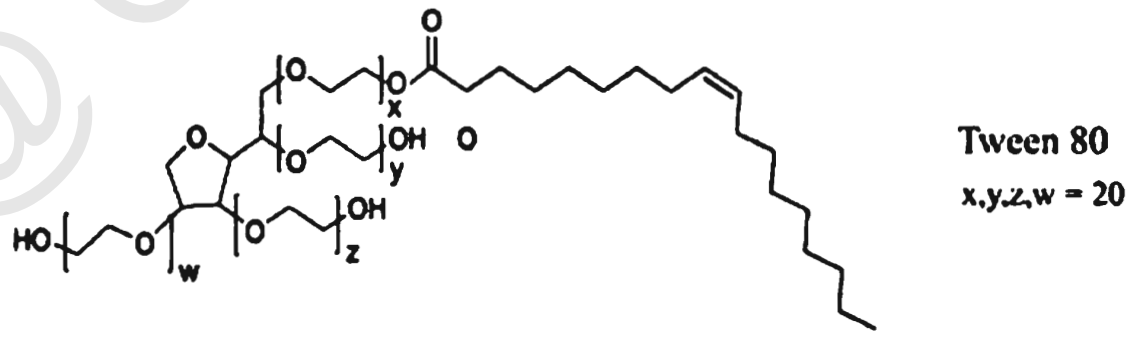

Figure 2-11: The chemical structure of tween 80 (PubChem, n.d.)

2.7.3 Distilled Monoglyceride (MDG)

Distilled monoglyceride of fatty acids is well known as the food emulsifier with e-number of E471.Based on product information from supplier, the type used in

this study is type P(V) which is a distilled monoglycerides made from edible, fully hydrogenated vegetable palm oil. The HLB value of this hydrophobic surfactant used is 5 and the chemical structure of MDG is shown as in Figure 2-12 which the tail group is larger than the head group. The molecular weight of this emulsifier based on the CAS-number (85251-77-0) and the chemical formula ($C_{21}H_{42}O_4$) is 358.55 g/mol.

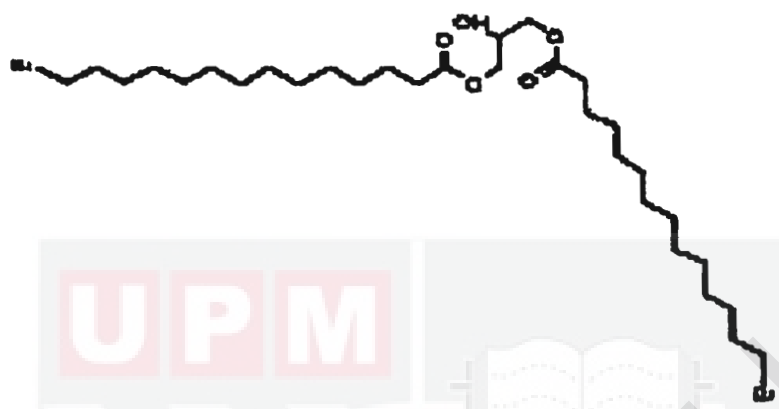

Figure 2-12: The chemical structure of distilled monoglyceride (European Chemical Agency, n.d.)

2.7.4 Lecithin (LCT)

Lecithin is a natural phospholipid surfactant derived from the cell membranes of soybeans and other compounds such as fatty acids, triglycerides and carbohydrates, and it had been used to establish and stabilize nanoemulsions (Wu et al., 2016). Concentrate of soybean lecithin consisting of more than 94% phosphatidylcholine and less than 2% triglycerides and composed mainly of linoleic acid (62 - 65%) and palmitic acid (15 - 17%) esters (Sigma-Aldrich, n.d.). Based on the CAS number (8002-43-5) and the molecular formula ($C_{42}H_{80}NO_8P$) of this lecithin, the molecular weight is 758.062 g/mol (ChemicalBook, n.d.-a). This type of natural food grade hydrophobic surfactant also is t widely used in food industry such as in margarine and chocolate. Other than that, this soy lecithin has a low HLB value which is 7. Soy lecithin has been frequently used individually or combined with other emulsifiers such as proteins to formulate emulsions or enhance emulsion stability (Xue, 2015). The

chemical structure as in Figure 2-13 shows this lecithin having the larger tail group (hydrophobic) compared to the head group (hydrophilic). The properties of soy lecithin show that it is good emulsifier and favour for water-in-oil emulsion.

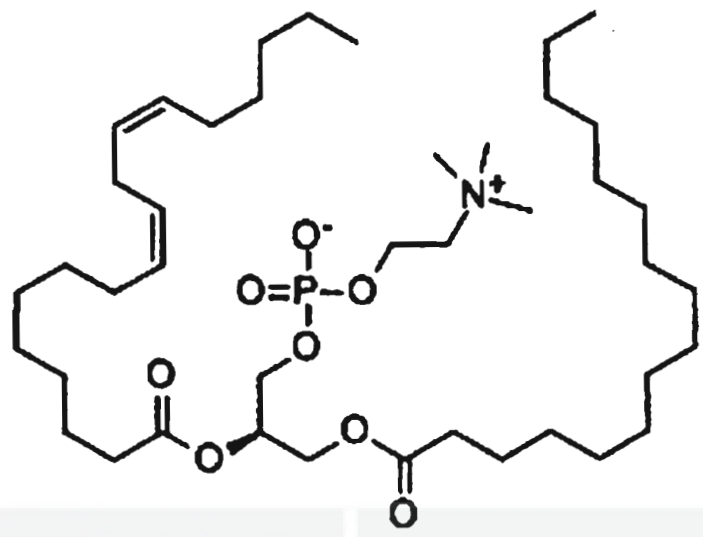

Figure 2-13: The chemical structure of soy lecithin (ChemicalBook, n.d.-a)

2.7.5 Polyglycerol Polyricinoleate (PGPR)

Polyglycerol Polyricinoleate (PGPR) is hydrophobic nonionic emulsifier and widely known as an excellent water-in-oil emulsifier in the food industry, because it forms very stable emulsions even when the water content is very high, such as 80% (Bastida-Rodríguez, 2013). This emulsifier usually used for chocolate making process in the food industry. PGPR is manufactured from polymerized glycerol and polymerized ricinoleic acid. The HLB value for PGPR used in this study is 4.0 as in the technical data sheet based (Cosmetic Ingredient Review, 2016). The molecular weight of this PGPR is 520.696 g/mol based on the chemical formula ($C_{27}H_{52}O_9$) from the CAS number (29894-35-7) the chemical structure is as in Figure 2-14.

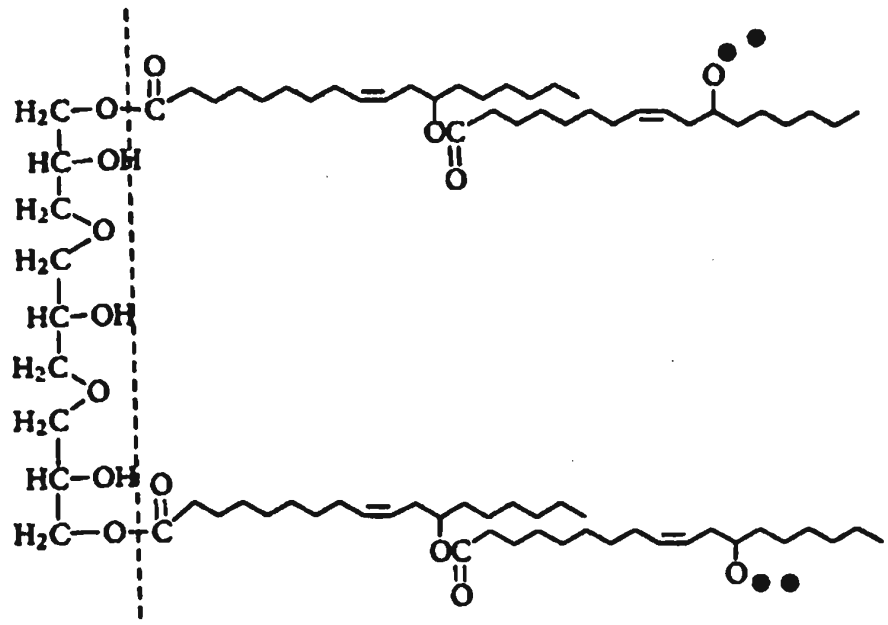

Figure 2-10: The chemical structure of polyglycerol polyricinoleate. Black dots denote polyricinoleic acid chains (Bastida-rodríguez, 2013)

Figure 2-11: The chemical structure of ricinoleic acid (Bastida-rodríguez, 2013)

2.8 Soxhlet Extraction Method

A solution to the problem of extracting "trapped lipids" is to use an extraction procedure such as the soxhlet that will help break up the polar barriers and allow the solvent to reach the non-polar compounds and extract them. A Soxhlet extractor is a piece of laboratory apparatus that invented in 1879 by Franz von Soxhlet which was originally designed for the extraction of a lipid from a solid material. Generally, a Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent (Jensen, 2007). If the desired compound has a significant solubility in a solvent, then a simple filtration can be used to separate the compound from the insoluble substance (Jensen, 2007). Before using the soxhlet extractor, the sample must be ground and prepared prior to the extraction

procedure in order to break up the cell membranes and other structures that would make extraction difficult (Abdolshahi et al., 2015).

2.9 Polydispersity Index (PDI)

With respect to particle size distribution characterization, a parameter used to define the size range of the lipidic nanocarrier systems is called the "polydispersity index" (PDI) (Danaei et al., 2018). The term "polydispersity" is used to describe the degree of non-uniformity of a size distribution of particles. This index is dimensionless and scaled such that values smaller than 0.05 are mainly seen with highly monodisperse standards (Danaei et al., 2018). PDI values bigger than 0.7 indicate that the sample has a very broad particle size distribution (Danaei et al., 2018). The most important characteristics of molecular weight distributions of polymer are briefly presented by PDI (Rogosic, Mencer, & Gomzi, 1996). PDI also can described the theoretical distribution functions for the description of molecular weight distributions of polymers (Rogosic et al., 1996).

2.10 Zeta Potential

Zeta potential is a measure of the magnitude of the electrostatic or charge repulsion/attraction between particles and is one of the fundamental parameters known to affect stability (Malvern Panalytical Ltd, n.d.). The understanding about the causes of dispersion, aggregation or flocculation can be achieved when performing the zeta potential measurement and can be applied to optimize the formulation of emulsion, dispersion and suspensions. Thus, it can be concluded that zeta potential measurements are to improve the formulation stability and shelf life and reduce formulation time and cost (Malvern Panalytical Ltd, n.d.). The following Table 2-1 summarizes the stability behaviour of the colloid particles with respect to zeta potential.

Table 2-1: Stability behaviour of the colloid particles with respect to zeta potential (Barron, 2019)

Zeta Potential (mV)	Stability behavior of the particles
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

CHAPTER 3: METHODOLOGY

3.1 Materials

Passion fruit seeds were purchased from Kelantan. For extraction of passion fruit oil from the seeds, hexane was used as the solvent. Tween 80 was purchased from the Evergreen Chemical (Malaysia). Lecithin was purchased from the Sigma–Aldrich Co. (St. Louis, MO). Sucrose ester was kindly provided by Dai-Ichi Kogyo Seiyaku Co., Ltd. (DKS) (Japan). Distilled monoglyceride were received as a gift sample from Rikevita Sdn Bhd (Malaysia) and polyglycerol polyricinoleate was purchased from Shanghai Sunwise Chemical Co., Ltd (China). Distilled water was used in the preparation of all solutions and emulsions.

3.2 Overall Methodology

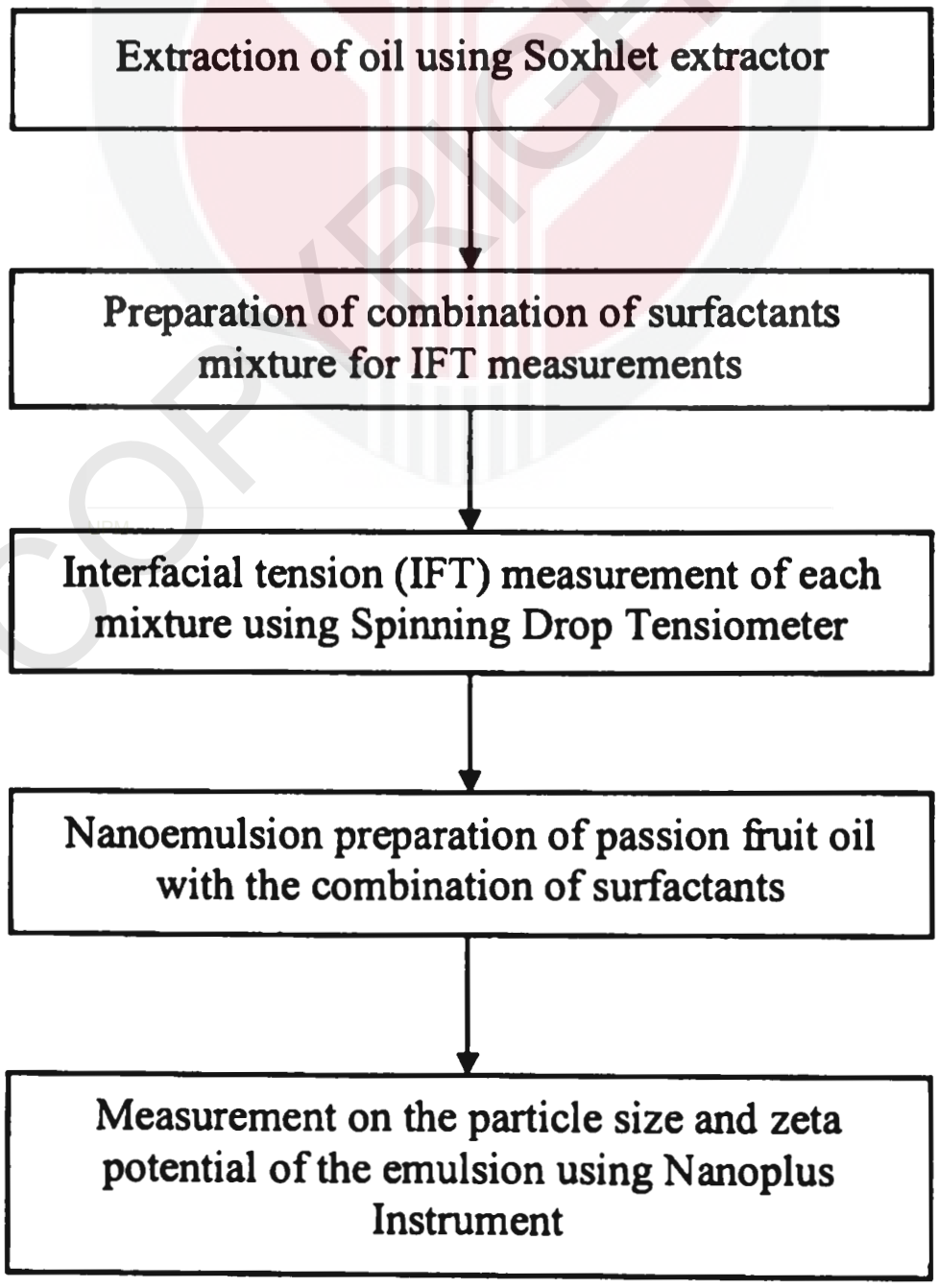

Figure 3-1: Overall methodology flow diagram

3.3 Extraction of Passion Fruit Oil

The Soxhlet extraction method was the extraction method used to extract the passion fruit oil from the passion fruit seeds in this study. In this extraction, the Soxhlet extractor apparatus was used at the Bioreactor Lab and hexane used as the solvent. Firstly, 30 grams of dried passion fruit seeds were crushed and grounded by the grinder and then the crushed seeds were put into the cleaned dried thimble and then the seeds were covered with cotton pads to avoid any foreign materials contaminate the samples. Then, round bottom flask was filled with 350 ml of hexane carefully and the thimble was inserted into the extractor of the Soxhlet apparatus. Next, the Soxhlet apparatus as shown in Figure 3-2 was put on the stove plate and the switched on. The time taken to extract the oils from the 30 grams of the seeds takes about 4 hours. After 4 hours, the round bottom flask was transferred to the rotary evaporator to evaporate the hexane from the mixture of extracted oil and hexane. The extracted oil was then left on the fume chamber overnight. Finally, the extracted passion fruit oil was ready to be used for interfacial tension measurement and emulsion.

Figure 3-2: Passion fruit oil extraction using Soxhlet method (Abdolshahi et al., 2015)

3.4 Sample Preparation: Varying Concentration and Ratio of Surfactants Combinations for Interfacial Tension Measurements

There are six types combinations of surfactants were used in this study which are Sucrose Ester:Lecithin (SE:LCT), Sucrose Ester:Distilled Monoglyceride (SE:MDG), Sucrose Ester:Polyglycerol Polyricinoleate (SE:PGPR), Tween 80:Lecithin (T80:LCT), Tween 80:Distilled Monoglyceride (T80:MDG) and Tween 80:Polyglycerol Polyricinoleate (T80:PGPR). For first IFT measurement, the aqueous solutions of these six combinations of surfactants were prepared at varying concentrations (0.5%, 1.0%, 1.5% and 2.0%, w/w) and constant surfactant ratio. After finding the concentration of each surfactants mixtures that gave the lowest IFT, that concentration was used to prepare the mixture of this six combinations of surfactants with the different weight ratio of surfactant to surfactant (100:0, 80:20, 50:50, 20:80 and 0:100 w/w).

These samples of mixtures were prepared by dissolved the combination of the surfactants in water as the continuous phase using magnetic stirrer for 5 minutes on the hot plate which take place at 55°C to dissolve the combination of sucrose ester and 35°C for the combination of Tween 80. Then after 5 minutes, the supply heat stopped, and the mixtures were continuously mixed for 15 minutes. Then the mixtures were then cooled down to room temperature at 25°C. The density of each mixture was measured using glass pycnometer. The combination of surfactants used in this study was listed in each schematic diagram figures below:

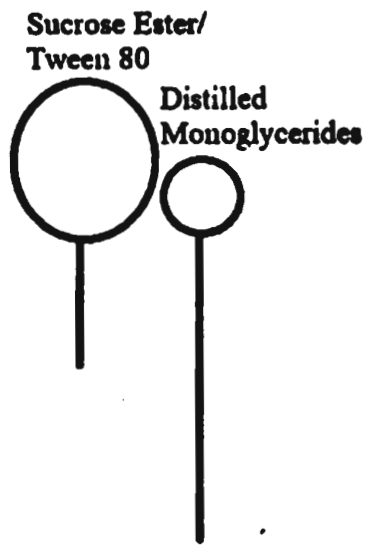

Figure 3-3: Combination of sucrose ester/Tween 80 with distilled monoglyceride (SE:MDG, T80:MDG)

Figure 3-4: Combination of sucrose ester/Tween 80 with lecithin (SE:LCT, T80:LCT)

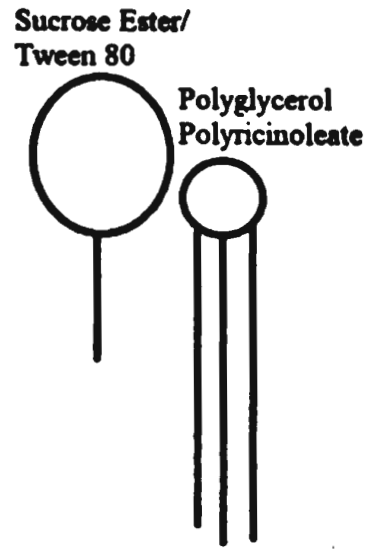

Figure 3-5: Combination of sucrose ester/Tween 80 with polyglycerol polyricinoleate (SE:PGPR, T80:PGPR)

3.5 Interfacial Tension Measurement

The interfacial tension (IFT) measurements of each mixture were performed using Spinning Drop Tensiometer (SVT 20N, Dataphysics Instruments, Germany) as shown in Figure 3-6. The capillary tube was filled with a mixture of surfactant combination until it seeps out the capillary end using syringe and needle. Then, a drop of passion fruit oil was added before the capillary end was closed. The filled capillary tube was put in the equipment. All parameters such as density, temperature and rotational speed for running the machine was inserted in the SVT20 software. In order to avoid error in the result of the measurement IFT value, no bubble presented in the oil and mixture of surfactant combination was ensured. Lastly, the IFT against time result was recorded and each the IFT reading for each mixture was repeated twice.

Figure 3-6: Spinning drop tensiometer

3.6 Preparation of Passion Fruit Oil Nanoemulsion Using Combination of Surfactants

Passion fruit oil nanoemulsion was prepared in the combination of surfactants mixture by using the spontaneous emulsification method. However, spontaneous emulsification method, mixed the oil with single hydrophilic surfactant (Komaiko & Mcclements, 2016). In this study, combination of hydrophobic and hydrophilic surfactant used, and there are two problems occurred when mixed them in oil. Sucrose

ester is in powder form and only can be dissolved in water and next is when the hydrophobic surfactant was mixed in the oil, when the oil was titrated into distilled water, formation of clumps of oil occurred. Thus, modification on the spontaneous emulsification used which mixed the combined surfactants in other phase rather than oil (Riehm et al., 2016).

Water at 90 wt%, passion fruit oil at 5 wt% and a combination of surfactants at 5 wt% was used in this study. The ratios of SE:LCT, SE:MDG, SE:PGPR, T80:LCT, T80:MDG and T80:PGPR in the mixtures, reported by weight, were the primary compositional variable of interest in this work.

Firstly, the combined surfactant was mixed with the distilled water using a magnetic stirrer on the stirring hotplate (FAVORIT, PLT Scientific Sdn Bhd, Malaysia) with the speed control pointing to 1 at 55°C (with the combination of sucrose ester) and at 35°C (with the combination of Tween 80) for 5 minutes. After 5 minutes, the mixture was stirred at 25°C with the same speed for 15 minutes. Then, passion fruit oil was slowly added at a rate of 1drop/20 seconds into the stirred combination of surfactants mixture. The mixture then was stirred with at the same speed at 25°C for 15 minutes to ensure the emulsion was properly homogenized. Each emulsion using this combination of surfactants were repeated twice.

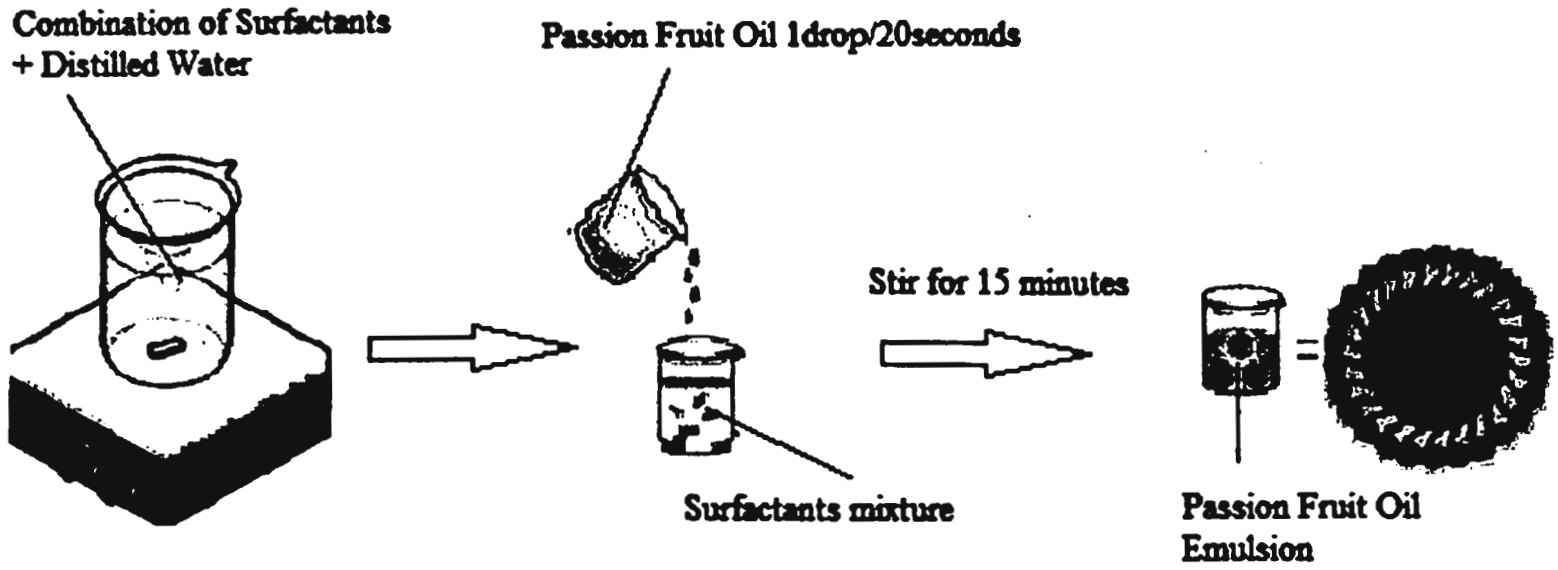

Figure 3-7: Schematic representation of preparation of passion fruit oil nanoemulsion

3.7 Particle Size and Zeta Potential Measurement

The measurement of the passion fruit nanoemulsion on the particle size and zeta potential were done using the dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instruments Ltd, Malvern, UK). The freshly prepared fine emulsions were diluted using distilled water at 25 °C in order to minimize the multiple scattering effects. A little amount of the diluted nanoemulsion was transferred into the particle size cell probe. The data were reported as the mean droplet diameter (nm) and polydispersity Index (PDI) also was measured to indicates the heterogeneity (monodisperse or polydisperse) of sizes of particles in a mixture (Malvern, 2011). While for zeta potential measurement, the diluted nanoemulsion was transferred into the zeta potential cell probe. The results are given as the average ± standard deviation of the three value obtained (Rao and McClements, 2013).

CHAPTER 4: RESULT AND DISCUSSION

PART A: Determination of interfacial tension (IFT) between passion fruit oil (PFO) and combination of food-grade surfactants at different packing geometry.

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

For each combination of surfactants, the concentration was varied at constant surfactant ratio to identify at which concentration give the lowest IFT. The mixture of surfactants and distilled water is called as continuous phase while passion fruit oil called as the oil phase. This study is important in order to find the concentration that give the lowest IFT.

4.1.1 Effect at Surfactant Concentration on Interfacial Tension for Combination of Sucrose Ester with Distilled Monoglyceride (SE:MDG), Lecithin (SE:LCT) and Polyglycerol Polyricinoleate (SE:PGPR)and Passion Fruit Oil

The results of IFT is shown in Figure 4-1, the combinations of surfactants show the good result on IFT reduction as the IFT was decreased from 2.76 mN/m at 0% surfactants concentration to 0.831 mN/m for SE:MDG, 0.423 mN/m for SE:LCT and 0.172 mN/m for SE:PGPR. These results showed that the function of the surfactant are to lower the interfacial tension between passion fruit oil and water.

Based on Figure 4-1, the lowest IFT was found is 0.101 mN/m at 1.0% concentration of SE:LCT, 0.465 mN/m at 1.5% concentration for SE:MDG and 0.046 mN/m at 1% concentration for SE:PGPR. This result shows that IFT reading is minimum value at critical micelle concentration (CMC) of the surfactant (Kumar,

Saxena, & Mandal, 2016). After CMC value, when the concentration of combined surfactant was increased, the IFT was found to be slightly increased.

These three combinations have similar HLB which is 10.5 for SE:MDG, 12.4 for SE:LCT and 10 for SE:PGPR according to the ratio used in this study. Overall, combination of SE:PGPR show the lowest IFT compared to other combinations. This is because, SE:PGPR might be able to promote an optimum packing geometry in oil-water phase which is crucial in producing nanoemulsion by low energy method compared to SE:LCT and SE:MDG. In addition, combination of SE:MDG have the highest IFT since both of the surfactant have a single tail geometry. Thus, the concentration at 1% for SE:LCT and SE:PGPR and at 1.5% for SE:MDG were used to identify the IFT among the ratios between surfactants in the sucrose ester mixtures.

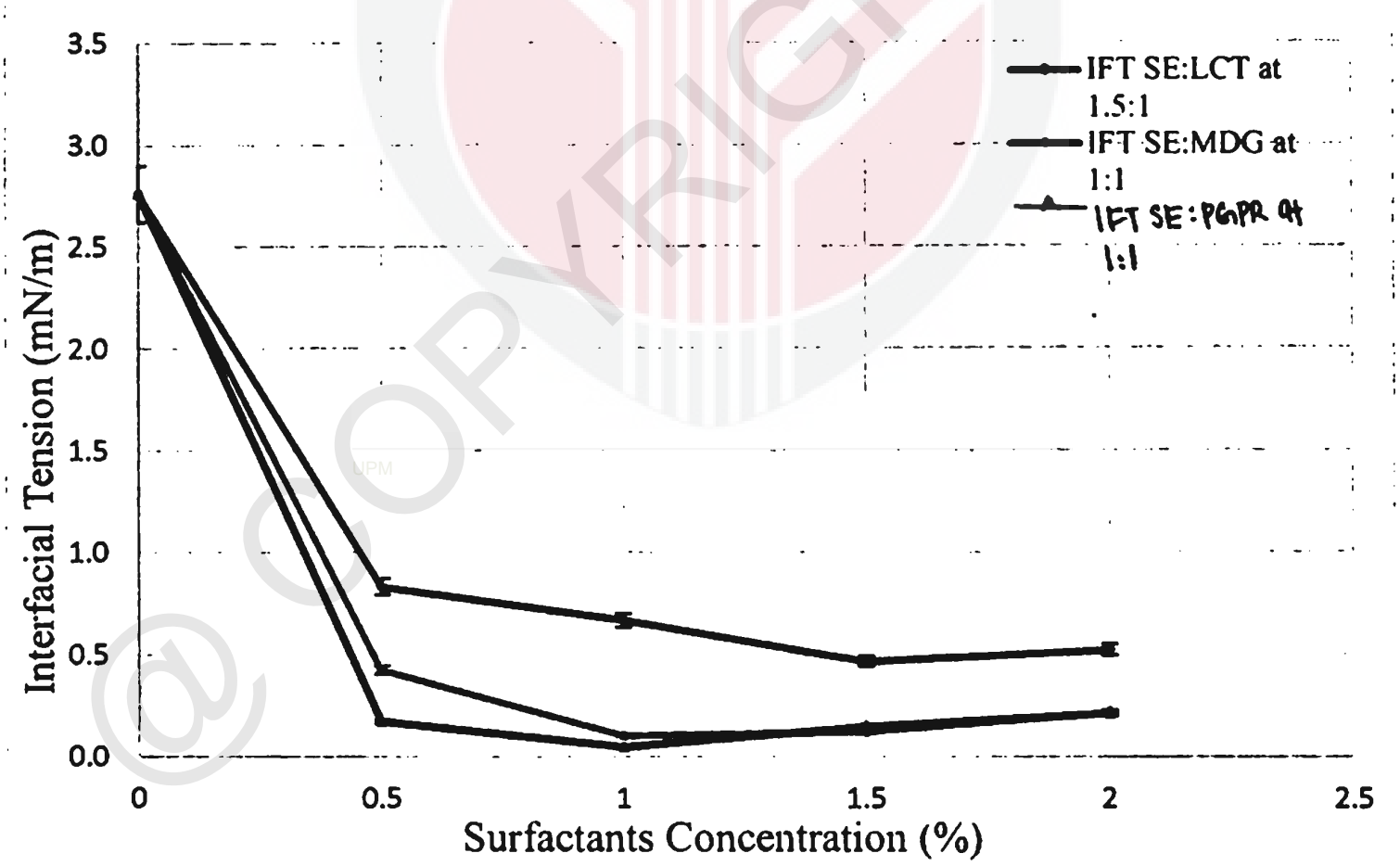

Figure 4-1: Graph of IFT against concentration for different combination of surfactant between sucrose ester:distilled monoglyceride (SE:MDG), sucrose ester:lecithin (SE:LCT) and sucrose ester:polyglycerol polyricinoleate (SE:PGPR) at constant ratio

4.1.2 Effect at Surfactant Concentration on Interfacial Tension for Combination of Tween 80 with Distilled Monoglyceride, Lecithin and Polyglycerol Polyricinoleate and Passion Fruit Oil

As shown in Figure 4-2, when there is surfactants mixture present in the continuous phase at concentration 0.5% of surfactants mixtures, the IFT was found to decrease to 0.604 mN/m for T80:LCT, 0.491 mN/m for T80:MDG and 0.063 mN/m for T80:PGPR. The IFT was continuously reduced until the point of CMC was reached. The lowest IFT for each combinations is 0.406 mN/m at 1.5% concentration of T80:MDG, 0.302 mN/m at 1% concentration of T80:LCT and 0.040 mN/m at 1% concentration of T80:PGPR.

The HLB for these three combinations is 11.8 for T80:LCT, 10 for T80:MDG and 9.5 for T80:PGPR. Between these combinations, the lowest IFT was obtained from the combination of T80:PGPR followed with T80:LCT and lastly T80:MDG. This is because of the packing geometry of T80:PGPR which is the same with the previous SE:PGPR which it from the optimum packing geometry of T80:PGPR. Tween 80 has a single unsaturated tail while Polyglycerol Polyricinoleate have polyricinoleic acid side chains which make the PGPR is lipophilic surfactant while Tween 80 is hydrophilic surfactant. Therefore, the concentration at 1% for T80:LCT and T80:PGPR and at 1.5% for T80:MDG were used to identify the IFT among the ratios between surfactants in the Tween 80 mixtures.

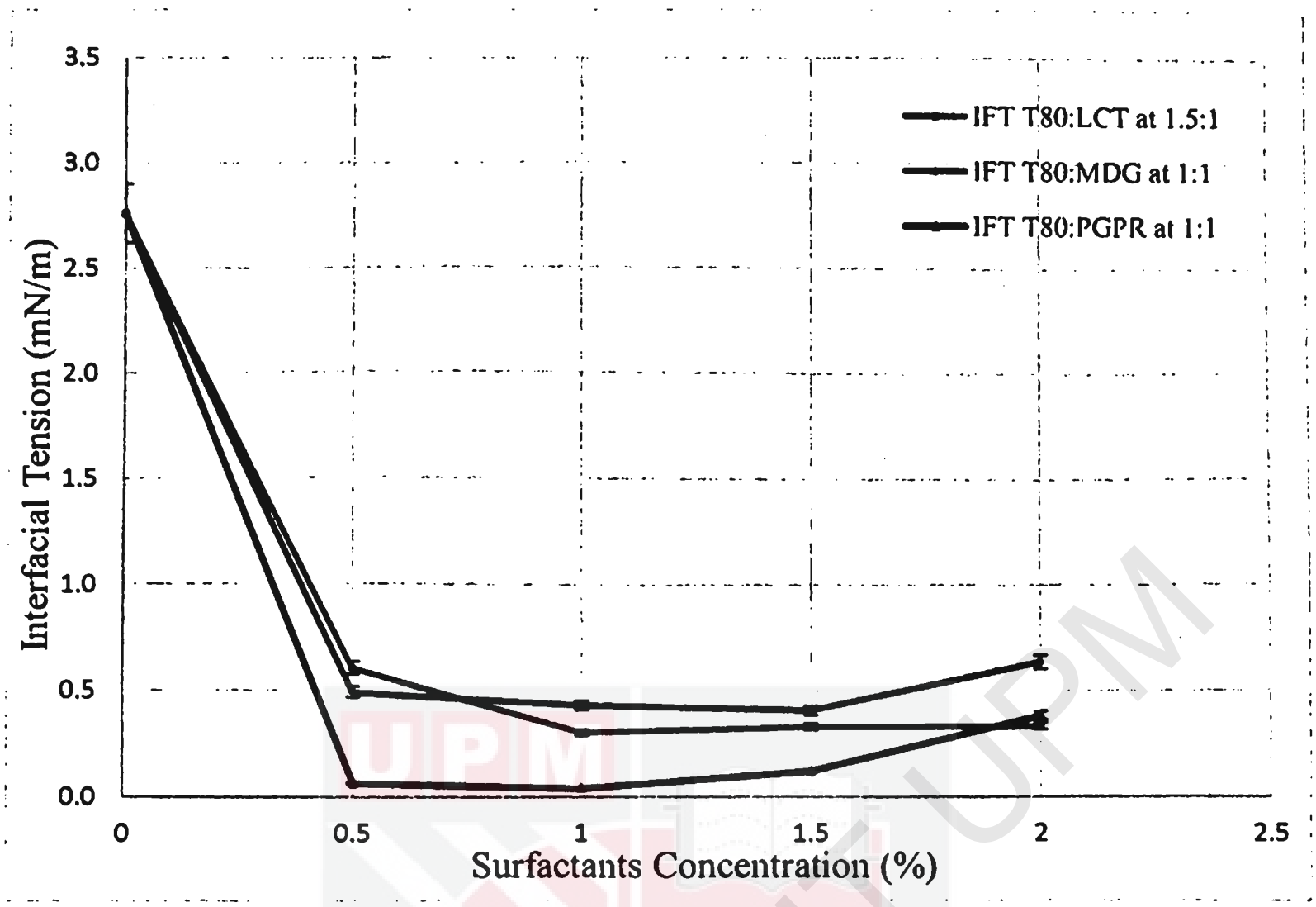

Figure 4-2: Graph of IFT against concentration for different combination of surfactant between Tween 80:distilled monoglyceride (T80:MDG), Tween 80:lecithin (T80:LCT) and Tween 80:polyglycerol polyricinoleate (T80:PGPR) at constant ratio

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

In each combination of surfactants, the weight ratio between surfactants in a combination was varied at the same concentration of surfactants mixture that shows the lowest IFT at the point of critical micelle concentration. The mixture of surfactants and distilled water is considered as the continuous phase while passion fruit oil is considered as the oil phase. This study is important in order to determine the surfactant ratio that will provide the lowest IFT. These obtained IFT can be related to the effect on the particle size.

4.2.1 Effect at Surfactant Ratio on Interfacial Tension for Combination of Sucrose Ester with Distilled Monoglyceride (SE:MDG), Lecithin (SE:LCT) and Polyglycerol Polyricinoleate (SE:PGPR) and Passion Fruit Oil

The results of IFT as shown in Figure 4-3 were obtained by having the same concentration surfactant mixtures with different ratio of sucrose ester with other surfactants in a mixture. The concentration used for the surfactants mixture was based on the previous result at CMC point that yields the lowest IFT among the range of concentration used. So, concentration used for SE:LCT and SE:PGPR is at 1%, while at 1.5% for SE:MDG.

Based on Figure 4-3, by using sucrose ester alone in the continuous phase, the lowest IFT can be obtained which is 0.014 mN/m at 1.5% SE concentration and 0.022 mN/m at 1% SE concentration. Sucrose ester has HLB value at 16 which is very hydrophilic that favour the emulsion of oil-into-water.

When the concentration of MDG was increased in SE:MDG, the IFT increased drastically as shown in Figure 4-3. This indicates that a single hydrophobic tail of MDG will not help to promote the nanoemulsion. For combination of SE:LCT, the ratio of 80:20 shows the similar value of IFT which only increase slightly when using sucrose ester alone. As the concentration of LCT increased, the IFT increased. Overall, combination of SE:PGPR shows the lowest IFT as the ratio from 80:20 to 20:80 is similar and drastically increased PGPR alone was used. Therefore it is believed that combination of SE:PGPR may improved the formation of nanoemulsion using low energy method as when the IFT of the oil-water system is low, the spontaneous emulsification can be done easily (Riehm et al., 2016).

Moreover, as shown in Figure 4-3, when the LCT and PGPR alone was used in the continuous phase, the IFT shows a higher value which indicates the high energy at the interface of the oil-water system by using these LCT and PGPR alone which can be concluded that the high chances of separation between PFO and water. This is because the HLB value of LCT and PGPR is low which is 7 and 3.5, contain a large group of tails which is hydrophobic that actually favours the formation of a water-in-oil emulsion. The IFT of using MDG or distilled monoglyceride alone cannot be done because the camera of spinning drop tensiometer do not detect the oil in MDG mixture due to its milky white appearance even when the illumination setting of the camera was increased. But it was confirmed that the by using MDG alone for the oil-water system will result in high IFT because of the MDG have a larger tail group which is hydrophobic and is usually used for water-into-oil emulsion. Therefore, among these three sucrose ester mixtures, the combination of SE:PGPR at 80:20 (HLB at 13.6) showed the lowest IFT followed by SE:LCT at 80:20 (HLB at 14.2) that leads to spontaneous emulsification.

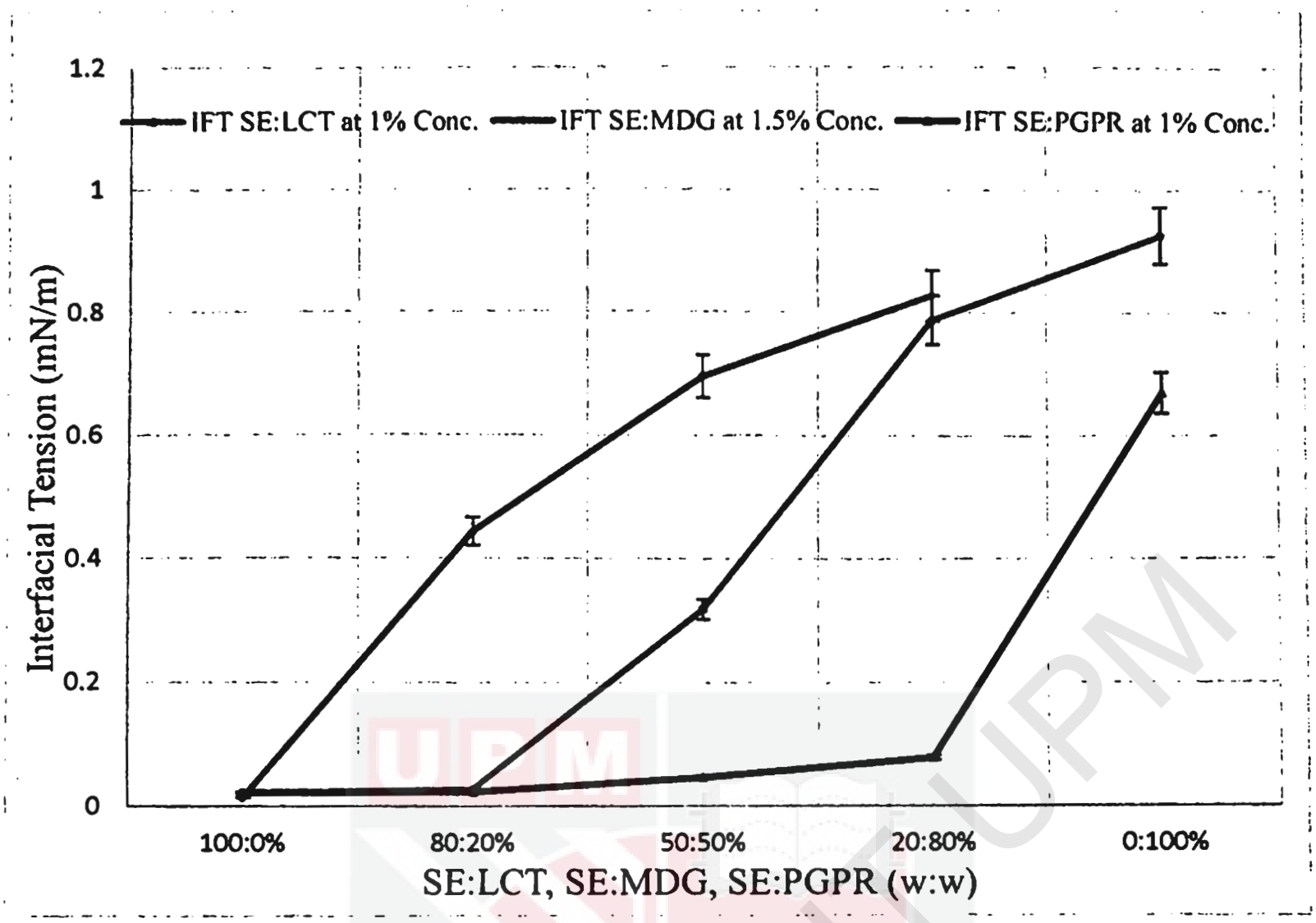

Figure 4-3: Graph of IFT against surfactant ratio for different combination of surfactant between sucrose ester:distilled monoglyceride (SE:MDG), sucrose ester:lecithin (SE:LCT) and sucrose ester:polyglycerol polyricinoleate (SE:PGPR) at constant concentration

4.2.2 Effect at Surfactant Ratio on Interfacial Tension for Combination of Tween 80 with Distilled Monoglyceride (T80:MDG) , Lecithin (T80:LCT) and Polyglycerol Polyricinoleate (T80:PGPR) and Passion Fruit Oil

Based on the Figure 4-4, the IFT trend was showed that by having the same concentration surfactant mixtures with different ratio of Tween 80 with other surfactants in a mixture. The concentration used for the surfactants mixture was based on the previous result at CMC point that yields the lowest IFT among the range of concentration used. The concentration used for T80:LCT and T80:PGPR is at 1%, while at 1.5% for T80:MDG. As shown in Figure 4-4, at every ratio using combination of T80:MDG, the IFT had increased which shows that T80:MDG was not a good

combination to produce lower IFT as the packing geometry of the tail to head might not be at the optimum level.

Other than that, it was found that IFT value decreases from using Tween 80 alone at 0.240 mN/m to 0.214 mN/m when combined with LCT at 80:20 at 1% concentration was used in the continuous phase. Then, by adding the concentration of LCT, the IFT was shown to increase. For combination of T80:PGPR, the IFT was reduced until ratio of 50:50 at 0.053 mN/m. Then, by increasing the PGPR, the IFT was increased drastically. This is because when the lipophilic surfactants are the dominant in the combination of surfactants, the hydrophobic chain will increased and it is only appropriate to be used for water-in-oil emulsion to produce reversed micelles in the emulsion system. Therefore, among these three Tween 80 mixtures, the combination of T80:PGPR at 50:50 (HLB at 9.5) show the lowest IFT followed by T80:LCT at 80:20 (HLB at 13.4) that can lead to spontaneous emulsification.

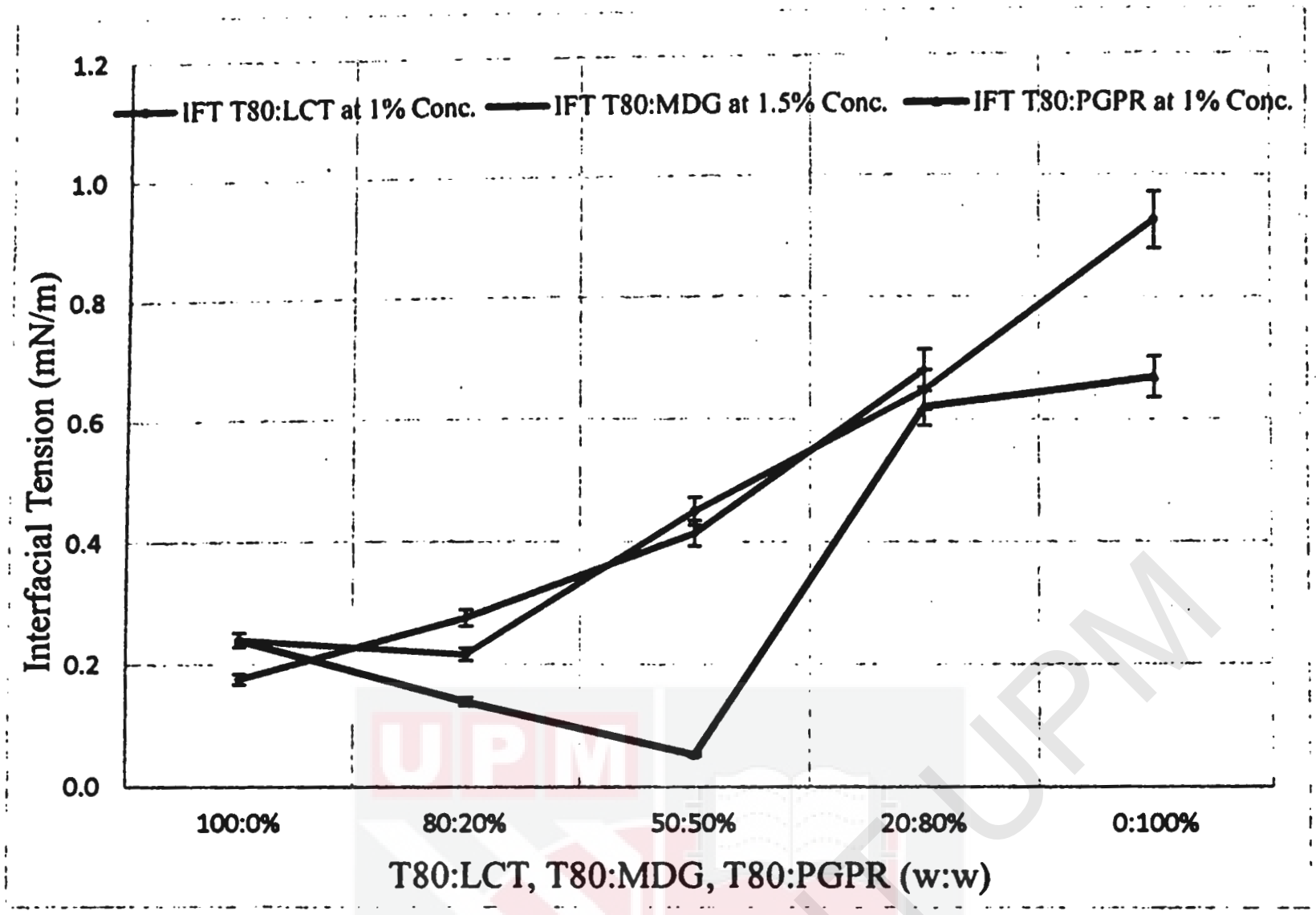

Figure 4-4: Graph of IFT against surfactant ratio for different combination of surfactant between Tween 80:distilled monoglyceride (T80:MDG), Tween 80:lecithin (T80:LCT) and Tween 80:polyglycerol polyricinoleate (T80:PGPR) at constant concentration

PART B: Determination of the effect on size particles and zeta potential of produced nanoemulsion using combination of food-grade surfactants at different packing geometry.

4.3 Effect on Particle Size and Zeta Potential of Produced Emulsion Using Passion Fruit Oil with Combination of Food-Grade Surfactants

One of the low energy method to prepare nanoemulsion is called spontaneous emulsification. This procedure was done to ensure that the surfactants and oil should be mixed together as an organic phase and was titrated into an aqueous phase which is water (Komaiko & Mcclements, 2016). However, the sucrose ester which is in powder form has to be dissolved with water as stated in the material data sheet provided by the supplier as in appendices. Other than that, even the lipophilic surfactant which is lecithin, distilled monoglyceride and polyglycerol polyricinoleate can be easily dissolve in PFO as an organic phase, the problem will only arise when this organic phase was titrated to aqueous phase that contains water and hydrophilic surfactant, where the lumpy mixtures will be produced. Thus, the methods to produce nanoemulsion for each combination of surfactants was synchronized by dissolving both of surfactants in water as aqueous phase and PFO was titrated into the aqueous phase. The concentration used in this emulsion system is at 5% of PFO, 5% of the combination of surfactants and 90% of distilled water. The ratios in this 5% of a combination of surfactants were varied to identify the effect of using the combination of surfactants on the particle size, polydispersity index and zeta potential.

4.3.1 Effect on Particle Size and Zeta Potential of Produced Emulsion Using Passion Fruit Oil with Combinations of Sucrose Ester

In this study, the effect on the particle size and zeta potential of the PFO emulsion by using the combination of sucrose ester which is SE:MDG, SE:LCT and SE:PGPR were studied. The constant concentration of combined surfactants used was 5% with different surfactant ratio. The reason why only 5% of surfactant used in this study is when any combination of sucrose ester used at 10%, it will produce thick and very viscous liquid. Thus 5% of concentration will be used for all combinations in order to observe and compare the pattern easily.

Based on Table 4-1 below, when the SE alone was used at 5% of concentration, the particle size is 254.6 nm. This can be considered very small because of insignificant amount of the surfactant used. The IFT obtained when using SE alone at 1% concentration was 0.022 mN/m. When the ratio of hydrophobic surfactants (MDG, LCT and PGPR) were increased as shown in Table 4-1, 4-2 and 4-3, the particle size was shown to increase. These results can be validated with the obtained IFT as by increasing the hydrophobic surfactant in the combination with sucrose ester, the IFT will also increased.

Among these three combinations with sucrose ester, the combination of SE:LCT produced the smallest particle size. Nano size of the particle was obtained at the ratio of 80:20 of SE:LCT which is 187.9 nm. The obtained IFT at this ratio also showed a lower IFT which is at 0.022 mN/m. Even when the IFT by using only SE was lower, an optimum packing geometry of the surfactants SE:LCT might be the reason why the nanoemulsion can be produced even at 5% of surfactants concentration. The balance interaction of SE:LCT might be obtained by having the same size ratio of the tail part to head part in the oil-water phase thus providing an

optimum packing geometry that can favour the production of nanoemulsion when compared to use only sucrose ester which only have one tail.

Furthermore, based on both Table 4-1, 4-2 and 4-3, when 100% hydrophobic surfactants were used in the emulsion, PFO was seen to separate from the water straight away as large viscous of oil clumps formed because the surfactant contained more hydrophobic part and it tends to attract to only PFO which is a favour to produce a water-into-oil emulsion.

From the previous result of IFT, overall the combination of SE:PGPR shows the lowest IFT compared to others. However, in this study, the smallest particle size or nanoemulsion could not be obtained. This is because, improper amount or concentration of surfactants (SE:PGPR) was used in the emulsion. As shown in Table 4-4, when using only 2.5% as the concentration of combined SE:PGPR at ratio 80:20, the smaller particle size can be obtained. Thick and viscous liquid was produced when using 5% of SE:PGPR at 80:20, and less viscous liquid was produced when using 2.5% of SE:PGPR at 80:20. The high level of surfactant may also increase droplet size due to the high viscosity of the liquid crystalline phase making it harder for the spontaneous formation of fine emulsion droplets (Komaiko & Mcclements, 2016). In summary, it is important to use enough surfactant to form small droplets, but not so much that there will be excess surfactant. Otherwise, thick and viscous liquid or large clumps will be formed.

Polydispersity index or PDI is a measurement of the distribution of molecular mass in an emulsion or dispersion. Based on these three combination as shown in Table 4-1, 4-2 and 4-3, the obtained PDI value was varied. The value of PDI ranges from 0 which is for a perfectly uniform sample with respect to particle size and to 1.0 for a

highly poly-disperse sample with multiple particle size populations (Ostrosky, Rocha-filho, & Veríssimo, 2015).

When using sucrose ester in the combinations, zeta potential was found to be high which is more than ±61 mV. This shows an excellent stability behaviour of the particles in the PFO emulsion using these combinations. As shown in Table 4-2, the combination of SE:LCT at 80:20 showed the highest zeta potential as the particle size obtained is the lowest compared to others.

Therefore in this study, by using 5% concentration of the combined surfactants and 5% of passion fruit oil, nanoemulsion can be produced using SE:LCT at 80:20 because of the optimum packing geometry. The combination with SE also show an excellent stability of the particles in emulsion and further study is needed to study the optimum concentration and ratio to produce nanoemulsion when using combination of SE:PGPR.

Table 4-1: Results of passion fruit oil emulsion using the combination of sucrose ester:distilled monoglyceride (SE:MDG)

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		SE	MDG			
5	90	100	0	254.6	0.261	-95.36
5	90	80	20	447.8	0.257	-88.02
5	90	60	40	separate		
5	90	50	50	separate		
5	9	20	80	2861.6	0.505	-65.98
5	90	0	100	separate		

Table 4-2: Results of passion fruit oil emulsion using the combination of sucrose ester:lecithin (SE:LCT)

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		SE	LCT			
5	90	100	0	254.6	0.261	-95.36
5	90	80	20	187.9	0.242	-102.15
5	90	60	40	769.8	0.416	-92.96
5	90	50	50	345.5	0.226	-81.15
5	9	20	80	454.4	0.314	-93.42
5	90	0	100	separate		

Table 4-3: Results of passion fruit oil emulsion using the combination of sucrose ester:polyglycerol polyricinoleate (SE:PGPR)

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		SE	PGPR			
5	90	100	0	254.6	0.261	-95.36
5	90	80	20	1306.0	0.621	-97.20
5	90	60	40	1518.3	0.498	-85.37
5	90	50	50	1941.5	0.741	-98.56
5	9	20	80	separate		
5	90	0	100	separate		

Table 4-4: Effect on size particle, PDI and zeta potential using different amount of concentration of sucrose ester:polyglycerol polyricinoleate (SE:PGPR)

Formulation	Measurements
- 5% concentration of PFO - 2.5% concentration of SE:PGPR - 80:20 of SE:PGPR	- Particle Size: 350.8 nm - PDI: 0.358 - Zeta Potential: -100.09 mV
- 2.5% concentration of PFO - 2.5% concentration of SE:PGPR - 80:20 of SE:PGPR	- Particle Size: 227.9 nm - PDI: 0.321 - Zeta Potential: -106. 08 mV

Other than that, as shown in Figure 4-5, the appearance of produced emulsion is milky white when the mixture was combined with sucrose ester. This is because sucrose ester itself was white in colour. The yellowish colour as shown in Figure 4-6 can be seen when the ratio of lecithin was increased in the emulsion as the lecithin is originally yellow in colour.

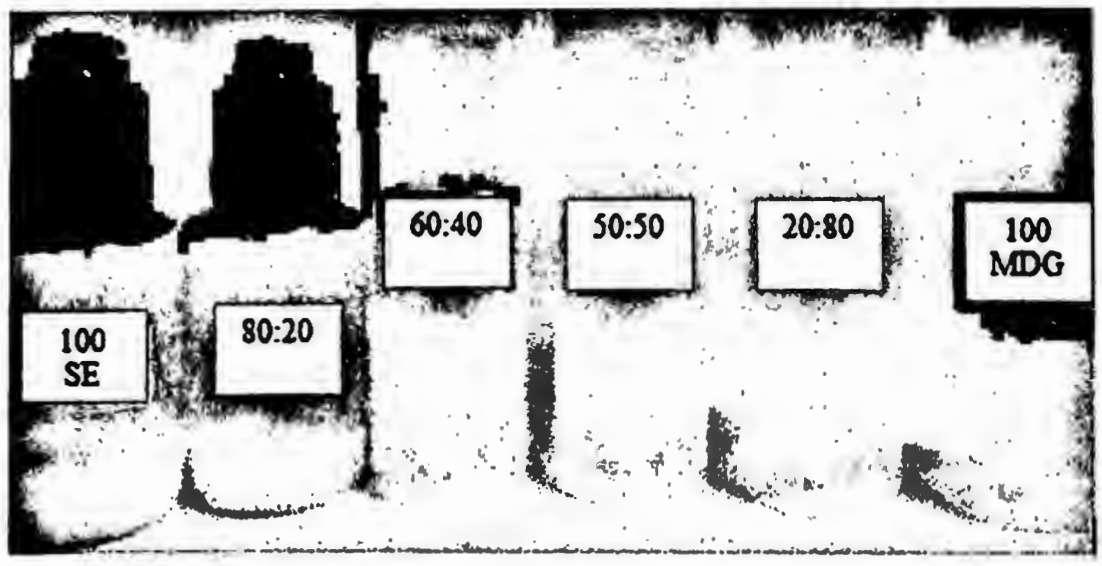

Figure 4-5: Appearance of produced emulsion of passion fruit oil using combination of sucrose ester:distilled monoglyceride (SE:MDG)

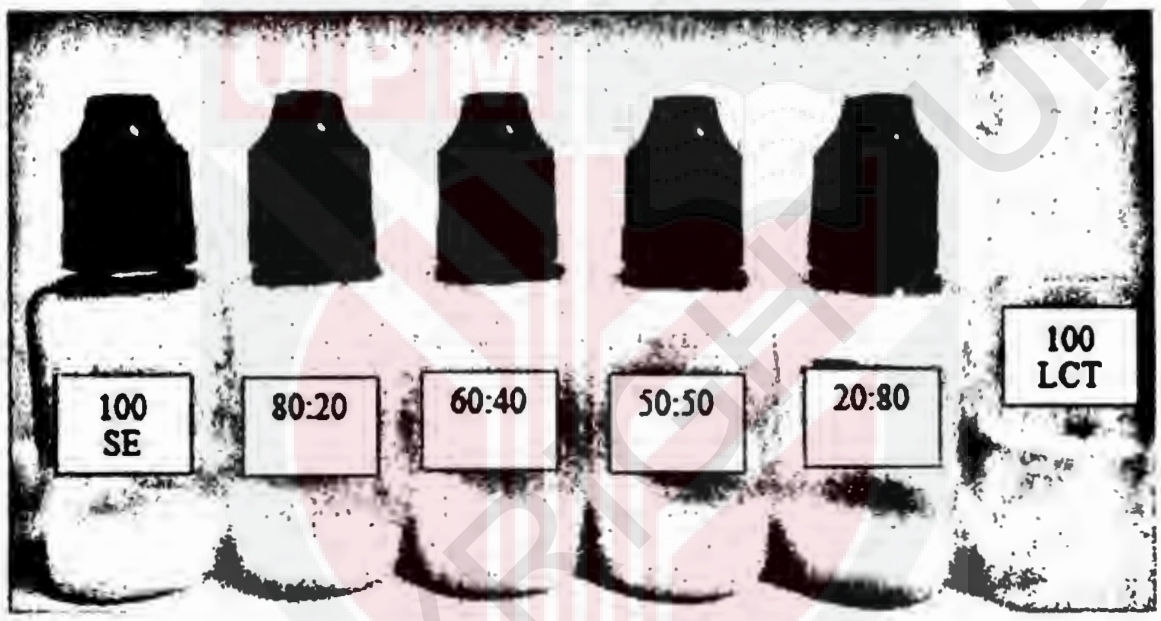

Figure 4-6: Appearance of produced emulsion of passion fruit oil using combination of sucrose ester:lecithin (SE:LCT)

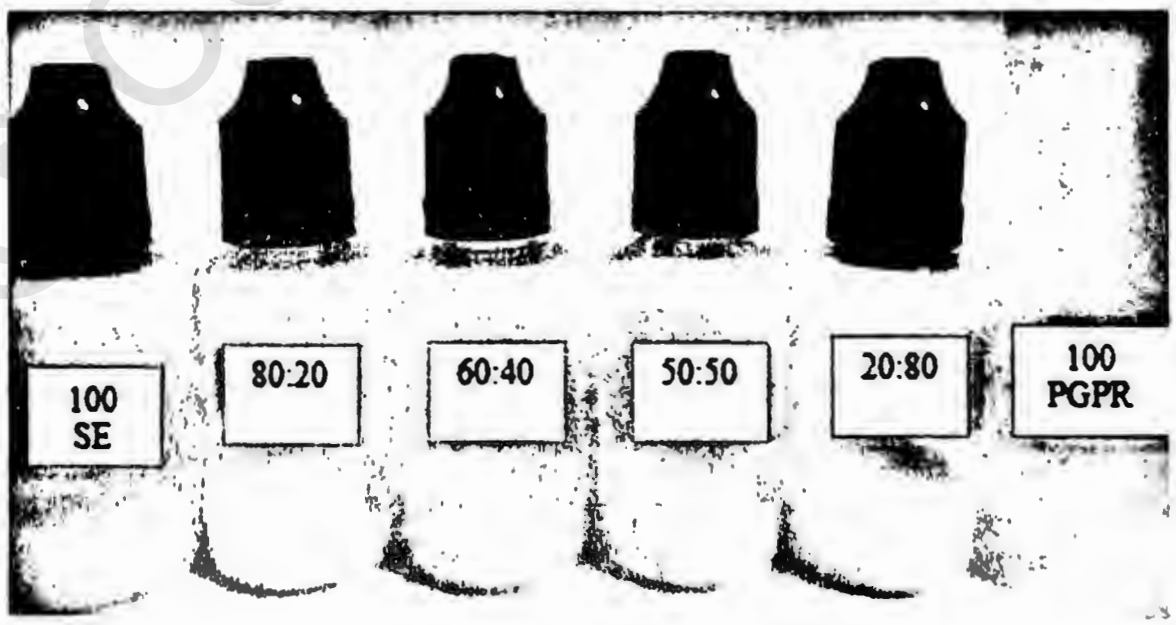

Figure 4-5: Appearance of produced emulsion of passion fruit oil using combination of sucrose ester:polyglycerol polyricinoleate (SE:PGPR)

4.3.2 Effect on Particle Size and Zeta Potential of Produced Emulsion Using Passion Fruit Oil with Combinations of Tween 80

Based on Table 4-5, 4-6 and 4-7, the effect on the particle size and zeta potential of the PFO emulsion by using the combination of Tween 80 which is T80:MDG, T80:LCT and T80:PGPR were studied. The constant concentration of combined surfactants used was 5% with different surfactant ratio.

When using only Tween 80 at 5% concentration, the phase separation occurred because the surfactant concentration used is not enough to emulsify 5% of PFO. When using Tween 80, at least 20% concentration should be used to produce emulsion with particle size is 425.4 nm. This is one of major drawbacks when using synthetic surfactant in low energy method as they need high amount of surfactant used in producing nanoemulsion (Komaiko & Mcclements, 2016).

Based on Table 4-5 and 4-6, when the hydrophobic surfactants (MDG and LCT) was increased in the surfactant ratio, the particle size was increased too. This result can be related with obtained IFT of T80:MDG as the IFT increased when the MDG increased.

However, using combination of T80:PGPR at every ratio as shown in Table 4-8, the passion fruit oil and water was separated directly even when previous IFT obtained was the lowest. This might be due to improper concentration and the amount of the surfactants used in producing emulsion. In order to produce emulsion especially in nano size, the correct amount and surfactant ratio of SE:PGPR should be used. As shown in Table 4-8, the emulsion can be produced at much lower size which is 326.3 nm when 20% of SE:PGPR (80:20) compared when using only 10% of T80:PGPR which produce 1104.8 nm as the particle size. Further study is needed to obtain the

optimum concentration and surfactant ratio of T80:PGPR to produce nanoemulsion using low energy method.

Other than that, the PDI value found is varied in each emulsion. The lower the PDI value which is near to zero shows a monodisperse droplet population, while the higher the PDI value which is near to one shows a wide range of droplet size in the emulsion (Ostrosky, Rocha-filho, & Veríssimo, 2015).

The zeta potential measurement between this three combinations showed different range of values. By using combination of T80:MDG, the zeta potential obtained is |-40.82 mV| to |-46.42 mV| while the combination of T80:LCT was |-64.87 mV| to |-66.78 mV|. This shows that the combination of T80:LCT have an excellent stability behaviour of the particle compared to T80:MDG.

Table 4-5: Results of passion fruit oil emulsion using the combination of Tween 80:distilled monoglyceride (T80:MDG)

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		T80	MDG			
5	90	100	0	separated		
5	90	80	20	separated		
5	90	60	40	1420.7	0.312	-40.82
5	90	50	50	1718.3	0.626	-46.42
5	9	20	80	2623.8	0.541	-45.78
5	90	0	100	separated		

Table 4-6: Results of passion fruit oil emulsion using the combination of Tween 80:lecithin (T80:LCT)

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		T80	LCT			
5	90	100	0	separated		
5	90	80	20	1036.8	0.534	-64.87
5	90	60	40	1311.8	0.541	-66.11
5	90	50	50	1463.9	0.551	-66.78
5	9	20	80	1903.4	0.713	-66.24
5	90	0	100	separated		

Table 4-7: Results of passion fruit oil emulsion using the combination of Tween 80:polyglycerol polyricinoleate (T80:PGPR)

PFO (%)	Water (%)	Ratio of Surfactant Combination at 5% Concentration (%)		Avg. Particle Size (nm)	Avg. PDI	Avg. Zeta Potential (mV)
		T80	PGPR			
5	90	100	0	separated		
5	90	80	20			
5	90	60	40			
5	90	50	50			
5	9	20	80			
5	90	0	100			

Table 4-8: Effect on size particle, PDI and zeta potential using different amount of concentration of Tween 80:polyglycerol polyricinoleate (T80:PGPR)

Formulation	Measurements
- 5% concentration of PFO - 20% concentration of T80:PGPR - 80:20 of T80:PGPR	- Particle Size: 326.3 nm - PDI: 0.298 - Zeta Potential: -36.33 mV
- 5% concentration of PFO -10% concentration of T80:PGPR - 80:20 of T80:PGPR	- Particle Size: 1104.8 nm - PDI: 0.339 - Zeta Potential: -49.57mV

As shown in Figure 4-8, when the ratio of MDG used in the combinations was increased, the appearance of the emulsion becomes more viscous and milky white in colour. This is because the MDG is white in colour. The phase separation on each ratio of combination T80:PGPR at 5% concentration can be clearly seen in Figure 4-10.

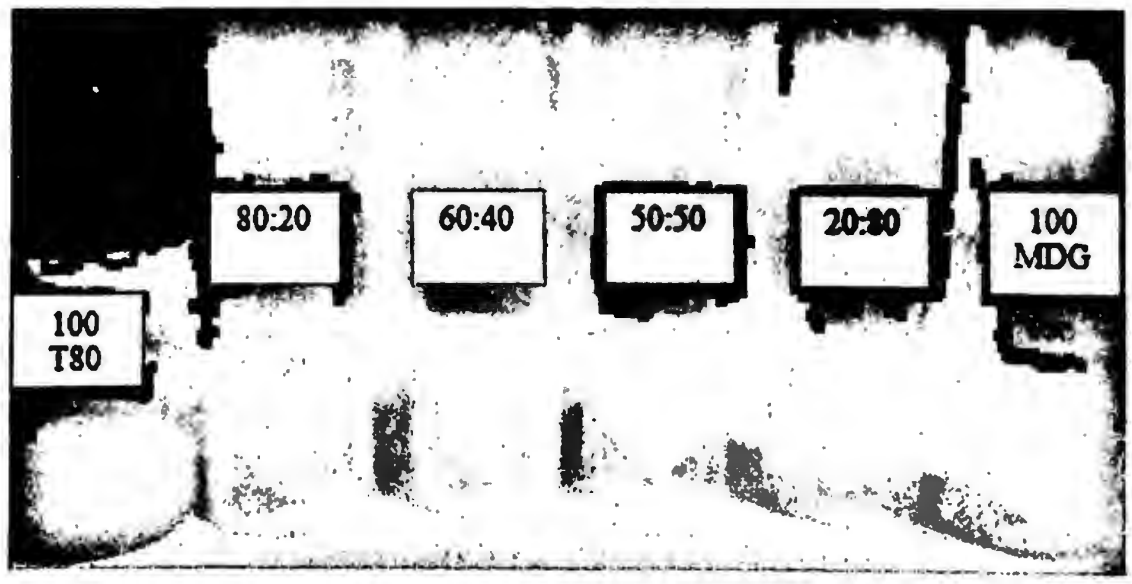

Figure 4-8: Appearance of produced emulsion of passion fruit oil using combination of Tween 80:distilled monoglyceride (T80:MDG)

Figure 4-9: Appearance of produced emulsion of passion fruit oil using combination of Tween 80:lecithin (T80:LCT)

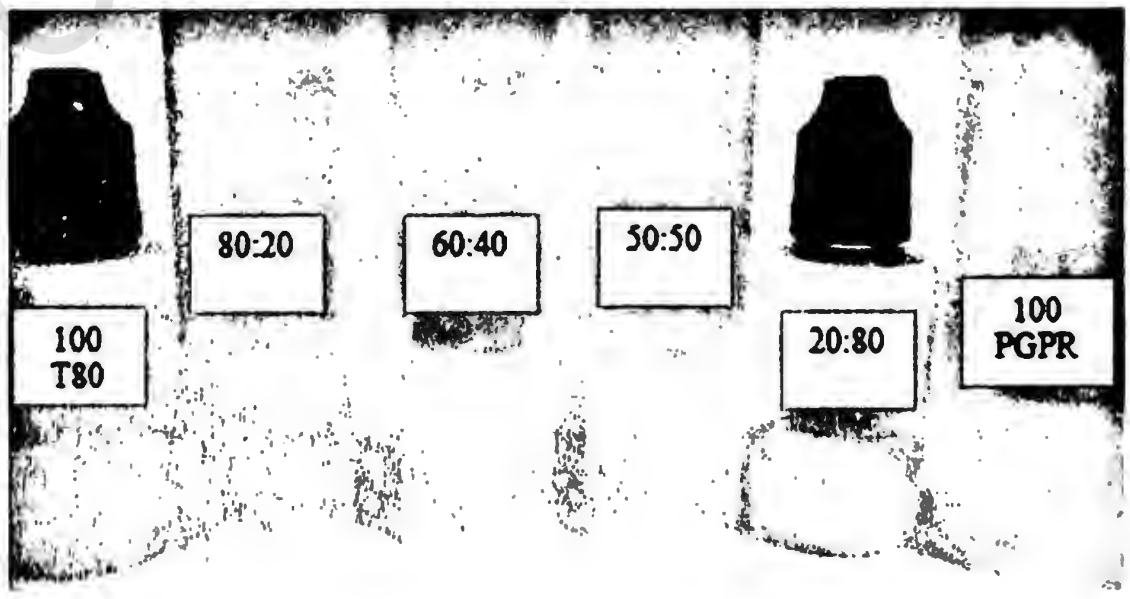

Figure 4-10: Appearance of produced emulsion of passion fruit oil using combination of Tween 80:polyglycerol polyricinoleate (T80:PGPR)

4.4 Effect of Addition Rate of Passion Fruit Oil into Combination of Surfactants Mixture

In this study, the comparison on the particle size when the addition rate of the PFO to the combination of surfactants with distilled water at 1 drop for 20 seconds, and pouring straight away all the PFO to the surfactants mixture were studied as shown in Table 4-9. The chosen formulation is SE:LCT with the weight ratio of 80:20 at the concentration of 5%, PFO at 5% of concentration and 90% concentration of distilled water. Based on Table 4-7, the particle size was obviously greater which is 931.1 nm when the PFO was poured straight away to the SE:LCT mixture compared to 183.4 nm of the particle size when the addition rate of the PFO is 1 drop/20 seconds. This result shown that it is crucial to control the addition rate of the organic phase into the aqueous phase when using the SE method (Komaiko & Mcclements, 2016). When the PFO was added quickly into the surfactants mixture, the viscous surfactant-oil-water clumps may form if other formulation used (such as T80:MDG) which are difficult to breakup and disperse even though there is stirring of the mixture from the magnetic stirrer.

Other than that, this addition rate also affects the PDI value for addition of PFO at 1 drop/20 seconds, the PDI value obtained is lower which indicates a homogeneous monodisperse droplet population while for pouring all the PFO in the surfactants mixture, the PDI value indicates that the emulsion has a very huge particle size distribution. Usually, between 5 and 15 minutes as the addition times was used by most of the researches used to form small oil droplets in the emulsion (Komaiko & Mcclements, 2016). Therefore, the longer the addition rate of organic phase to aqueous phase, the smaller the particle size in emulsion can be obtained.

Table 4-9: Effect on addition rate of PFO to sucrose ester:lecithin (SE:LCT)

Addition Rate	Particle Size (nm)	PDI
1 drop of PFO per 20 seconds	183.4	0.242
Poured straight away all of the PFO	931.1	0.556

CHAPTER 5: CONCLUSION AND RECOMMENDATION

In this study, the effect of using combination of food grade surfactants which are Sucrose Ester:Distilled Monoglyceride, Sucrose Ester:Lecithin, Sucrose Ester:Polyglycerol Polyricinoleate, Tween 80: Distilled Monoglyceride, Tween 80:Lecithin and Tween 80:Polyglycerol Polyricinoleate with the formation and stability of passion fruit oil nanoemulsion using low energy method were studied.

The combination of surfactant that produces the lowest IFT is sucrose ester with polyglycerol polyricinoleate, follow by sucrose ester with lecithin and finally sucrose ester with distilled monoglycerides. Similar trend was found with the combination using Tween 80. Based on the combination of surfactant used, the lowest IFT achieved is 0.034 mN/m using Tween 80: polyglycerol polyricinoleate which indicates the possibility to produce nanoemulsion. This indicate that the surfactant which contain more tail group produce the lowest IFT when combine with a single short tail of high HLB surfactant.

The combination of sucrose ester and lecithin at the only 5% concentration with 5% concentration of passion fruit oil can produce passion fruit oil nanoemulsion which is 187.9 nm at surfactant ratio 80:20 (SE:LCT) at low interfacial tension of oil-water phase using low energy method. However, based on 5% concentration of surfactant used, nanoemulsion cannot be produced with the combination of sucrose ester:polyglycerol polyricinoleate and Tween 80:polyglycerol polyricinoleate although the obtained IFT is low. This is due to improper concentration of surfactant used because when only 2.5% concentration at ratio 80:20 of sucrose ester:polyglycerol polyricinoleate, the particle size drop to 350.8 nm and when 20% concentration at ratio 80:20 of Tween 80:polyglycerol polyricinoleate, the particle size drop to 326.3 nm. In this study, combinations with sucrose ester have an excellent

stability behaviour of the particle size in the emulsion compared to the combinations with Tween 80 even the particle size obtained is macromolecule.

Future work should seek to identify the optimum concentration/ amount and surfactant ratio of the combination of sucrose ester:polyglycerol polyricinoleate and Tween 80:polyglycerol polyricinoleate that can produce nanoemulsion using low energy method because the IFT obtained is approaching to ultra-low IFT which indicates the nanoemulsion can be easily produced.

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APPENDICES

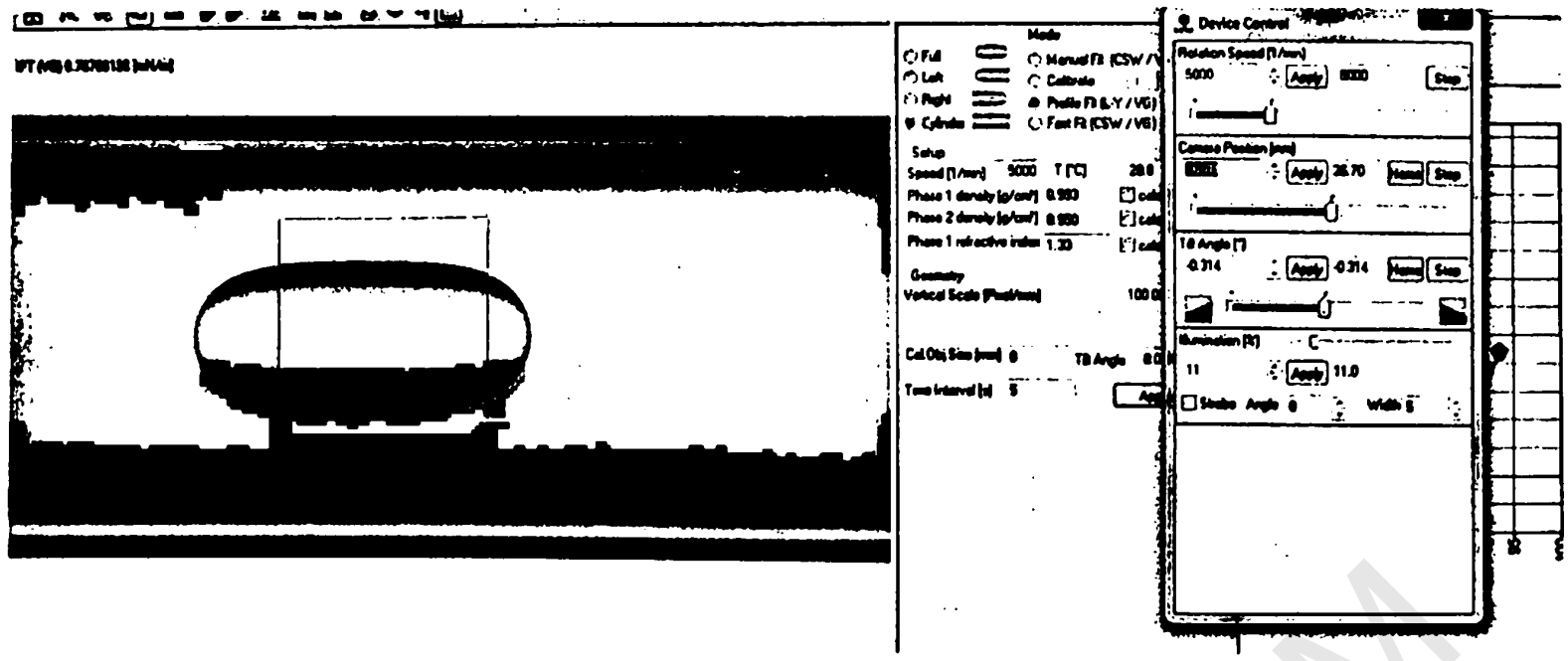

Figure A-1: IFT measurement using spinning drop tensiometer

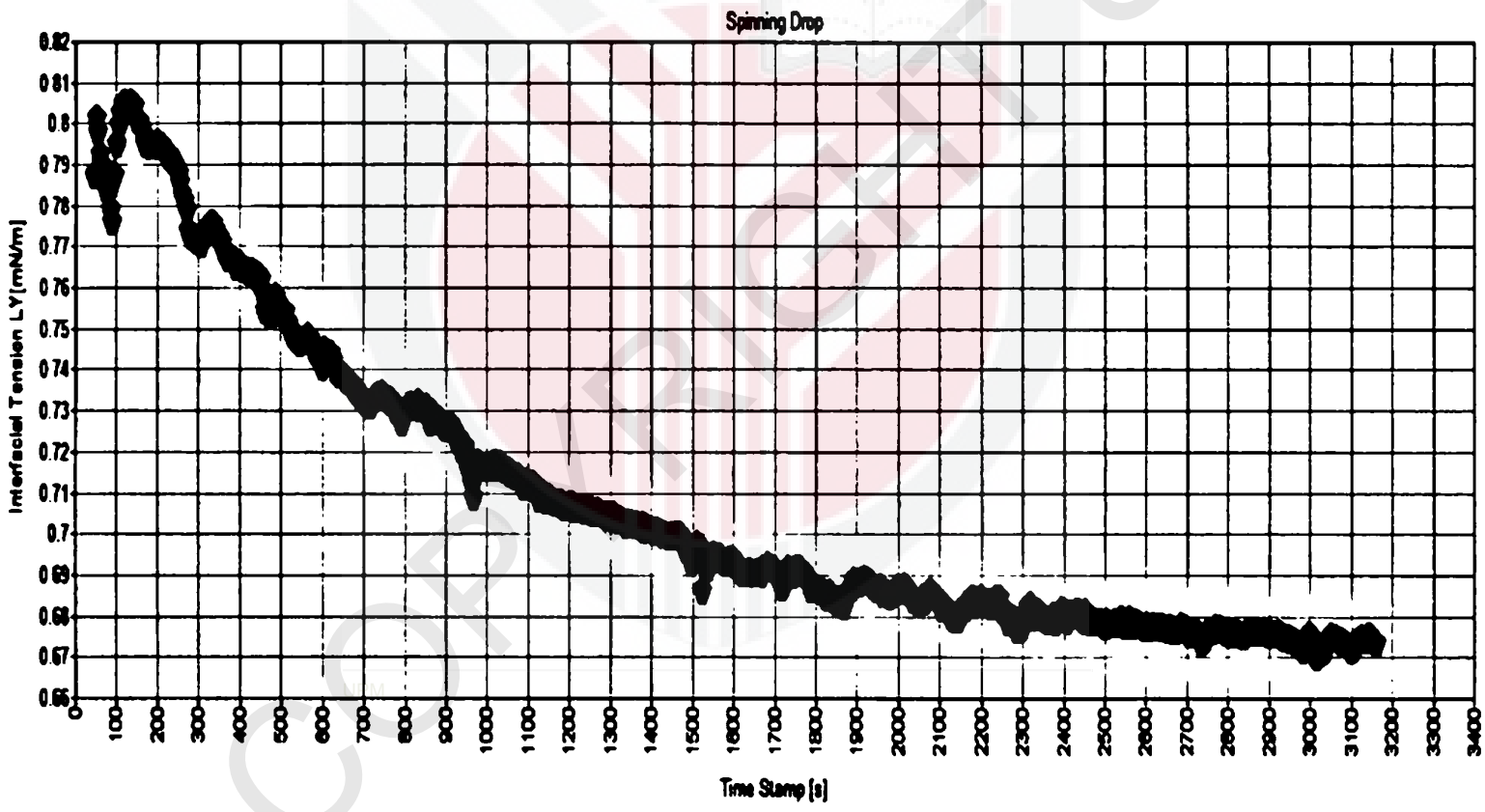

Figure A-2: Graph of interfacial tension against time

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

Code	Surfactant Combination	Surfactant Concentration	Ratio	Density (g/cm^3)	RPM	IFT Value (mN/m)		
						First	Second	Mean
1	-	-	-	0.950	8000	2.66	2.86	2.76
1A	SE:MDG	0.5%	0.25:0.25	0.994	5000	0.825	0.836	0.831
1B	SE:MDG	1.0%	0.5:0.5	0.993	5000	0.679	0.652	0.666
1C	SE:MDG	1.5%	0.75:0.75	0.995	5000	0.476	0.453	0.465
1D	SE:MDG	2.0%	1.0:1.0	0.996	4000	0.523	0.521	0.522
1E	SE:MDG	0.1%	0.05:0.05	0.991	5000	0.829	0.901	0.865
2A	T80:MDG	0.5%	0.25:0.25	0.995	5000	0.487	0.495	0.491
2B	T80:MDG	1.0%	0.5:0.5	1.000	5000	0.437	0.427	0.432
2C	T80:MDG	1.5%	0.75:0.75	1.010	5000	0.402	0.409	0.406
2D	T80:MDG	2.0%	1.0:1.0	1.030	5000	0.626	0.642	0.636
3A	SE:LCT	0.5%	0.3:0.2	0.993	6000	0.413	0.432	0.423
3B	SE:LCT	1.0%	0.6:0.4	0.994	6000	0.110	0.091	0.101
3C	SE:LCT	1.5%	0.9:0.6	0.995	6000	0.124	0.119	0.122
3D	SE:LCT	2.0%	1.2:0.8	0.996	6000	0.189	0.242	0.216
4A	T80:LCT	0.5%	0.3:0.2	0.991	8000	0.615	0.592	0.604
4B	T80:LCT	1.0%	0.6:0.4	0.995	7000	0.292	0.312	0.302
4C	T80:LCT	1.5%	0.9:0.6	0.993	7000	0.326	0.330	0.328
4D	T80:LCT	2.0%	1.2:0.8	0.996	5000	0.341	0.352	0.333
5A	SE:PGPR	0.5%	0.25:0.25	0.991	4000	0.169	0.175	0.172
5B	SE:PGPR	1.0%	0.5:0.5	0.992	5000	0.049	0.043	0.046
5C	SE:PGPR	1.5%	0.75:0.75	0.992	6000	0.139	0.158	0.149
5D	SE:PGPR	2.0%	1.0:1.0	0.992	5000	0.195	0.221	0.208
6A	T80:PGPR	0.5%	0.25:0.25	0.990	6000	0.054	0.071	0.063
6B	T80:PGPR	1.0%	0.5:0.5	0.991	6000	0.047	0.033	0.053
6D	T80:PGPR	1.5%	0.75:0.75	0.991	6000	0.120	0.122	0.121
6E	T80:PGPR	2.0%	1.0:1.0	0.992	5000	0.370	0.395	0.383

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

Code	Surfactant Combination	Surfactant Concentration	Ratio	Density (g/cm^3)	RPM	IFT Value (mN/m)		
						First	Second	Mean
7A	SE:LCT	1.0%	1.0:0	0.994	4000	0.026	0.017	0.022
7B	SE: LCT	1.0%	0.8:0.2	0.991	3000	0.025	0.029	0.027
7C	SE: LCT	1.0%	0.5:0.5	0.993	5000	0.330	0.306	0.318
7D	SE: LCT	1.0%	0.2:0.8	0.994	7000	0.785	0.789	0.787
7E	SE: LCT	1.0%	0:1.0	0.995	5000	0.866	0.986	0.926
8A	T80:LCT	1.0%	1.0:0	0.989	5000	0.236	0.244	0.240
8B	T80: LCT	1.0%	0.8:0.2	0.990	4000	0.230	0.204	0.217
8C	T80: LCT	1.0%	0.5:0.5	0.990	5000	0.432	0.468	0.450
8D	T80: LCT	1.0%	0.2:0.8	0.993	5000	0.658	0.636	0.647
8E	T80: LCT	1.0%	0:1.0	0.995	5000	0.866	0.986	0.926
9A	SE:MDG	1.5%	1.5:0	0.996	2000	0.013	0.014	0.014
9B	SE:MDG	1.5%	1.0:0.5	0.996	4000	0.426	0.459	0.443
9C	SE:MDG	1.5%	0.75:0.75	0.995	5000	0.679	0.710	0.695
9D	SE:MDG	1.5%	0.5:1.0	0.998	500	0.811	0.843	0.827
10A	T80:MDG	1.5%	1.5:0	0.990	4000	0.180	0.172	0.176
10B	T80:MDG	1.5%	1.0:0.5	1.000	4000	0.300	0.253	0.276
10C	T80:MDG	1.5%	0.75:0.75	1.010	5000	0.402	0.426	0.414
10D	T80:MDG	1.5%	0.5:1.0	1.010	4000	0.671	0.689	0.680
11A	SE:PGPR	1.0%	1.0:0	0.994	4000	0.026	0.017	0.022
11B	SE:PGPR	1.0%	0.8:0.2	0.991	5000	0.029	0.017	0.023
11C	SE:PGPR	1.0%	0.5:0.5	0.992	5000	0.049	0.043	0.046
11D	SE:PGPR	1.0%	0.2:0.8	0.992	5000	0.075	0.081	0.078
11E	SE:PGPR	1.0%	0:1.0	0.993	5000	0.665	0.673	0.669
12A	T80:PGPR	1.0%	1.0:0	0.989	5000	0.236	0.244	0.240
12B	T80:PGPR	1.0%	0.8:0.2	0.992	5000	0.133	0.146	0.140
12C	T80:PGPR	1.0%	0.5:0.5	0.992	5000	0.047	0.058	0.053
12D	T80:PGPR	1.0%	0.2:0.8	0.992	5000	0.615	0.626	0.621
12E	T80:PGPR	1.0%	0:1.0	0.993	5000	0.665	0.673	0.669

Table A-3: Results of passion fruit oil emulsion using the combination of sucrose ester:distilled monoglyceride

Ratio of Surfactant		Particle Size (nm)			PDI		Zeta Potential (mV)	
SE	MDG	1st	2nd	Avg.	1st	2nd	1st	2nd
100	0	243.2	266.0	254.6	0.255	0.267	-96.23	-94.49
80	20	415.4	480.2	447.8	0.237	0.277	-89.83	-86.21
60	40	separate						
50	50	separate						
20	80	2521.2	3202.0	2861.6	0.447	0.563	-66.69	-65.27
0	100	separate						

Table A-4: Results of passion fruit oil emulsion using the combination of sucrose ester:lecithin

Ratio of Surfactant		Particle Size (nm)			PDI		Zeta Potential (mV)	
SE	LCT	1st	2nd	Avg.	1st	2nd	1st	2nd
100	0	243.2	266.0	254.6	0.255	0.267	-96.23	-94.49
80	20	179.4	196.4	187.9	0.212	0.272	-99.81	-104.49
60	40	804.5	735.1	769.8	0.520	0.312	-95.12	-90.80
50	50	421.1	269.9	345.5	0.275	0.177	-77.06	-85.24
20	80	312.7	596.1	454.4	0.296	0.332	-97.64	-89.20
0	100	separate						

Table A-5: Results of passion fruit oil emulsion using the combination of sucrose ester:polyglycerol polyricinoleate

Ratio of Surfactant		Particle Size (nm)			PDI		Zeta Potential (mV)	
SE	**PGPR**	**1st**	**2nd**	**Avg.**	**1st**	**2nd**	**1st**	**2nd**
100	0	243.2	266.0	254.6	0.255	0.267	-96.23	-94.49
80	20	1464.6	1147.4	1306.0	0.681	0.561	-98.21	-96.19
60	40	1470.0	1566.4	1518.3	0.442	0.554	-89.50	-81.24
50	50	1831.8	2051.2	1941.5	0.722	0.760	-100.86	-96.26
20	80	separate						
0	100	separate						

Table A-6: Results of passion fruit oil emulsion using the combination of Tween 80:distilled monoglyceride

Ratio of Surfactant		Particle Size (nm)			PDI		Zeta Potential (mV)	
T80	**MDG**	**1st**	**2nd**	**Avg.**	**1st**	**2nd**	**1st**	**2nd**
100	0	separate						
80	20	separate						
60	40	1107.9	1733.5	1420.7	0.240	0.384	-36.93	-44.71
50	50	2365.2	1074.4	1718.3	0.711	0.541	-43.22	-46.42
20	80	2891.0	2356.6	2623.8	0.631	0.451	-39.40	-48.16
0	100	separate						

Table A-7: Results of passion fruit oil emulsion using the combination of Tween 80:lecithin

Ratio of Surfactant		Particle Size (nm)			PDI		Zeta Potential (mV)	
T80	LCT	1st	2nd	Avg.	1st	2nd	1st	2nd
100	0	separate						
80	20	935.2	1138.4	1036.8	0.442	0.626	-67.43	-62.31
60	40	1172.6	1451.0	1311.8	0.560	0.522	-62.74	-69.48
50	50	1747.8	1180.0	1463.9	0.522	0.580	-69.76	-63.80
20	80	2263.1	1543.7	1903.4	0.722	0.704	-65.62	-66.86
0	100	separate						

Appendix A: Technical data sheet of distilled monoglyceride

RIKEVITA (MALAYSIA) SDN BHD (210381-U)

1/1
Creation Date: 04 Mac 2002
Revision Date: 30 Nov 2016
SDS No. UN-RM-D-0004(11)

SAFETY DATA SHEET

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

PRODUCT NAME	**TYPE P(V)**
CHEMICAL NAME	**Mono- and diglycerides of fatty acids**
APPLICATION	**Food emulsifier**

MANUFACTURER

Company name	**RIKEVITA (M) SDN BHD**
Address	**No.11, Jalan Bayu, Tampoi, 81200, Johor Bahru, Johor, Malaysia**
Phone No.	**+60-7-2381733**
Fax No.	**+60-7-2381737**
E-mail	**pt@rikevita.com.my / qadept@rikevita.com.my**

SUPPLIER

Company name	**Riken Vitamin Europe GmbH**
Address	**Friedrich-Ebert-Strasse 1, 40210 Duesseldorf Germany**
Phone No.	**+49-211-863240-0**

EMERGENCY CONTACT

Riken Vitamin Europe GmbH	**+49-211-863240-0**
Riken Vitamin USA INC.	**+1-310-294-5290**
RIKEVITA (SINGAPORE) PTE. LTD.	**+65-6298-3505**
RIKEVITA ASIA CO., LTD.	**+886-2-3765-6008**
RIKEVITA FINE CHEMICAL & FOOD INDUSTRY (SHANGHAI) CO., LTD.	**+86-21-34976619**

2. HAZARDS IDENTIFICATION

CLASSIFICATION

Physical	**Not classified.**
Health	**Not classified.**
Environmental	**Not classified.**

3. COMPOSITION / INFORMATION ON INGREDIENTS

Substance / Mixture: Substance

Chemical name	Composition (%)	CAS number
Glycerides, C16-C18 mono-	100%	91052-47-0

Other identification number **EEC No. : E 471** **FDA No. : 21 CFR 184.1505**

Appendix B: Technical data sheet of polyglycerol polyricinoleate

SUNWISECHEM

Reliance Value Innovation

SHANGHAI SUNWISE CHEMICAL Co., Ltd.

ISO9001:2008 certified
www.sunwisechem.com

TECHNICAL DATA SHEET

Product Name Polyglycerol polyricinoleate
Product Type Emulsifier, food grade

Identification
Synonyms PGPR
CAS No. 29894-35-7
Chemical Formula $C_{27}H_{52}O_9$

Properties
Appearance Yellow to brown viscous liquid
Stability stable under ordinary conditions

Specification

Item	Specification
Appearance	Yellow to brown viscous liquid
Acid value (mg KOH/g)	≤ 6.0
Iodine value(gI2/100g)	72-103
Hydroxyl value(mgKOH/g)	80-100
Di-, Tri, and Tetraglycerol content,%	≥75
Polyglycerols equal to or higher than Heptaglycerol content, %	≤10
Arsenic (As) (mg/kg)	≤3
Lead (Pb) (mg/kg)	≤2

Heavy Metals Specifications

Item	Specification

: Classification not possible.

Hazardous to the aquatic environment - Long-term hazard

: Not classified.

Hazardous to the ozone layer : Classification not possible.

GHS label elements

Pictograms :

Signal words : None.

Hazard statements : None.

Precautionary statements

[Prevention] : P201, P202: Obtain special instructions before use. Do not handle until all safety precautions have been read and understood.
P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.
P264: Wash hands thoroughly after handling.

[Response] : P264: Wash hands after handling.
P310, P314, P333, P305: Immediately call a POISON CENTRE or doctor/ physician and get medical advice/attention if any of the following occurred:
(Skin irritation, rash, in eyes, feeling unwell, or any other abnormality found in body)

[Storage] : P403+P233: Store in a well-ventilated place. Keep container tightly closed.
P235: Keep cool.

[Disposal] : P501: Dispose of contents/ container in accordance with local/ regional/ national/ international regulation.

Other hazards not applicable to GHS to classification

Physical and chemical risk : There is a possibility of dust explosion under specific circumstance.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Substance or mixture : Substance

Chemical name : Sucrose fatty acid ester

Chemical formula or structural formula

: $C_{33}H_{62}O_{12}$

Official gazette notification reference number (ENCS)

: 8-67

Official gazette notification reference number (ISHL)

: Listed.

CAS Registry Number : Trade secret.