# CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Emulsion and Emulsifiers

An emulsion is a heterogeneous system, which consists of immiscible lipophilic phase dispersed in aqueous phase, or vice versa, in form of droplet. Emulsion may be typical emulsions (water-in-oil or oil-in-water, commonly abbreviated as W/O or O/W) or multiple emulsions (water-in-oil-in-water or oil-in-water-in-oil, abbreviated as W/O/W or O/W/O). Emulsion is widely used in various processes for example paint [12-14], coating [15-17], cosmetic [18-21], food [22-25] and pharmaceuticals [26-29]. A dispersion of immiscible liquid can be formed with mechanical energy input resulting in a metastable dispersion. Homogenization is a well known process for converting two separate immiscible liquids into an emulsion or reducing the size of droplets in pre-existing emulsion [30]. These systems are thermodynamically unstable owing to unfavorable contact, and different densities between oil and water phase, resulting in phase separation [31-34].

Emulsifiers are surface-active molecules that are widely added into emulsion for improving the stability of emulsion [32]. Most emulsifiers are amphiphilic molecules having polar and non-polar regions in the same molecules. They were absorbed at the surface of emulsion droplets [35] in order to reduce interfacial tension. Moreover, they can reduce tendency of droplets aggregation by steric or electrostatic repulsion. [30,33,36-37]. They increase interfacial viscosity and interfacial elasticity, which makes break-up of droplet more difficult [38]. Furthermore, Good emulsifiers need following properties; (I) able to form and stabilize emulsions, (II) quick adsorption around the dispersed drop during emulsification, (III) relatively inexpensive, (IV) non-toxic, and (V) save to handle [39].

Various kinds of synthetic and natural emulsifiers are widely used in emulsification. Normally, emulsifiers can be categorized into two types – small molecular surfactants and polymeric surfactants.

Each type of emulsifier has its own particular advantages and disadvantages. Some small molecular surfactants (Figure 2.1) such as, sorbitan monostearate, glyceryl stwa, stearyl alcohol etc., are highly effective in generating small emulsion droplets during emulsification because of their rapid adsorption rates. Nevertheless, they do not provide strong enough droplet-droplet repulsion, resulting in poor long-term stability against droplet aggregation [30,40].


Sorbitan monostearate

Stearyl alcohol

Glyceryl sterate
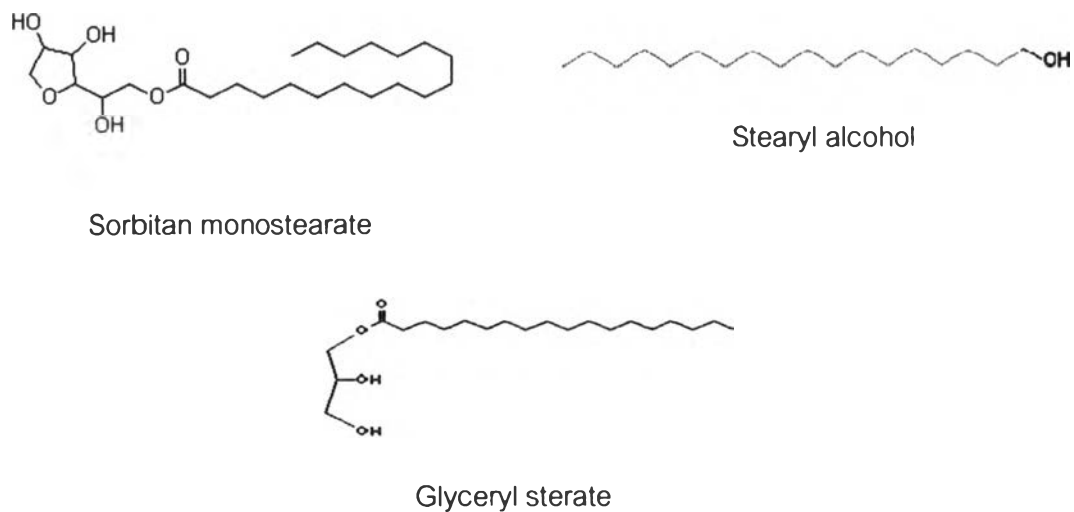
Figure 2.1 Example of small molecular surfactants.

On the other hand, polymeric surfactants, such as polysaccharides, and proteins are highly effective at providing long term stability, but inefficient on creating small droplets during emulsification [30,40].

The emulsifier molecules, such as phospholipids, proteins, or polysaccharide, coat emulsion droplets in form of a thin layer. Nevertheless, the orientation of emulsifier at an oil-water interface depends on emulsifier type. The characteristic of emulsifier at the interfacial membrane depend on structure of emulsifier (Figure 2.2). For small molecular surfactants, nonpolar segments orient to oil phase whereas polar segments orient to water phase. Meanwhile, polymeric surfactants show

a similar characteristic but form like a tail-train-loop and globular at oil-water interface for flexible and globular polymer, respectively.
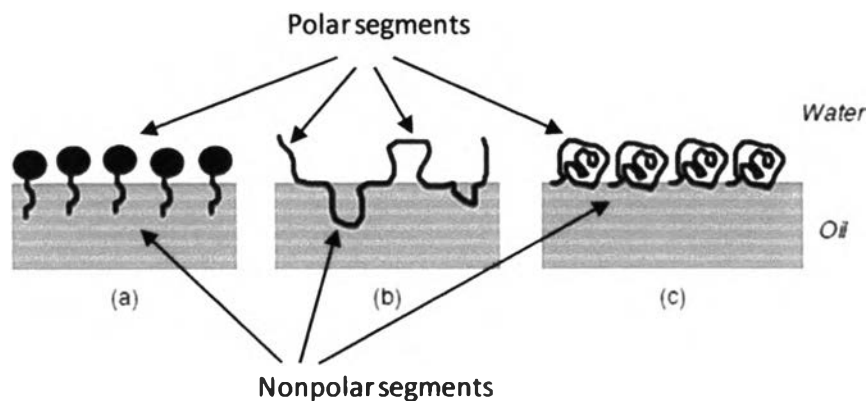
Figure 2.2 Characteristic of the interfacial emulsifier membrane between oil and water phase depending on structure of emulsifiers a) small molecular surfactant, b) flexible polymer, and c) globular polymer [30].

2.1.1 Natural polymeric emulsifier

To date, natural polymers were widely used as emulsifiers or surfactant in colloidal system to replace synthetic emulsifiers or surfactants because of good health records [41]. Macromolecular emulsifier, which extracted from natural occurring materials, can be divided into two types, protein-based and polysaccharide-based emulsifies. In this section, we will review about properties of them to use as emulsifier.

2.1.1.1 Proteins

Many proteins are highly effective emulsifiers due to their amphiphilic property i.e. the molecules containing both hydrophobic and hydrophilic regions resulting in reducing the interfacial tension and interacting at oil-water interface [42].

Additionally, the interfacial membranes formed by proteins usually exhibit electrically charged, hence the major mechanism of proteins to prevent droplet flocculation is electrostatic repulsion [43]. Several researches have been studied emulsifying properties of protein-based emulsifiers in various conditions.

K. Demetriades and D.J. McClements [44] studied influence of pH and heating to physical properties of O/W emulsions stabilized by whey protein isolate and emulsified with nonionic surfactant. In the O/W emulsion system that had only whey protein, it was found that the surface of emulsion droplets showed negatively charged when pH value over than isoelectric point (pI) of protein whereas positively charged surface was detected at pH below pI. Due to electrostatic repulsion between the droplets, the emulsions were prevented to form flocculation. Around pI, the electrostatic repulsive forces were reduced leading to droplet flocculation. This phenomenon was detected around pH 4-6 using the light scattering technique and showed that proteins lose their colloidal stabilizing characteristics at pH values close to their pI [45].

Furthermore, proteins are induced to denature by heat and some chemical substances. The denaturation of proteins with chemicals might enhance protein flexibility. S. Poon et al. [46] studied the relationship between protein flexibility and emulsifying activity for O/W emulsion. The enhancement of emulsify activity was determined from the decrease in mean droplet size of O/W emulsions. The model proteins (lysozyme, apomyoglobin, casein, bovine serum albumin, and lactoglobulin) were denatured by disrupting disulfide bond or noncovalent bond with 0.05 M dithiothreitol or 8 M urea, respectively. It was found that after denaturation of proteins with dithiothreitol, lysozyme markedly enhanced the emulsify activity more than the others. It was believed that the location of the disulfide bond may be an important factor for this enhancement. On the other hand, denaturation of proteins with urea enhanced the emulsify activity of apomyoglobin, casein and lysozyme by disrupting noncovalent interactions. These denaturants allowed greater freedom movement of protein in a consequence of changing the orientation of the hydrophobic domains. This flexible orientation could enhanced the emulsify activity.

Consequently, proteins lose their colloidal stabilizing characteristics at high concentrations of electrolytes. T. Tokle and D.J. McClements [47] studied effect of salt concentration (0-200mM) on physicochemical properties of lactoferrin stabilized O/W emulsion. Lactoferrin is a globular glycoprotein derived from secretory fluids such as milk, tears, saliva, and nasal secretion. They found that the stability of emulsions depend on additional salt concentration. Containing 100 mM NaCl and 150 mM at pH 6, the emulsions were relatively stable to droplet aggregation due to mean particle diameter below 300 nm and 350 nm, respectively. However, mean particle diameter increased to 400-500 nm at high salt concentration (200 mM). It was attributed to counter-ion binding and electrostatic screening effects.
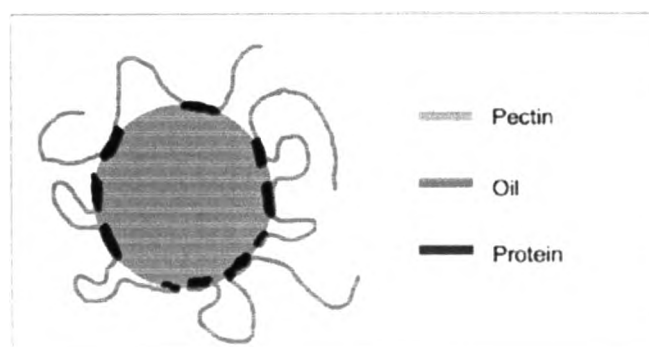
2.1.1.2 Protein-conjugated polysaccharide

As mention above, protein-based emulsifiers are sensitive to pH and ionic strength effect. Nowadays, there are several efforts to improve the protein solubility and interfacial functionality for as emulsifier via the covalent linking of proteins to polysaccharides, for examples ovabumin-dextran conjugates [1], whey protein isolate-maltodextrin conjugates [2], caseinate-dextran conjugates [3].

Additionally, gum arabic, a complex mixture of polysaccharides and glycoproteins, can be defined as a naturally occurring proteins-polysaccharides emulsifier [5]. Unfortunately, it is necessary to use high concentration of gum arabic to emulsify O/W emulsion. Since, the emulsifying property of gum arabic at the oil-water interface is attributed to a small amount of protein which is covalently bound to a highly branch polysaccharide structure [48].

Similarly to gum arabic, pectin is one more example of protein-polysaccharide complex emulsifier. Pectin is extracted from the plant cell wall, especially citrus peels, apple pomace and sugar beet pulps. It should be noted that the plant primary cell wall contains proteins and particularly hydroxyproline-rich protein. The extraction and purification can not completely remove these proteins from pectin. Kravtchenko et al. [49] analyzed various industrial samples. They reported the presence

of hydroxyproline-rich protein, which was not completely removed by copper purification from pectin.

Leroux *et al.* [50] extracted pectin from dried citrus peels or sugar beet pulp by hydrolysis. Protein exists in pectin around 0.77-2.28%. Furthermore, they suggest that emulsifying properties of pectin are most probably due to the protein residues present within the pectin, while polysaccharides is more clearly with its ability to stabilize emulsion than with the performance to form emulsion. Figure 2.3 showed hypothetical model of oil-in-water emulsion stabilized by pectin, proposed by Leroux *et al.* In order to provide more information about the contribution of the acetyl groups to emulsifying capacity, pectin was acetylated. It was found that the higher in acetyl groups, the more emulsifying capacity which is due to the adherence of acetyl groups to oil phase. H. U. Endreβ [51] also mentioned that high acetyl content in the chemical structure of pectin could enhance the hydrophobicicity of pectin followed by increase the emulsion stability.

Figure 2.3 Hypothetical model of oil-in-water emulsion stabilized by pectin [50].

E. Dickinson [52] suggested that the proteins-polysaccharides conjugates combine the properties of a hydrophobic protein, i.e. being adsorbed strongly to the oil-water interface, with the properties of a hydrophilic polysaccharide, i.e. being highly solvated by the aqueous medium. Besides, it is believed that
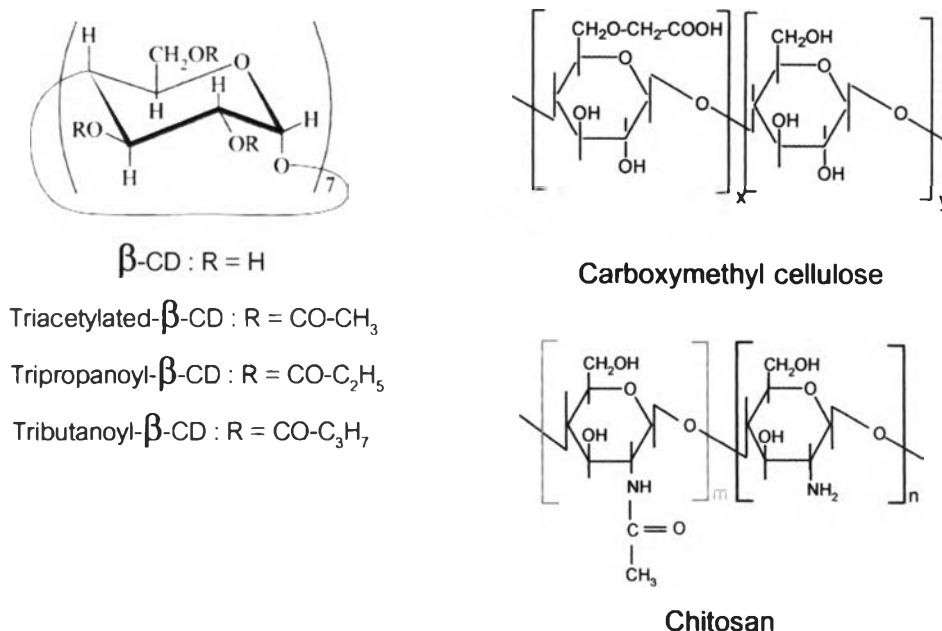
polysaccharides enhance emulsifying properties of proteins through steric stabilization. Since polysaccharide introduces a bulky polymeric layer to the droplet surface, avoiding droplet aggregation and coalescence [53].

C.A. Dunlap and G.L. Côté [53] proposed characteristic of protein-polysaccharide conjugate bound to an oil droplet concerning with size of polysaccharide in stabilizing emulsions. From a theoretical point of view, a larger polysaccharide should produce a thicker polymeric layer and provide greater stability to the emulsion. From a practical truth, as polysaccharides become lager, their size may prevent the droplet surface from becoming saturated with protein. Due to the polysaccharides would occupy a much greater volume than allowed protein to pack tightly on the droplet surface. Therefore, the greater density and thickness of the polysaccharide layer are able to compensate for the decease in density of the protein layer bound to oil droplets.

2.1.1.3 Polysaccharide

It is rarely to find the research studies focused on polysaccharide-based emulsifiers. Polysaccharide also functions as emulsifier due to its viscosity and amphiphilic properties. Viscous polysaccharides used to stabilize emulsion by thickening the aqueous phase. However, polysaccharides mostly have hydrophilic regions, but less hydrophobic regions for interaction with the oil phase of emulsion leading to ineffective emulsifying. Nevertheless, polysaccharides are relatively insensitive to pH change and salt.

Polysaccharides e.g. β-cyclodextrin (β-CD) and its derivatives [54], carboxylmethyl cellulose (CMC) [55] and chitosan [56] were found to be used as emulsifiers. The structure of β-CD and its derivatives (triacetylated-β-CD, tripropanoyl-β-CD and tributanoyl-β-CDs), carboxymetyl cellulose and chitosan were shown in Figure 2.4.

β-CD : R = H

Triacetylated-β-CD : R = CO-CH₃

Tripropanoyl-β-CD : R = CO-C₂H₅

Tributanoyl-β-CD : R = CO-C₃H₇

Carboxymethyl cellulose

Chitosan

Figure 2.4 Structure of β-cyclodextrin and its derivatives, carboxymetyl cellulose and chitosan (Chitin is composed predominantly of (m) units. Chitosan is composed predominantly of (n) units).

Cyclodextrins have a hydrophobic cavity and form an inclusion complex with a hydrophobic guest molecule. M. Inoue *et al.* [54] and K. Hashizaki et al. [57] reported about the preparation and formation mechanism of emulsions using CDs as an emulsifier. It was found that the emulsions were formed a kind of Pickering emulsion as shown in Figure 2.5, which was stabilized by solid particles. To clarify types of emulsion, contact angle of emulsion was measured. The emulsion was centrifuged and the obtained precipitate was molded into pellet. A small droplet of water was dropped onto the pellet. The contact angle was measured through microcamera at 25°C. An O/W emulsion showed contact angle less than 90°, where as W/O emulsion showed the contact angle over than 90°. It was cleared that as prepared emulsion was O/W emulsion. Subsequently, M. Inoue *et al.* [54] prepared and studied the formation mechanism of W/O emulsions using natural β-cyclodextrin (β-CD) and triacyl modified β-CDs (triacetyl-, tripropanoyl- and tributanoyl-β-CDs) as emulsifiers.

The stable W/O emulsion was formed by using tripropaonyl β-CD as emulsifier. Whilst, only unstable W/O emulsion was formed by using tributanoyl-β-CDs.
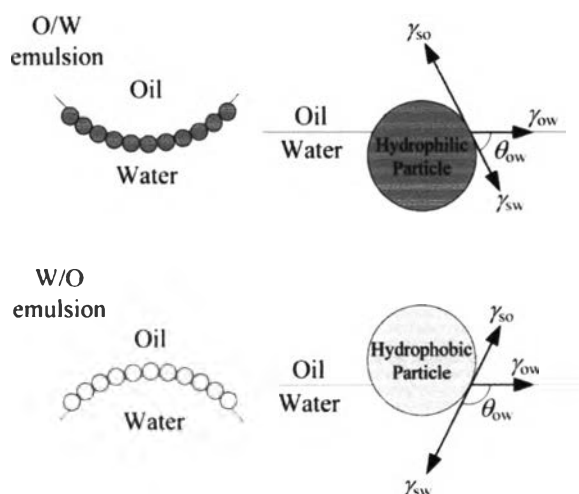

Figure 2.5 Formation mechanism of Pickering emulsion. [54]

Carboxymethylcelloulose (CMC), is a water soluble cellulose and biodegradable polymer, which used as emulsifier. M. Mirhosseini *et al.* [24] studied influence of pectin and CMC on physical stability of orange beverage emulsion. CMC (0.1%, 0.3% and 0.5% w/w) or pectin (1.5%, 3%, 4.5% w/w) was added into continuous phase for using as emulsifier in O/W emulsion. It was found that the stability of all orange beverage decreased during storage period but pectin showed more effective on physical stability than CMC except for the emulsion containing 1.5 %w/w. Pectin acted as emulsifier due to its own small amount of protein as mentioned above [50], whereas CMC acted as emulsifier because of its own hydrophobic at main chain and hydrophilic at side chain and hydrophilic carboxylmethyl groups. The emulsion can be stabilized by electrostatic repulsion.

Chitosan is a modified natural polymer prepared by the partial deacetylation of chitin. The name, chitosan, does not refer to a uniquely define compound but rather define a family of copolymers with various fraction of degree of deacetylation. It is called chitosan when degree of deacetylation of chitin is over 50% [58-59]. Chitosan acts as emulsifier without adding any surfactant, because chitosan is

composed of mixture molecules with different degrees of deacetylation (DD). Therefore, chitosan behaves as a mixture of surfactants with different hydrophilic-lipophilic balance (HLB). Molecules with high DD promoted the formation of O/W emulsion, whereas those of lower DD promoted the formation of W/O emulsion [6-7].

Rodriguez *et al.* [6] studied emulsification of sunflower oil by using chitosan with DD between 75 and 95% as emulsifiers at concentration 0.2, 1.0, 2.0% in 0.1 M hydrochloric acid. It was found that chitosan can produced stable W/O/W emulsions by one-step emulsification. At low DD and high DD of chitosan produced unimodal of the droplet size distribution. At intermediate DD, the droplet size distribution was unimodal only when used the most concentrated solutions.

2.2   Characteristic of emulsifier

2.2.1   Molecular characteristics

Emulsifiers or surfactants are amphiphilic which have one part contacting with non-polar media and the other part contacting with polar media. The energetically most favorable orientation for these molecules is at interface so that each part of molecule can orient in an environment for which it has greatest affinity. Figure 2.6 depicts the location of the anionic emulsifier components at equilibrium above its critical micelle concentration. These molecules orient mono-layers at air-liquid interface (surface of solution) and liquid-solid interface (beside container), including micelle form in solution to cover immiscible liquid in emulsion. This characteristic shows ability of emulsifier or surfactants to alter surface, interfacial properties, and to cover immiscible liquid in micelles for applied emulsifiers or surfactants in many applications such as detergency and wet-ability.
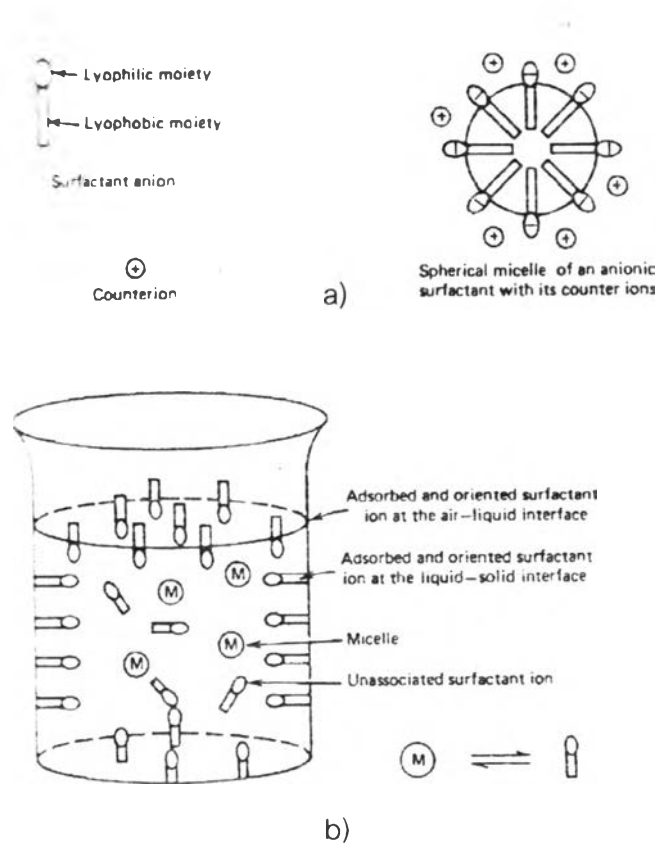
Figure 2.6 Illustration of anionic surfactant solution at equilibrium above its critical micelle concentration, showing the components (a) and the locations of the components at equilibrium (b). [60].

Generally, the high energy required to produce emulsion especially for nano-emulsion. It can be described in terms of the Laplace pressure, p, which is the difference in pressure between inside and outside of the droplet. Laplace pressure can be calculated from equation (2.1) [61],

$$p = \gamma\left(\frac{1}{R_1} + \frac{1}{R_2}\right)$$ (2.1)

Where γ is surface tension, R_1 and R_2 are the principal radii of curvature of the drop.

For a spherical drop, $R_1 = R_2 = R$ and,

$$p = \frac{2\gamma}{R}$$

(2.2)

Therefore, surfactants or emulsifier plays an important role in formation of emulsion by lowering interfacial tension as a result of reducing p and reducing in the stress needed to break up a drop. This indicates that emulsion can be formed easier when emulsifier is added into oil and water mixture.

2.2.2 The hydrophilic-lipophilic balance (HLB)

The HLB is the most empirical predictive for determination of emulsifying characteristic of emulsifier [36]. The HLB is described by a number which indicates the relative affinity of emulsifier for the oil and aqueous phase [62]. A molecule with a high HLB number has a high ratio of hydrophilic groups to lipophilic groups, and vice versa. Furthermore, the HLB number of emulsifier is a useful factor to indicate its solubility in oil or water phase and to predict type of emulsion formed by that emulsifier [63]. HLB ranges and their application are shown in Table 2.1

Table 2.1 HLB ranges and their applications.

HLB Range	Application
3-6	W/O emulsion
7-9	Wetting agent
8-18	O/W emulsion
13-15	Detergent
15-18	Solubilizer

However, it should be noticed that emulsion properties are not only depended on physicochemical variables (such as nature of components, temperature, and etc.) but also depended on addition order of the components [64-67].

2.2.3   Critical Micelle Concentration (CMC)

At very low concentration, most emulsifier molecules are soluble in water to form solutions. Some of the emulsifier molecules preferably laid at the surface of the solution (i.e. air-solution interface). As the concentration increased to reach critical concentration, the emulsifier molecules are packed close together to form spherical aggregates or micelles in which the core is populated with hydrophobic chains and the corona is polar groups in case of O/W emulsion system. The concentration which emulsifiers start to form micelle is called Critical Micelle Concentration (CMC).

Many methods use to determine CMC of emulsifier, such as osmotic pressure, conductivity, surface tension and interfacial tension, by investigation of physical properties of aqueous surfactant solution as a function of surfactant concentration. The physical properties of emulsifier change vary sharply above or below the CMC value because the changes in behaviors correspond with the formation of micelle. Figure 2.7 shows physical properties of aqueous surfactant solution as a function of surfactant concentrations. The breakpoint in the physical properties versus surfactant concentration curve is the evident critical concentration and thus corresponds to the CMC of surfactant. It is obvious that the formation of micelles must occur at some specific concentration of surfactant.

CMC

Conductance

Surface tension

Conductivity

Osmotic pressure

Turbicity

Solubility

Interfacial tension

Surfactant concentration
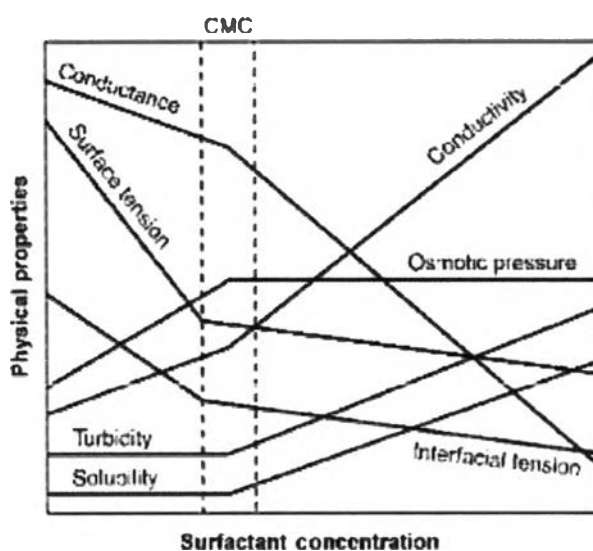
Figure 2.7 Physical properties of aqueous surfactant solution as a function of surfactant concentrations [68]. The breakpoint in the physical properties versus surfactant concentration curve is the evident critical concentration and thus corresponds to the CMC of surfactant.

2.3 Nano-emulsions

Nano-emulsions are fine dispersions possess droplet covering the range of size around 100-600 nm [69-70]. Nano-emulsions are also referred to as mini-emulsions [71-72], finely dispersed emulsion [73], submicron emulsion [74-75], translucent emulsion [76] or ultrafine emulsions [77-78].

The advantages of nano-emulsion were shown in Table 2.2

Table 2.2 The advantages of nano-emulsion.

Application of nano-emulsion	Advantages	References
Personal care product and cosmetics	- Using low surfactant concentration - Suitable for efficiently delivery of active gradients through the skin - Deposit uniformly on the skin	[61, 79-80]
	- Good sensorial properties (rapid penetration, merging texture)	[79]
Miniemulsion polymerization	- Consistency of size and composition of each droplet during the formation of particles	[81]
Drug delivery system	- Excellent vehicles for incorporation of lipophilic compounds due to contain the high oil core fraction	[82]

From those advantages, nano-emulsion is a good candidate for application in the pharmaceutical field as drug delivery system, personal care product and cosmetics, including miniemulsion polymerization. Furthermore, nano-emulsion may be applied as a substitute for liposome and vesicles which are less stable [61].

2.3.1 Formation of nano-emulsion

Nono-emulsion can be prepared by two main methods which are low energy emulsification and high energy emulsification. Low energy emulsification takes the advantage of the phase transition during emulsification process as a result of a

change in the spontaneous curvature of the surfactant. Two different routes are used: a change in temperature, while composition is maintained (refer as phase inversion temperature method, PIT) [83-84], or a change in composition at constant temperature (refer as phase inversion composition method, PIC) [85]. On the other hand, high energy emulsification is a method using a high mechanical energy input, i.e. high shear device, such as static mixers, a high-intensity homogenizer [86], high pressure homogenizer [87], ultrasonic transducer [88-91], etc.

The PIT emulsion was first introduced by Shinoda and Saito [83]. It is based on the change in physicochemical of emulsifier with changing temperature from a coarse O/W emulsion at high temperature into a fine O/W emulsion at low temperature. The temperature at an emulsifier solution converts from a micellar to a reverse-micellar system or an O/W emulsion changes to a W/O emulsion is known as PIT.

Polyoxyethylene is an example of nonionic surfactant which become lipophilic when increase temperature due to dehydration of its chain. At low temperature, the polyoxyethylene monolayer has a large positive spontaneous curvature forming oil-swollen micellar solution phase (or O/W emulsions). At high temperature, the spontaneous curvature become negative or in other words, the hydrophilic head groups of the emulsifier molecules become progressively dehydrated and form water-swollen reverse micelles (or W/O emulsions) [92] .

Emulsions prepared by PIC are similar to those observed in the PIT emulsification at constant temperature. It also took place by changing composition during the emulsification process [93]. Example of emulsifier used in this system are a combination of sorbitan momooleate (Span 80) with a low hydrophilic-lipophilic balance (HLB =4.3) and sorbitan monooelate ester with a high HLB (HLB =15). As these two emulsifiers are chosen due to possess the same backbone leading to a controlled changed in the final HLB and can mix easily [94].

Preparation of nano-emulsion by using high shear device has advantages over than other methods due to a better control of the droplet size and a large choice of emulsifier can be used [78-79]. Furthermore, emulsions prepared by the PIT emulsification showed extremely fast coalescence rate [78].

2.4   General characteristic of unstable emulsion

The term emulsion stability refers to the ability of emulsion to resist changes in its properties over time [30]. However, an emulsion may become unstable due to many phenomena such as creaming, sedimentation, and syneresis. These phenomena occur from various mechanisms such as flocculation, coalescence, and Ostwald ripening. In this section, we will describe about these phenomena and physical mechanisms.

2.4.1   Phenomena of unstable emulsion

As mention above, an emulsion may become unstable due to many phenomena such as creaming, sedimentation, and syneresis. Creaming and sedimentation are caused by simple gravitational separation. These phenomena were induced by a density difference between dispersed and continuous phases [95], resulting in two layers of dispersion. The droplets have tended to rise or settle according to stokes's law. Creaming describes the upward movement of droplets due to the fact that the dispersed phase has a lower density than the surrounding liquid. Furthermore, it should be mention that the emulsion layer that is more concentrated is the "cream" layer. However, when droplets move upward sometime, the bottom phase shows clear lower serum (or syneresis) due to less of emulsion droplets.

Whereas sedimentation describes the downward movement of droplets due to the fact that the dispersed phase has a higher density than the surrounding liquid and may exhibit clear upper serum at the top of container. Thus, O/W emulsion tend to occur creaming, whereas W/O tend to occur sedimentation [30]. Figure 2.8 shows phenomena of unstable emulsion describing above.
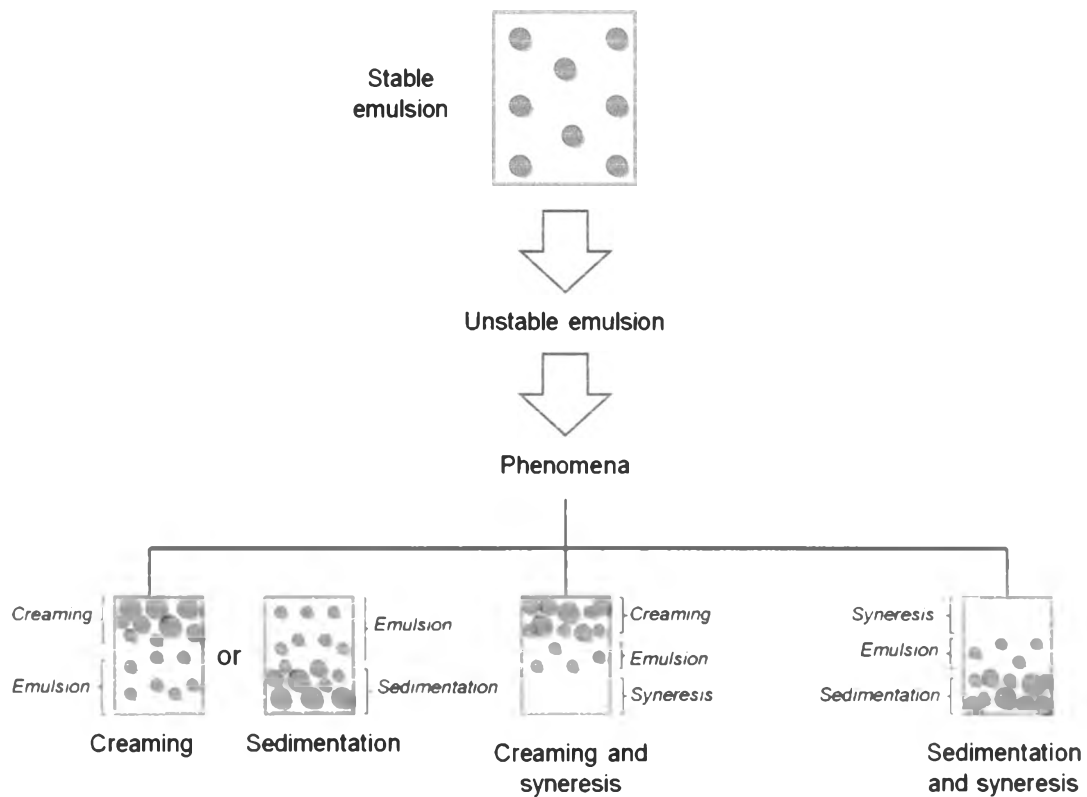
Figure 2.8 Phenomena of unstable emulsion.

The movement of creaming or sedimentation, movement upward or downward, may be considered according to Stokes' law. Stokes' law was used for very small spherical objects dispersed in a continuous viscous fluid. The particle's velocity upward or downward movement of emulsion droplets corresponds to friction force which is given by;

$$F_d = 6\pi\eta RV \tag{2.3}$$

where F_d is the frictional force acting on the interface between the fluid and the particle (N),

η is the fluid's viscosity (kg m^{-1} s^{-1}),

R is the radius of the spherical object (m) and,

V is the particles's velocity (m/s)

If the particles are falling in the fluid due to gravity by their own weight, then terminal velocity, also known as the settling velocity, is reached when this frictional force combined with the buoyant force (opposite force exerted by a fluid) exactly balance the gravitational force. The resulting settling velocity is given by:

$$V_s = \frac{2(\rho_p - \rho_f)gr^2}{9\eta} \tag{2.4}$$

Where V_s is the particles' settling velocity (m/s) (move vertically downwards when $\rho_p > \rho_f$, or move upwards when $\rho_f > \rho_p$),

g is the gravitational acceleration (m/s^2),

ρ_p is the mass density of the particles (kg/m^3), and

ρ_f is the mass density of the fluid (kg/m^3)

However, it should be noted that creaming or sedimentation are complicated when the droplets are electrically charged because the smaller counter-ion sediments at a slower rate than the colloidal species. Moreover, Stoke's law fails to apply well for particles or droplets size larger than 180 μm [36].

Furthermore, creaming process which caused from flocculation and coalescence of the emulsion resulting in increased the droplet size [96].

2.4.2 Physical mechanism of unstable emulsion

There are a variety of physicochemical mechanisms which corresponded to the alterations in emulsion properties. These mechanisms are the main causes of emulsion instability. The most common physical mechanisms are shown schematically in Figure 2.9. These physical mechanisms can be categorized into 2 types which are reversible process and irreversible process. Reversible physical

mechanisms consist of flocculation. While, irreversible physical mechanisms consist of coalescence and Ostwald ripening.

Physical mechanism of unstable emulsion
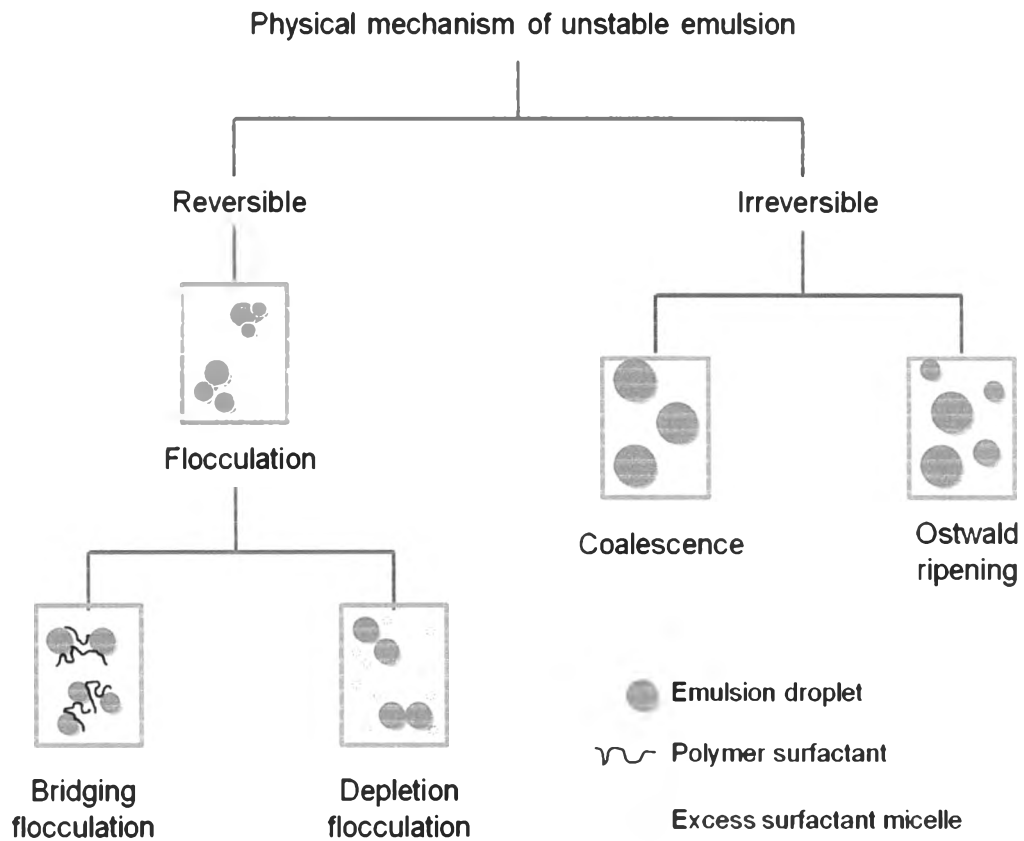
Figure 2.9 Physical mechanisms caused to destabilization of emulsions.

2.4.2.1 Reversible process

❖ Flocculation

Flocculation occurs from any of Brownian motion, sedimentation, or stirring , resulting in two or more dispersed species clumping together, possibly touching at some points without any change in total surface area. Therefore, the dispersed species retain their identity but lose their kinetic independence since the

aggregate moves as a single unit. Flocculation can be divided into 2 types, depletion flocculation and bridging flocculation.

- Depletion Flocculation

Depletion flocculation can be induced in surfactant or emulsifier-stabilized emulsion system by the presence of excess surfactant micelles in the aqueous continuous phase and form aggregates [97] which may be called as sub-micelles [98]. Sub-micelles are excluded from the gab between emulsion droplets at a separation less than the micelles diameter, with the resulting osmotic pressure gradient. It is lead to attraction between droplets and refers to as depletion flocculation [99]. Figure 2.10 illustrates mechanism of depletion flocculation that mention above.
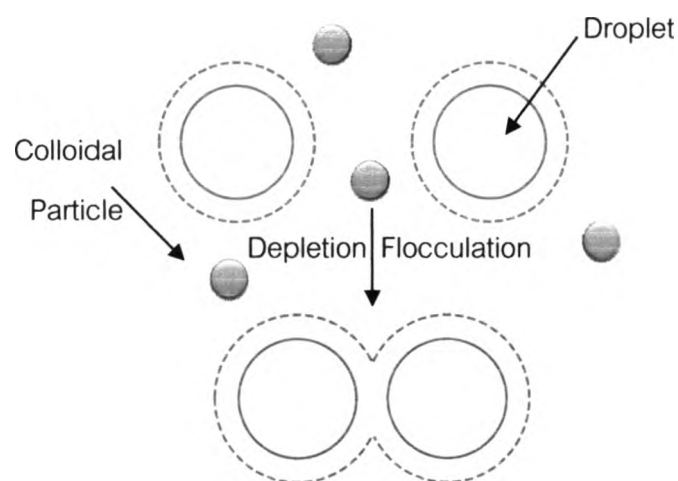
Figure 2.10 An attractive depletion interaction between emulsion droplets and surrounded by small non-adsorbing colloidal particles [30].

E. Dickinson and M. Golding [98] studied influence of unadsorbed sodium caseinate on the stability of O/W emulsion. The depletion flocculation has been provided by creaming profile, changed in apparent viscosity of emulsion when applied shear stress. The creaming profile of 2%w/w sodium caseinate-stabilized emulsion

showed cream layer around 20 mm after keeping emulsion for 30 days. However, the creaming profile for the emulsion containing 4%w/v sodium caseinate-stabilized emulsion has reach 160 mm after keeping emulsion for 30 days due to more extensive flocculation at the higher protein concentration. Furthermore, the change in apparent viscosity of the emulsion containing 2 and 4%w/v sodium caseinate showed newtonian and shear-thinning behaviour, respectively. They were observed distinct pseudoplastic behaviour due to flocculate emulsion droplet at the high protein concentration (4%w/v). Further evidence of the dependence of the state of flocculation was affirmed by light microscopy. At a protein concentration 3.2 %w/w, there was significant flocculation of the emulsion with the formation of a continuous network clearly visible (Figure 2.11). These results allowed to postulate that excess protein formed sub-micelles and saturated within emulsion droplets. Depletion flocculation will be induced due to exclusion of sub-micelles from the gap between adjacent droplets.


Figure 2.11 Light micrograph of flocculated O/W emulsion (10%v/v oil, pH 6.8) containing 3.2% w/w caseinate. Bar denotes a length of 20 μm [98].

● Bridging Flocculation

Bridging flocculation may occur in emulsion system in two ways [67]: First, the emulsion system emulsified by using polymeric surfactant which contained functional groups in various sites on the same molecule. Those polymeric surfactants will be adsorbed on the surface and exposed their functional groups on the surface of the droplets. The polymeric surfactants on the adjacent droplets (with two or more droplets) may bind together in a loose arrangement.

Second, surfactant molecules are adsorbed onto droplet and extended chain into the liquid phase. These extended portions are capable to interact with neighbor droplet resulting in bridging by interaction of the extended portion attached to each droplet. This type of bridging frequently occurred with long polymeric surfactant molecule. Furthermore polymer chains easily stretch out at the low polymer concentration resulting in increased opportunities to occur bridging of the particles [100].

Figure 2.12 shows illustrations of bridging flocculation, steric stabilization and depletion flocculation.

|	Attraction	Repulsion
	Bridging flocculation	**Steric stabilization**
Adsorbing polymer		
Nonadsorbing polymer	**Depletion flocculation**	
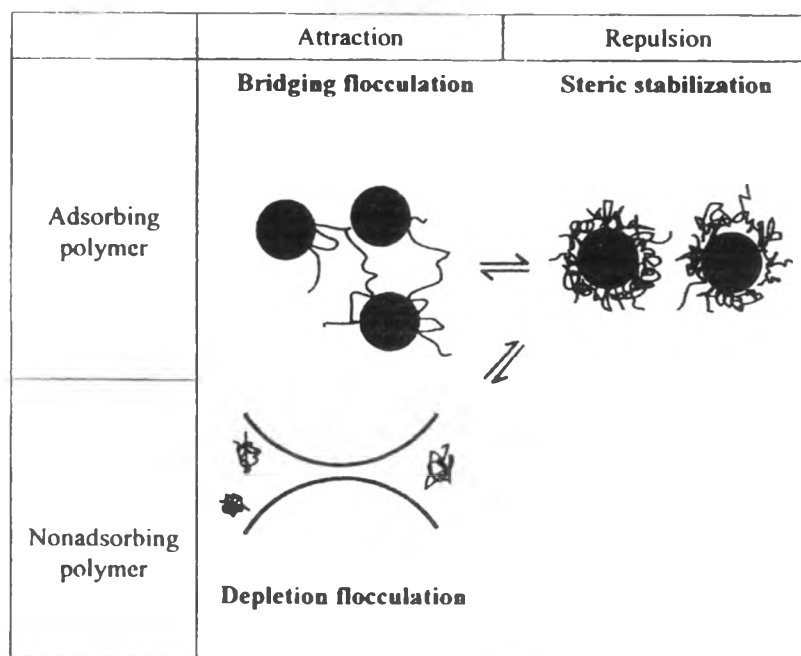
Figure 2.12 Illustration of bridging flocculation (left) and steric stabilization (right) due to adsorbed polymer molecules, and depletion flocculation (bottom) due to non-adsorbed polymer molecules [101].

The flocculation of emulsion can be proved by investigation of emulsion viscosity or shear stress under applied various shear rates. The emulsion system with low droplet-droplet interaction for each individual emulsion droplets (non-flocculation) would exhibit Newtonian behaviors. In contrast, the shear-thinning behavior of emulsions could be attributed to the formation of clusters or flocculation [102-103].

The shear-thinning behavior of droplet flocculation can be explained as follows; when apply low shear rate to the emulsion, shear forces may not high enough to disrupt flocs, resulting in constant viscosity. As increasing shear rate, the shear forces could high enough to deform and disrupt flocs. The elongation and alignment of emulsifier under the shear field cause of the deformation of flocs, resulting in decrease in viscosity (Figure 2.13)
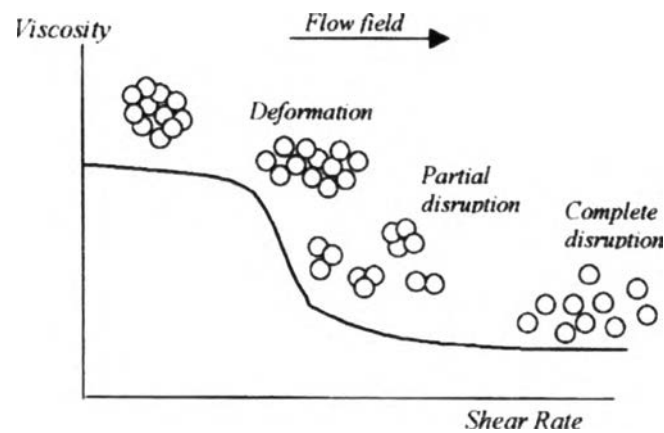
Figure 2.13 Emulsion containing flocculated droplets exhibits shear thinning behavior because the flocs are deformed and disrupted under increasing shear rate [30].

Rheological property of emulsions in term of shear stress (τ), which was a function of shear rate ( ), co $\dot{\gamma}$ be described by the power law equation.

$$\tau = m\dot{\gamma}^{n} \tag{2.5}$$

Where τ is the shear stress, $\dot{\gamma}$ is shear rate, m and n are constants of consistency index and power index, respectively. The consistency index represents the viscosity of the system. The power index represents fluid behaviors. The power index equals to 1 indicates the Newtonian behavior or that of less than 1 indicates the shear thinning behavior [32].

2.4.2.2 Irreversible process

❖ Coalescence

Coalescence can occur when droplets close with each other due to Brownian motion and van der Waals attraction [Sing, A.J.F., Gracia, A., Lachaise, J., 1999]. Furthermore, thin film of emulsifier between the droplets distorts, forms a flat lamella and the liquid inside drains, leading to rupture of separating film. The original species lose their identity and become part of a new species that droplets are fuse

together to form a single larger unit, reducing the total surface area [104]. Figure 2.14 shows coalescence mechanism.
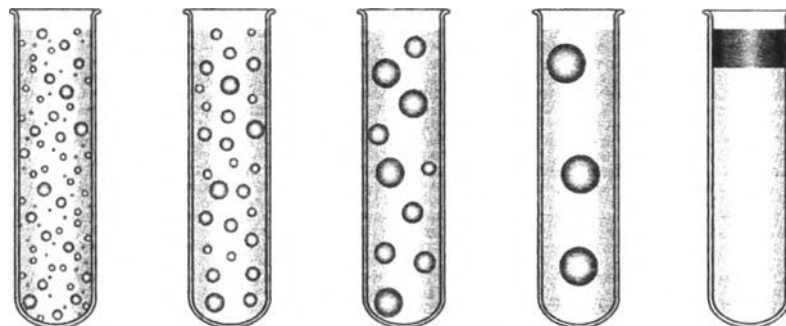
Figure 2.14 Droplet coalescence leads to a growth in the mean droplet diameter and may eventually lead to complete separation of the oil and aqueous phase [30].

Sing and co-workers [76] reported that the coalescence rate of emulsion decreases when the molecular weight of surfactant increases due to a high steric repulsive potential of the surfactant.

Furthermore, Barad and co-workers [96] produced O/W emulsion using sodium dodecyl sulphate as emulsifier with various concentrations: 0.5%, 1.0%, 1.5%, 2.0 and 2.5% w/v. It was founded that an emulsion containing low surfactant concentration (0.5% w/v) was not stabled and occurred phase separation in short period of time. While, the mean droplet diameter declines from around 20 nm to 15 nm as the surfactant concentration increased from 1.0 up to 2.5% w/v, including no change in mean droplet diameter and droplet size distribution. It was attributed that the surfactant concentration in the membrane phase significantly affected the emulsion stability. It may be because high surfactant concentration increased interfacial surfactant film strength and enhanced resistance to coalescence.

❖ Ostwald ripening or molecular diffusion

Ostwald ripening is the main mechanism for nano-emulsion destabilization [61,69]. Ostwald ripening occurs from the different pressure of internal phase within the small and big droplets contained in the emulsion. As a result, internal phase tends to diffuse from small droplets into big one leading to a progressive displacement of the droplet size distribution up to higher average sizes [105].

Ostwald ripening rates,ω, were determined according to the Lifshitz-Slezov and Wagner (LSW) [106-107] theory. The theory predicts a linear relationship between the cube of droplet radius, r^3, and time, t, with the slope being the Ostwald ripening rate follow;

$$\omega = \frac{dr^3}{dt}$$

(2.6)

The cube of radius can be determined from;

$$r^3 = \frac{8}{9}\left[\frac{C(\infty)\gamma V_m D}{\rho RT}\right]t$$

(2.7)

So, from equation 2.6, we will obtain

$$\omega = \frac{dr^3}{dt} = \frac{8C(\infty)\gamma V_m D}{9\rho RT}$$

(2.8)

Where $C(\infty)$ is the bulk phase solubility (the solubility of an infinite large droplet), γ is the interfacial tension, V_m is the molar volume of the oil, D is the diffusion coefficient of the dispersed in the continuous phase, ρ is the density of the oil, R is the gas constant, and T is the absolute temperature.

It should be noted that, the LSW theory assumes that the droplet of dispersed phase are spherical and the distance between droplets is higher than droplet diameter [78].

Furthermore, some research was reported that   stwald ripening and depletion flocculation were two major mechanisms to induce instability of emulsion [79].

2.5   Interaction between the droplets

Several kinds of forces involving in emulsion stability are mentioned below.

2.5.1   Electrostatic repulsion

Ionic emulsifiers (cationic or anionic) would get involved in electrostatic forces.   In aqueous solution, an ionic emulsifier, such as sodium lauryl sulfate, is ionized into two components: the anionic portion (e.g. lauryl sulfate) and the cationic portion (e.g. sodium). For o/w emulsion, the hydrophobic tails of the emulsifier are dissolved in the oil droplet leaving the ionized heads on the emulsion droplet surface. This molecular orientation results in a net negative charge on the surface of the droplets. This surface charge leads to the formation of a secondary shell of dissolved counter ions which is known as "electrical double layers". The oil droplet is prevented from coalescing by their repulsive forces of electrical layers when the droplet surface possesses the same charges.

The surface charge of droplets can be determined by zeta potential which is the potential at the slipping plane. The general dividing line between stable and unstable emulsion with zeta potential is taken as +30 mV or -30 mV that droplets possess zeta potentials outside these range normally considered as stable emulsion [108]. An expended set of guidelines, develop for particles suspensions, emulsion was given in Table 2.3. It should be noted that similar trend would also be observed for positive zeta potential.

**Table 2.3** Stability criteria based on zeta potential [109].

Stability characteristic	Zeta potential (mV)
Maximum agglomeration and precipitation	+3 to zero
Excellent agglomeration and precipitation	-1 to -4
Fair agglomeration and precipitation	-5 to -10
Agglomeration threshold (agglomerates of 2-10 particles)	-11 to -20
Plateau of slight stability (few agglomerates)	-21 to -30
Moderate stability (no agglomerates)	-31 to -40
Good stability	-41 to -50
Very good to stability	-51 to -60
Excellent stability	-61 to -80
Maximum stability – for solids	-81 to -100
- for emulsions	-81 to -125

However, the double layers repulsion is screened when in system possess high salt concentration resulting in colloid particles eventually coagulate [110].

2.5.2 Steric repulsion

At high polymer-dispersed species ratio, long-chain surfactant or high molecular weight emulsifier or surfactants adsorbed at the surfaces of droplet in form of loops, trains, or tails configuration. If the adsorbed polymer extends from droplet surface, then the bristles on a hair brush preclude two brushes pushed together resulting in

prevent the oil droplets from coalescence (Figure 2.15). This is known as steric repulsion or steric stabilization [36].
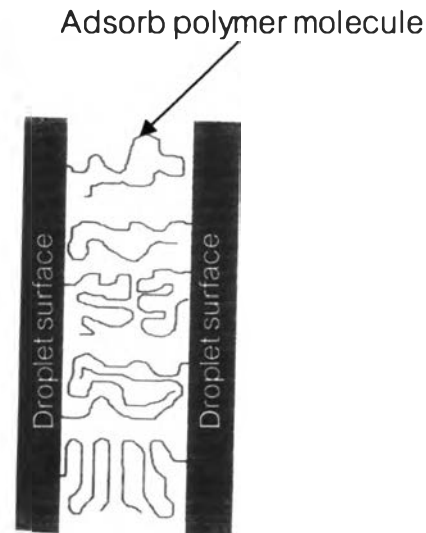
Figure 2.15 Illustration of volume-restriction steric repulsion between droplets by adsorbed polymer molecules [36].

However, it should be noted that droplet surfaces should be fully covered by the adsorbed emulsifier for effective stabilization, otherwise uncovered regions of adjacent droplets may come into contact with each other, or bridging flocculation between droplets may occur.

The thickness of polymer chain, stabilized droplet or micelle through steric, is determined in term of hydrodynamic thickness or hydrodynamic radius. The hydrodynamic radius (R_H) is calculated from the diffusion coefficient (D) using the Stokes-Einstein equation follow [110];

$$D = \frac{kT}{6\pi\eta R_H}$$

Where k is the Boltzman constant, T is the absolute temperature, η is the viscosity of the suspension medium.

Moreover, environmental condition has effective to steric stabilization. Horne and Davidson [110] studied effect of environmental condition in the diluting buffer on hydrodynamic thickness in bovine milk proteins. Bovine milk protein consists of casein, a group of phosphoproteins. It demonstrated that the hydrodynamic radius of casein micelles gradually decreased in size from 112 to 97 nm with increased ethanol content in the diluting buffer from 5-20%v/v. It was because in the poor solvent in aqueous alcohol mixture, the protein chain providing the steric stabilization through hydrodynamic radius in the casein micelle. The system has been forced to collapse leading to instability.

Electrostatic (ionic repulsion) and steric mechanism are two main mechanisms to stabilize droplet [111]. It is also possible that emulsion droplets was stabilized both electrostatic and steric stabilization; these are said to be electrosterically stabilization [36]. Electrostatic and steric are stabilized emulsion through repulsive force. This stabilized mechanisms help to prevent or slow down the destabilization of emulsion such as flocculation, and coalescence [112].

Nevertheless, each type of stabilization has its own advantages and disadvantages as shown in Table 2.4.

Table 2.4 Comparison of the advantages and disadvantages of electrostatic and steric mechanism to stabilize emulsion [adapted from 113].

Polymeric steric stabilization	Electrostatic stabilization
Insensitive to pH	pH-dependent; aggregation tends to occur when emulsifier loses its charges
Insensitive to electrolyte	Aggregation tends to occur at high electrolyte concentration (> critical flocculation concentration)
Large amounts of emulsifier needed to cover droplet surface	Small amounts of emulsifier needed to cover droplet surface
Weak flocculation (easily reversible)	Strong flocculation (often irreversible)
Good freeze-thaw stability	Poor freeze-thaw stability

2.5.3   Van der Waals attractions [30]

Van der Waals interactions act between all type of colloidal particles, and always be considered when calculating the overall interaction potential between emulsion droplets [31]. Furthermore, van der Waals interactions are always attractive force leading to association of emulsion droplets with each other in the absence of any other interaction. Van der Waals interactions can be divided into three categories as follows (Figure 2.16).
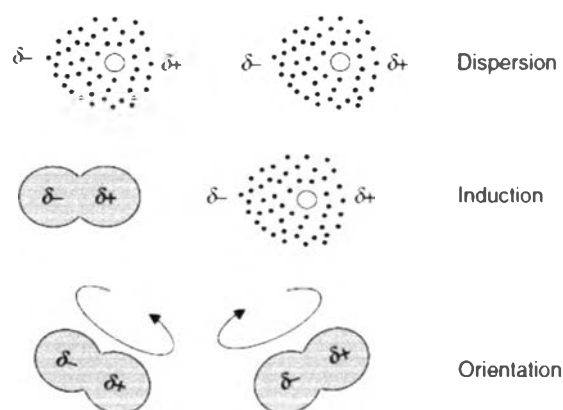
Figure 2.16 Schematic representation of three categories of Van der Waals intermolecular interactions [30].

2.5.3.1 Dispersion forces

This force occurs from their interaction between a temporary dipole and a dipole induced in the neighboring droplets by the presence of temporary dipole. For a moment that an uneven distribution of the negatively charged electrons around positively charged nucleus and then temporary dipole is formed. This temporary dipole produces an electrical field that induces a dipole in a neighboring molecule. Consequently, there is an instantaneous attractive force between the two dipoles.

2.5.3.2 Induction force

This force occurs from the interaction between a permanent dipole and a dipole induced in a neighboring droplet by the presence of the permanent dipole. A permanent dipole causes an alteration in the distribution of electrons of a neighboring molecule that leads to the formation of an induced dipole.

2.5.3.3 Orientation force

This force occurs from the interaction between two permanent dipoles that are continuously rotating. On average each individual rotating dipole has no net charge, but there is still a weak attractive force between different dipoles because the

movement of one dipole induces some correlation in the movement of a neighboring dipole. When the interaction between the two dipoles is strong enough to cause them to be permanently aligned, this contribution is replaced by the electrostatic dipole-dipole interaction

The overall intermolecular pair potential due to van der Waals interactions (W_{VDW}) is given by

$$W_{VDW} = \frac{-(C_{disp} + C_{ind} + C_{orient})}{(4\pi\varepsilon_o\varepsilon_R)^2 S^6}$$

Where C_{disp}, C_{ind}, C_{orient} are positive constants that depend on the dispersion, induction, and orientation contributions, respectively. ε_o is the dielectric constant of a vacuum (8.85×10^{-12} $C^2J^{-1}m^{-1}$), ε_R is the relative dielectric constant of the intervening medium, S is the center-to-center distance between the charges [114].

However, van der Waals interactions are considerably weaker than electrostatic interactions. For this reason, they are almost important in determining interactions between nonpolar molecules, where electrostatic interactions do not make a significant contribution. Furthermore, it should be noted that van der Waals interaction becomes increase as the droplet size increases and the strength of the interaction decreases as the concentration of electrolyte in O/W emulsion increases due to electrostatic screening.

2.5.4 Hydration interactions

Ever since 1970s, scientists have been trying to investigate the cause of repulsive force occurring between different electrostatic charged molecules, for example DNA and biochemical species, when they closed to each other in aqueous system. This force knows as hydration interactions [115]. They occur from the structuring of water molecules around dipolar and ionic surface groups (or the "hydration" of surfaces) [30] of emulsion droplets but it does not occur in emulsion using

nonionic as emulsifier due to nonionic emulsifier do not usually bind highly hydrated ions[30].

Generally, when the electrolyte concentration is adequately high to screen the repulsion force generated by the surface charge, most colloids coagulate. However, if the hydration forces are strong enough, it will prevent the coagulation of colloids [116].

It should be noted that hydration interactions are dependent on the pH of aqueous phase because the electrolyte ions have to compete with the H^+ or OH ions in water [117]. For example, at relatively high pH and electrolyte concentration (>10 mM), it has been observed that Na^+ ions can adsorbed to negatively charged surface groups and prevent droplets from aggregation through hydration repulsion. However, the droplets aggregate when the pH of the solution is decreased because the high concentration of H^+ ions displace the Na^+ ions from the droplet surface [31].

2.6 Characterization technique for emulsion

To evaluate the stability of emulsion, surface charge of droplet was determined by zeta potential measurement, droplet size and droplet size distribution was determined by dynamic light scattering technique. The position of PCTS-labeled with fluorescein isocyanate dye though was observed by confocal laser scanning technique in order to determine the physical mechanisms for describing the unstable emulsion. These techniques were described in detail in this section.

2.6.1 Zeta potential measurement

Zeta potential measurement is sometimes called as a "charge" measurement. Zeta potential used to evaluate the charge stability of disperse system [118-119] The zeta potential is the potential of charge at slipping plane. In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle [119]. It should be noted that the charge at slipping plane will be very sensitive to the concentration and type of ions

in solution [120]. Schematic representation of zeta potential which determined at slipping plane (an outer surface of electrical double layer) is illustrated in Figure 2.17.
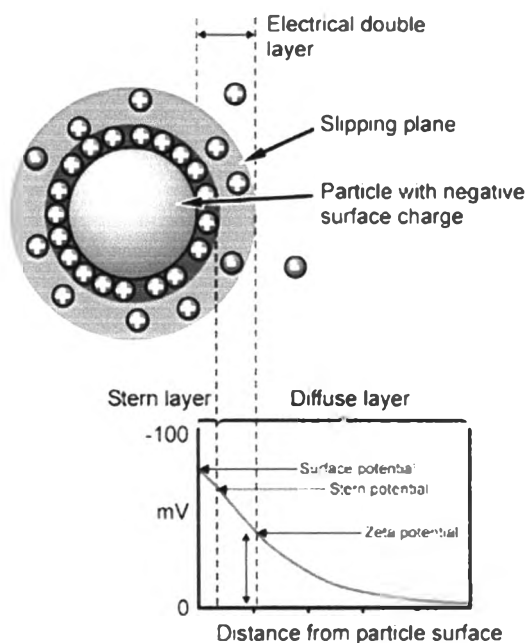
Figure 2.17 Schematic representation of zeta potential which determined at outer surface of electrical double layer. This figure shows for particle contained negative charge [120].

Zeta potential is determined by applying an electric field to the dispersion and measuring the velocity of the moving particles. This moving velocity of the particles is related to the electrical field strength and to the particles' electric potential at the boundary between the moving particle and liquid. This velocity is measured using the technique of laser Doppler anemometry. The frequency shift or phase shift of an incident laser beam caused by these moving particles is measured as the particle mobility. This mobility is converted to the zeta potential by inputting the dispersant viscosity and calculating according to the Smoluchowski or Huckel theories [118].

In this work, zeta potential was used to determine the surface charge of emulsion droplets with using various degree of phosphate substitution of PCTS as

emulsifiers. Those various kinds of emulsion droplets were expected to show different in surface charge.

2.6.2 Dynamic light scattering

One technique widely used to measure the emulsion droplet size and size distribution was dynamic light scattering technique (which is also known as photon correlation spectroscopy). In this technique, the intensity fluctuation of scattered light was measured by the droplet as they undergo Brownian motion [121]. When the light hits the moving particles, the wavelength of the incoming light was changed. This change is related to the size of particle. It is possible to compute the sphere size distribution and give a description of the particles' motion in the medium, measuring the diffusion coefficient of the particle and using the autocorrelation function.

However, the droplets in emulsion are not perfectly mono-disperse (usually with a narrow size distribution), so the light scattering results are analyzed as a polydispersity index that gives information on the deviation from the average size.

In this work, dynamic light scattering was used to investigate the emulsion droplet size and size distribution. Emulsion droplet size and size distribution are the main factors for considering the stability of emulsion.

2.6.3 Confocal Laser Scanning Microscopy (CLSM) [122]

Confocal laser is a technique for obtained high-resolution optical images or out-of-blur images with depth selectivity [123]. Furthermore, the key feature of CLSM is its ability to acquire in-focus images from selected depth, a process known as optical sectioning.

The confocal principle in fluorescence laser scanning microscopy is diagrammatically presented in Figure 2.18. Coherent light emitted by the laser system (excitation source) passes through a pinhole aperture that is situated in a conjugate plane (confocal) with a scanning point on the specimen and a second pinhole aperture positioned in front of the detector (a photomultiplier tube). As the laser is reflected by a dichromatic mirror and scanned across the specimen in a defined focal plane,

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secondary fluorescence emitted from points on the specimen (in the same focal plane) pass back through the dichromatic mirror and are focused as a confocal point at the detector pinhole aperture.
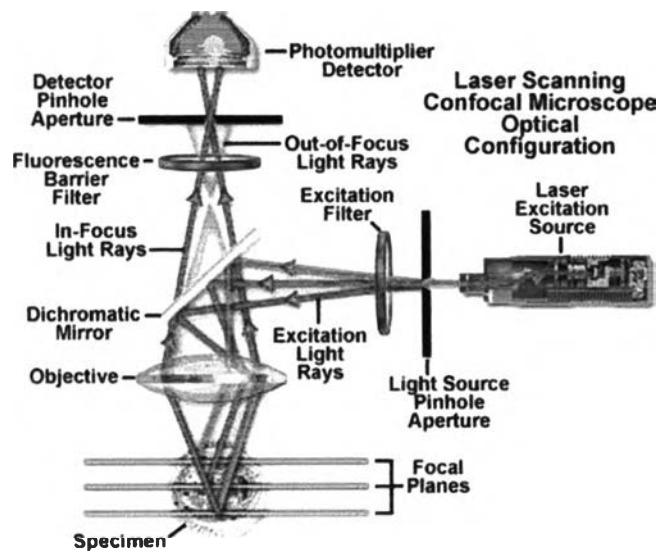

Figure 2.18 Schematic drawing of CLSM device [122].

The significant amount of fluorescence emission that occurs at points above and below the objective focal plane is not confocal with the pinhole (termed Out-of-Focus Light Rays in Figure 2.18) and forms extended Airy disks in the aperture plane. Because only a small fraction of the out-of-focus fluorescence emission is delivered through the pinhole aperture, most of this extraneous light is not detected by the photomultiplier and does not contribute to the resulting image. The dichromatic mirror, barrier filter, and excitation filter perform similar functions to identical components in a widefield epi-fluorescence microscope. Refocusing the objective in a confocal microscope shifts the excitation and emission points on a specimen to a new plane that becomes confocal with the pinhole apertures of the light source and detector.

In traditional widefield epi-fluorescence microscopy, the entire specimen is subjected to intense illumination from an incoherent mercury or xenon arc-

discharge lamp, and the resulting image of secondary fluorescence emission can be viewed directly in the eyepieces or projected onto the surface of an electronic array detector or traditional film plane. In contrast to this simple concept, the mechanism of image formation in a confocal microscope is fundamentally different. As discussed above, the confocal fluorescence microscope consists of multiple laser excitation sources, a scan head with optical and electronic components, electronic detectors (usually photomultipliers), and a computer for acquisition, processing, analysis, and display of images.

In this work, the confocal laser microscopy is used to observe the position of PCTS-labeled with fluorescein in order to determine the physical mechanisms for describing the unstable emulsion. Those physical mechanisms consist of coalescence, fluctuation, Ostwald ripening.