

Effect of Electrolytes on the Formation and Stability of n-Dodecane Nanoemulsions by the Phase Inversion Temperature (PIT) Method

By

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Dedicated to my beloved father

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17/9/1951 – 20/7/2008

DECLARATION

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ABSTRACT

This research focuses on the effect of sodium chloride (NaCl) or calcium chloride (CaCl₂) on the formation and stability of n-dodecane/non-ionic surfactant/aqueous nanoemulsions produced by the Phase Inversion Temperature (PIT) method. It is because there are only few works done to investigate the effect of electrolyte on the production of nanoemulsions, especially those produced by the PIT method. Furthermore, in this research, the ability for ageing nanoemulsions to reverse their physical properties to freshly-prepared state has also been investigated..

The nanoemulsions were produced by heating aqueous-continuous emulsions (O/W) to oil-continuous (W/O) emulsions, followed by a quenching process to produce O/W nanoemulsions. Pure milliQ water and concentration from 0.001M to 0.1M NaCl or CaCl₂ were used as continuous phase. The non-ionic surfactants used were polyoxyethylene (4) lauryl ether (Brij30) and sorbitan monooleate (S80). The stability was determined by dynamic light scattering technique by measuring the growth of the droplet size and size distribution (PDI) before the nanoemulsions were phase separated, which was determined by visual observation. The reversibility testing was done by measuring the droplet size and PDI as a function of temperatures for a three-day ageing nanoemulsion system.

Droplet size as small as 65nm was produced from a brine system while there was 77nm in a pure milliQ water system, with PDI lower than 0.2. The PIT temperature was found to be depressed when: (1) oil or surfactant concentration increased; (2) HLB number of surfactant system decreased; and (3) NaCl or CaCl₂ was added. The transitional temperature (ΔT_{trans}), a temperature difference between the temperature for producing O/W and W/O emulsions, has linked with the stability of the nanoemulsions. The largest ΔT_{trans} in a emulsion system with low oil concentration (R=0.2) appeared at 7wt% Brij30 at pure milliQ water system but at 6wt% Brij30 at NaCl system, showing that the surfactant concentration used to produce the most stable nanoemulsions was reduced by the aid of compression effect from NaCl. When the oil concentration increased, more NaCl was needed to produce stable nanoemulsions. Nanoemulsions produced by a mixture of hydrophilic and hydrophobic non-ionic surfactant was extremely unstable, with a big difference in PIT temperature and ΔT_{trans} compared to a pure surfactant system. It was found that NaCl was a better electrolyte than CaCl₂ to produce nanoemulsions with smaller droplet sizes and PDI and higher stability. For the most stable nanoemulsions, 20°C was better than 10°C, to keep the sample from phase separation for more than 30 days. Additionally, the addition of CaCl₂ was found to have no difference in the production of nanoemulsions by adding it either before or after the emulsification process.

It has been found that only system with NaCl appeared to have the ability to revert the droplet size and PDI, from ageing to freshly-prepared nanoemulsions. Furthermore, the reversibility ability was governed by phase inversion process as only system experiencing heating process could be reverted. However, nanoemulsions with high stability were partially reversible, where only droplet size was reversible.

The positive effects (increasing stability, smaller droplet size and PDI, and reversibility ability) from the addition of NaCl could only be achieved at certain electrolyte and surfactant concentrations.

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1.0 Introduction

Nanoemulsions, also referred to as miniemulsions, sub-micron emulsions, and unstable microemulsions (Sadurni *et al.*, 2005), which contain two immiscible liquids are an emulsion system with droplet sizes ranging from 20 to 200nm. They are classified between macroemulsions and microemulsions. Unlike microemulsions, nanoemulsions are only kinetically stable but they have long-term physical stability due to their ultra-small droplet sizes (Maestro *et al.*, 2008). Nanoemulsions have a wide-range usage such as in cosmetics, pharmaceuticals, drug delivery, and food industries (Sadurni *et al.*, 2005; Maestro *et al.*, 2008).

As nanoemulsions are non-equilibrium systems, energy is required for their production. There are low- and high-energy methods to produce nanoemulsions. High energy methods make use of mechanical devices and low energy methods make use of the physicochemical property of an emulsion system. By low-energy method, nanoemulsions are produced as a result of phase inversion, which can be either done by constant temperature (emulsion inversion point (EIP) method) or by applying phase inversion temperature (PIT) concept (Shinoda and Saito, 1968; Izquierdo *et al.*, 2002; Maestro *et al.*, 2008). By EIP method, low interfacial tension can be created, which is critical to produce nanoemulsions, by changing the curvature of a surfactant layer from positive to negative or vice versa. The change of the curvature can be achieved by a step-wise increasing in the volume fraction of continuous phase at constant temperature. PIT method has received more interest during the past few years due to its low energy consumption and low surfactant usage. Therefore, this method has been used in this research to produce aqueous-continuous (O/W) nanoemulsions. By the PIT method, the O/W nanoemulsions can be produced by heating the system followed by a quenching process, to create an extreme low interfacial tension (at the PIT temperature) and promote the formation of emulsions with ultra small droplet size.

The addition of electrolyte (salt) to stabilize emulsions is a common practice in emulsion industries, especially in the cosmetic, pharmaceutical and food industries. In the formation of emulsions, the addition of electrolyte will depress the PIT temperature and phase inversion will occur at a crucial electrolyte concentration when the system is at a constant temperature (Shinoda and Takeda, 1970; Wasan *et al.*, 1988; Anton *et al.*, 2007). For O/W emulsions with droplets larger than 1 μ m, coalescence will be enhanced by the addition of electrolyte (Baloch and Hameed, 2005), however, it has been reported that there is an optimum salt concentration to produce the most stable emulsions (Binks *et al.*, 2000). For the nano-sized O/W emulsions produced by high energy methods, the addition of electrolyte has been found to have no effect on droplet size but enhance the stability against creaming (Srinivasan *et al.*, 2000; Morais *et al.*, 2006; Martinez *et al.*, 2007). The improved stability is a result of the increasing interdroplet interaction and the increasing viscosity in the continuous medium (Morais *et al.*, 2006). To date, few studies have been conducted to investigate the effect of electrolyte on the formation and stability of O/W nanoemulsions, especially those produced by the PIT method. Therefore, the present research is important. In this research, sodium chloride (NaCl) and calcium chloride (CaCl₂) were chosen as they are the most common salt to be added into the production of emulsions.

Nanoemulsions produced by the PIT method are very sensitive to temperature (Ee *et al.*, 2007). They need to be handled at their optimum temperature in order to prolong the shelving life. However, it is very hard to store and deliver nanoemulsions at their optimum state for practical purposes. If an ageing or destabilised nanoemulsion system can be reverted back to its freshly-prepared state in terms of the droplet size and the size distribution, it may be said that the nanoemulsions have the reversibility ability. The reversibility ability plays an important role in nanoemulsion industries as it will allow no restriction on storage and delivery of nanoemulsions. The reversibility ability of different nanoemulsions was investigated in this research.

In this study, n-dodecane/non-ionic surfactant/aqueous emulsion system was chosen. n-Dodecane was chosen as a dispersed phase due to the incomplete understanding about n-dodecane nanoemulsions. The non-ionic surfactant was polyoxyethylene (4) lauryl ether (Brij30) or a mixture of Brij30 and sorbitan monooleate (S80). The aqueous phase was either water or salt water (NaCl and CaCl₂ with different concentrations). It has been found that the production and stability of nanoemulsions are mainly dependent on the concentration of surfactant, type of surfactant and concentration of dispersed phase. Therefore, the effect of the addition of NaCl or CaCl₂ has been investigated along with those factors. The following sets of experiment have been carried out to develop the knowledge of the effect of the addition of NaCl or CaCl₂ on the formation and stability of n-dodecane nanoemulsions produced by the PIT method.

1. Effect of [NaCl]¹ in fixed R² and [Brij30]³;
2. Effect of [NaCl] in different [Brij30] but fixed R;
3. Effect of [NaCl] in different R but fixed R_{so}⁴ and with Brij30 as surfactant;
4. Effect of [NaCl] in a mixture of surfactants (Brij30 and S80) at fixed R and total surfactant concentration;
5. Effect of different electrolyte (CaCl₂ and NaCl);

An optimum surfactant concentration to produce the stable n-dodecane nanoemulsions (with R=0.2) in pure milliQ water and brine systems was found from Experiment 2. In Experiment 3, 4 and 5, only systems with the optimum surfactant concentration were investigated by changing certain conditions (which have been shown above). In addition to the experiments above, a phase diagram for 0.01M NaCl/ 6wt% Brij30/ n-dodecane with R=0.2 was determined, and effects of different

¹ Sodium chloride concentration

$$^2 R = \frac{\text{oil}}{\text{oil} + \text{aqueous}} (\text{wt}\%)$$

³ Surfactant concentration

⁴ Surfactant-to-oil ratio (weight)

quality of water, rate of heating, different dilution agents and storage temperatures were investigated for some selected systems in order to provide a better understanding about the effect of NaCl or CaCl₂ in the production of aqueous-continuous nanoemulsions.

The stability of the nanoemulsions was determined by dynamic light scattering technique by measuring their droplet sizes and size distribution as a function of time before the nanoemulsions were phase separated, which was determined by visual observation. The structures of the nanoemulsions were determined by scanning electron microscopy (SEM) and optical microscopy, while the phases involved in the phase inversion process were determined by polarising microscopy.

A literature review is presented in Section 2 which describes the background and the previous works in the areas related to this study. In section 3, the materials used in this study and the reasons why the materials were chosen are discussed. Furthermore, the methods to produce n-dodecane nanoemulsions and to determine the stability of the nanoemulsions are presented in the section. After the necessary background has been developed and the methods to produce the nanoemulsions has been utilised, the results of the formation and stability of the nanoemulsions are described in Section 4. In the section, the results were discussed and compared with the previous works mentioned in Section 2. In addition to the formation and stability of the nanoemulsions, the reversibility ability of the nanoemulsions was also investigated and some interesting results were found. There are 11 sub-sections in Section 4, starting from the investigation of system consistency by heating and cooling a same system, to the investigation of the effect of storage temperature. Within the sub-sections, different results of different factors (eg. electrolyte/surfactant/oil concentration, types of surfactant) in the formation of the nanoemulsions were discussed. After the detailed result presentations and discussions in Section 4, the conclusion about the effect of electrolyte in the formation and stability of n-dodecane nanoemulsions are drawn in Section 5. As this study only provides some preliminary knowledge about the effect and the investigation was done in some limited conditions, some recommendations or future works are suggested in Section 6 for future research.

2.0 Literature Review

Emulsions are a heterogeneous system, which contains at least one immiscible liquid dispersed as globules in the others. The liquid which is finely broken up into globules is called the dispersed or internal phase; whilst the liquid which forms the matrix in which these globules are suspended is known as the continuous or external phase (Clayton, 1923; Becher, 1957). Generally, there are oil-in-water (water as continuous phase, which is abbreviated as O/W) and water-in-oil (oil as continuous phase and is abbreviated as W/O) emulsions. As emulsions are non-equilibrium and tend to be phase separated to achieve the lower interfacial tension, they have minimal stability. To increase the stability (to produce low interfacial tension), a third agent/agents is/are added to an emulsion system which is/are known as emulsifiers or emulsifying agents. Surfactants are commonly used in emulsion industries as emulsifying agents to stabilise emulsions, these are ionic surfactants (eg. sodium dodecyl sulphate (SDS) – anionic surfactant) and non-ionic surfactants (eg. sorbitan monooleate (Span 80)), where the structures of those two surfactants are presented in Figure 2.1.

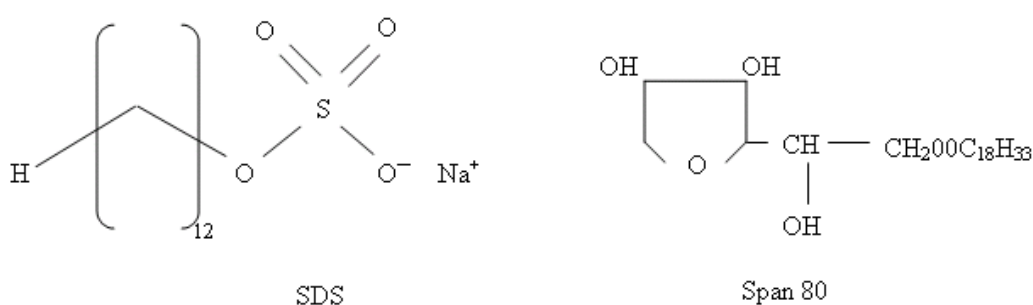


Figure 2.1: The structures of sodium dodecyl sulphate (SDS) and sorbitan monooleate (Span80).

To date, researchers have classified emulsions into three categories: macroemulsions, nanoemulsions and microemulsions, in regard to their droplet sizes and properties (Table 2.1). Among these emulsions, nanoemulsions have been receiving more attention due to the low energy consumption and low surfactant usage. Furthermore, they have higher stability than macroemulsions. Although microemulsions are more stable than nanoemulsions, their high surfactant usage makes them less economic effective in emulsion industry. To produce nanoemulsions, energy is required to create a low interfacial tension system to promote the formation of ultra small-sized droplets. There are generally two methods to supply the energy: high energy methods which make use of the mechanical devices such as high pressure homogenizer and high shear stirring; and low energy methods which make use of the physicochemical property of an emulsion system. By low energy methods, nanoemulsions are produced as a result of phase inversion. Phase inversion is an effect caused by interchanging internal phase to external phase such as O/W emulsions to W/O emulsions or vice versa. Phase inversion in an oil/non-ionic surfactant/water emulsion system can be controlled by several factors: type of oil, surfactant type and concentration, temperature of the system, water to oil ratio, additives in the oil and water phases, mixing condition and rate of order of additions of the different components. Among the factors, the first five factors are based on the change of affinity in surfactant while

the latter two factors are dynamic variables (Brooks *et al.*, 1998). The most common methods to produce nanoemulsions by using phase inversion are by changing the water to oil ratio at a constant temperature or by applying phase inversion temperature (PIT) concept (Shinoda and Saito, 1968; Izquierdo *et al.*, 2002; Maestro *et al.*, 2008), these methods will be further discussed in the following sections.

Table 2.1: Categorisation of emulsions.

	Macroemulsions	Nanoemulsions	Microemulsions
Droplet size	>1 μ m	20-200nm	Nano-size range
Stability	Unstable	Kinetically stable	Thermodynamically stable
Formation	Easy	Low/high energy	Spontaneously
Surfactant concentration	Low	Low	High
Appearance	Milky	Milky/ transparent	Transparent/ translucent

The attraction of nanoemulsions for application in emulsion industries has been well described in pharmaceuticals (Sadurni *et al.*, 2005; Morais *et al.*, 2006; Gutierrez *et al.*, 2008), oil refineries, food industries (Gutierrez *et al.*, 2008), and cosmetics (Bouchemal *et al.*, 2004; Sonnevile-Aubrun *et al.*, 2004; Solans *et al.*, 2005; Gutierrez *et al.*, 2008).

2.1 Nanoemulsions by low energy methods

Due to the energy consumption consideration in producing nanoemulsions, low energy methods are always preferred in emulsion industries. In order to utilise low energy methods, the surfactant shape for the phase behaviour needs to be studied in different systems. When phase behaviour is changed from one to another one, the interfacial tension is changed followed by the change in the curvature of the surfactant monolayer. The change of the interfacial tension is a crucial stage for producing the nanoemulsions by the low energy methods.

The behaviour of a surfactant system with temperatures is shown in Figure 2.2 with an oil/water/non-ionic surfactant system. As the significance of the behaviour of a surfactant system was recognised first by Winsor in 1954, most of the phase behaviours were named after him. The spontaneous curvature of surfactant monolayer in a non-ionic surfactant emulsion system can be easily controlled by varying the temperature. The surfactant need to be assembled in such a way that the bending energy is minimized when oil, water and surfactant are mixed together. At low temperature, the spontaneous curvature is large and positive, micelles are formed in water. The oil is solubilised by the micelles and it separates from the system as second phase if excess oil is added. Winsor I (WI) equilibrium describes the equilibrium of an aqueous micellar solution with excess oil (Figure 2.2(a)). Similarly, the spontaneous curvature is large (in absolute value) and negative at high temperature,

water is solubilised in micelles which are formed in oil. When more water is added, the excess water separates as a second phase and the Windsor II (WII) equilibrium is formed (Figure 2.2(e)).

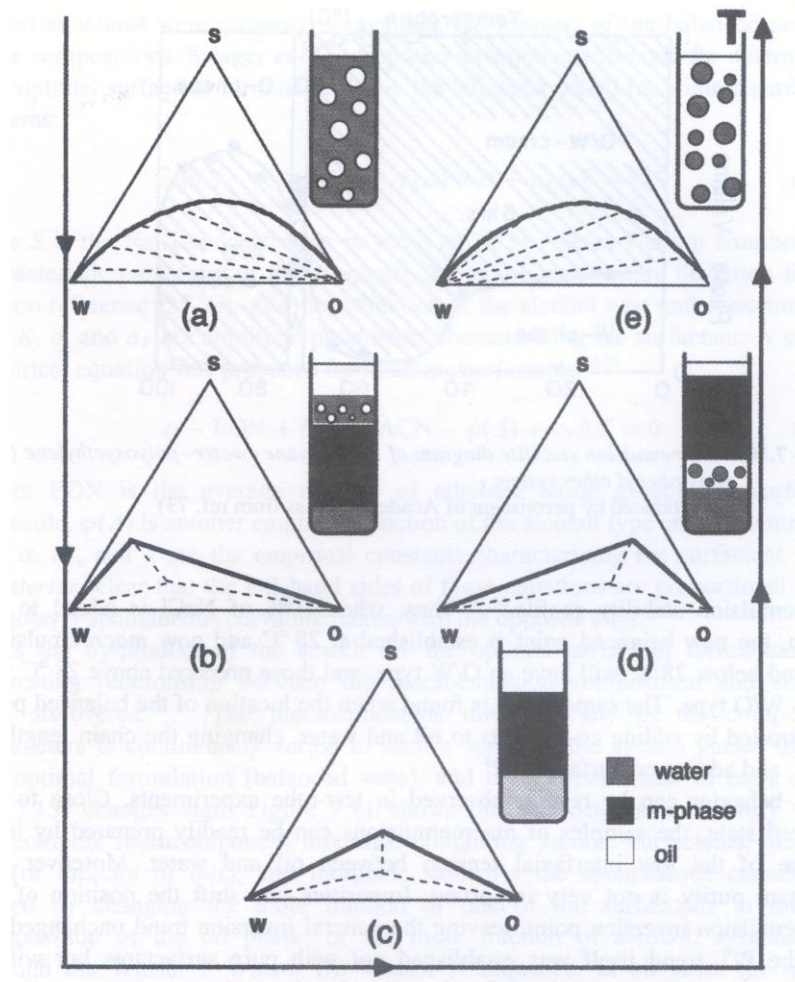


Figure 2.2: Schematic changes in the phase equilibrium of an emulsion system on raising the temperature. As the temperature increases, the emulsions are changed from O/W over the Windsor I region (a) to W/O over the Windsor II region (e), through the Windsor III region (b), (c), and (d) (Kabalnov, 1998).

In between the WI and WII regions, the phase behaviour is complicated and normally consists of „oil“ (upper), „water“ (lower), and „microemulsions (m-phase)“ (middle) phases. This three-phase equilibrium is called Windsor III (WII) equilibrium (Figure 2.2 (b), (c) & (d)). The WIII equilibrium is separated from the WI and WII equilibria by two end-points, in which two of the WIII phases become critical with respect to each other. At lower end point (Figure 2.2(d)), the lower and middle phases are critical to each other, while the upper phase is non-critical to them. At the upper end-point (Figure 2.2(b)), the upper and middle phases are critical and the lower phase is the non-critical „spectator“.

In the WIII state, the structure of the surfactant is intriguing and the „oil“ and „water“ phases represent weak molecular solutions of the surfactant in the solvents. Due to the small spontaneous curvature, the surfactants associate into structures of zero, or low, curvature, which are either lamellar liquid crystals (LLC) or a sponge-like continuous

microemulsion phase (L_3), where the surface has a locally saddle shape. It is believed that LLC phase is favoured for rigid surfactant monolayers while L_3 phase is favoured for flexible monolayers.

As the surfactant curvature is decreased with temperature (from positive to negative), the curvature is close to zero in WIII region. The zero surfactant curvature increases the mutual solubility between oil and water phase by creating a flat-direct continuous phase (LLC or L_3 phase while surfactant monolayer is neither concave to water nor oil). The decrease (or increase) of the curvature of the spontaneous surfactant monolayers is due to the affinity of surfactant to either water (at low temperature) or oil (at high temperature) was changed from one to the other when the hydrophilic tail of a non-ionic surfactant is dehydrated (or dehydrated) by increasing (or decreasing) the temperature.

From WI to WII region, the process for the emulsion system changes from water-continuous to oil-continuous and is called phase inversion process. During the phase inversion process, the system experiences WIII equilibrium. In WIII region, the formation of the LLC and L_3 phases have been discussed by researchers (Kunieda *et al.*, 1996; Anton *et al.*, 2008). In Figure 2.3, the phase inversion process is illustrated from O/W emulsions to LLC phase followed by L_3 phase, when temperature is increased. If the temperature is further increased, oil-continuous emulsions are formed. It can be noticed that LLC is a sandwich-like structure where water is trapped inside the surfactant layers while L_3 phase is a flexible structure and the mobility of water inside the structure is higher than in LLC phase. According Kunieda *et al.* (1996), the LLC phase was suggested be formed before L_3 phase in WIII region, however, a totally different suggestion was made by Anton and co-workers (2008), where L_3 phase was formed before LLC phase in WIII region. This argument has not been clearly justified and so exact phase behaviours involved in WIII region are as yet unclear. The understanding in the change of curvature of surfactant monolayer in the phase inversion process, to create extreme low interfacial tensions, is critical in the production of nanoemulsions by low energy methods.

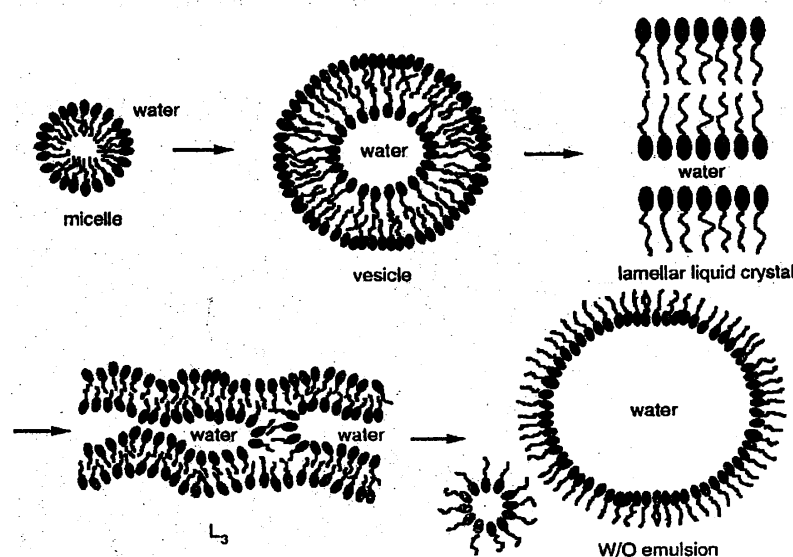


Figure 2.3: Schematic change in spontaneous curvature of surfactant layers in the process of phase inversion from O/W to W/O emulsions (Kunieda *et al.*, 1996)

2.1.1 Emulsion Inversion Point (EIP) Method

Low interfacial tensions, which are needed in the production of O/W nanoemulsions by low energy method, can be achieved by creating a transition in the curvature of surfactant monolayer by changing the water volume fraction. The transition occurred at constant temperature, and the method to produce nanoemulsions by this transition is called emulsion inversion point (EIP) method. In EIP method, water droplets are initially produced in a continuous oil phase by adding water into oil. By increasing the water volume fraction (step-wise addition of water into oil phase), the curvature of surfactant monolayer will change from concave towards water to concave towards oil at the inversion point. A bicontinuous microemulsion is formed at the inversion point for a short-chain surfactant emulsion system. During the transitional process, minimal interfacial tensions are achieved and promote the formation of emulsions with small droplet size (Fernandez *et al.*, 2004). Low energy methods conducted at a constant temperature involve catastrophic phase inversion (CPI), in which the O/W and W/O emulsions are not interchangeable by reversing the conditions.

The formation and stability of nanoemulsions produced by EIP method is highly dependent on the hydrophilic-lipophilic balance (HLB) number of a non-ionic surfactant (Sadurni *et al.*, 2005; Liu *et al.*, 2006). HLB number defines the polarity of a non-ionic surfactant in term of an empirical quantity. At 25°C, non-ionic surfactants are from lipophilic to hydrophilic on a scale of 1 to 20. Table 2.2 shows that the applications of a surfactant based on its HLB number (Brooks *et al.*, 1998). In this research, the HLB number for W/O and O/W emulsifier will be focused on.

Table 2.2: Applications of surfactants based on their HLB number (Brooks *et al.*, 1998)

HLB number range	Application
3-6	W/O emulsifier
7-9	Wetting agent
8-18	O/W emulsifier
13-15	Detergent
15-18	Solubiliser

O/W nanoemulsions produced by EIP method, by either non-ionic or ionic surfactant, have been performed widely by researchers (Forgiarini *et al.*, 2001; Sadurni *et al.*, 2005; Liu *et al.*, 2006; Sole *et al.*, 2006). In order to obtain nanoemulsions, a bicontinuous microemulsion/lamellar liquid crystal phase needs to be achieved in a non-ionic surfactant system (Forgiarini *et al.*, 2001; Sadurni *et al.*, 2005), whilst a cubic liquid crystal phase needs to be achieved in an ionic surfactant system (Sole *et al.*, 2006; Maestro *et al.*, 2008), during the emulsification process. By EIP method, the phase equilibria involved in the production of nanoemulsions is dependent on the HLB number (of surfactant) at a fixed water and oil concentration, because it is a

constant temperature process. An optimum HLB number has been found to produce the smallest and most stable nanoemulsions, and the optimum HLB number decreases with the increase of temperature (Sadurni *et al.*, 2005; Liu *et al.*, 2006). To produce nanoemulsions, a phase equilibrium consisting O/W microemulsions, lamellar liquid crystalline and oil, and a system with low interfacial tensions are essential. However, the kinetics of the emulsification process is the key issue to production of nanoemulsions, with the existing of direct or flat phase (LLC) and without excess oil phase.

The EIP method is also applicable in the production of W/O nanoemulsions by stepwise addition of oil into a mixture of water and surfactant. W/O nanoemulsions was firstly produced by low-energy emulsification method by Uson and co-workers (2004). Generally stable W/O nanoemulsions are produced by a mixed surfactant system with a low HLB number. To date, by using EIP method, a stable W/O nanoemulsions could be produced only with a very low water concentration (maximum 4.5wt% of dispersed phase). The phase equilibria needed to produce the nanoemulsions is multiphase regions with one of the phases being lamellar liquid crystalline phase (Uson *et al.*, 2004).

2.1.2 Phase Inversion Temperature (PIT) Method

PIT is a transitional phase inversion (TPI) process, where the O/W and W/O emulsions are interchangeable by tailoring the temperature of an emulsion system (Sajjadi, 2006). PIT method was first introduced in 1969 (Shinoda and Saito, 1969), since then, it has received more attention in the past few years due to its advantages such as low cost over other high energy methods. The PIT method works on the basis of the changes in the affinity of a polyoxyethylene-type non-ionic surfactant with temperatures (Figure 2.2). The curvature of the surfactant monolayer is more convex towards water at low temperatures but more concave towards water at high temperatures due to the dehydration of hydrophilic tail in a non-ionic surfactant. Therefore, O/W emulsions are preferably formed at low temperatures and W/O emulsions at high temperatures. When the curvature is neither concave nor convex towards water, this is where the PIT temperature existed. The interfacial tension of oil against water in the presence of a non-ionic surfactant is decreased with temperatures and reaches a minimum at a PIT temperature before it increases again if temperatures are further increased (Shinoda and Saito, 1968; Tadros *et al.*, 2004). Therefore, emulsions with very small droplet sizes can be produced when the emulsification process is carried out near the PIT temperature, however, they are very unstable at that temperature (Shinoda and Saito, 1969). Thus, a rapid cooling (quenching) process and with a storage temperature far away from the PIT temperature are needed to produce fine and stable O/W nanoemulsions. The initial droplet size is independent on storage temperature and the PIT temperature as long as the nanoemulsions are produced by the PIT method, however, the stability of those is highly dependent on quenching (Taisne and Cabane, 1998) and storage temperatures (Shinoda and Saito, 1969; Ee *et al.*, 2007). A storage temperature with 20-65°C lower than the PIT temperature is suggested to prolong shelving period of the nanoemulsions (Shinoda and Saito, 1969).

Phase behaviour with continuous phase (D) or continuous phase with excess water (D+W), was found as a critical initial phase for producing nanoemulsions before the quenching process (Morales *et al.*, 2003). Additionally, the mutual solubility between

oil and water phase increases in a sandwich-like structure between surfactant, water and oil layers (Morales *et al.*, 2003) and when WIII formulation conditions are approached (Salager *et al.*, 2002). Therefore, oil phase is required to be completely solubilised in a bicontinuous phase before the quenching process in order to produce O/W nanoemulsions, independently of whether the initial phase equilibria is single or multiphase (Morales *et al.*, 2003). The liquid crystal (lamellar type) is suggested to stabilise emulsions due to the formation of an onion skin multilayer structure between oil and water interface (Marquez *et al.*, 2003). A hysteresis zone, caused by the formation of bicontinuous phase, will create a delay in the phase inversion process, and so to give an incorrect PIT temperature (Marquez *et al.*, 2003). A real PIT temperature must be obtained from the phase equilibrium experiments, which emulsions are conducted in a series of temperatures and well equilibrated in those temperatures. However, a more conventional way to determine the PIT temperature is by measuring the dynamic conductivity of an emulsion system with temperatures, where a high conductivity is obtained at low temperatures (aqueous-continuous) and a low conductivity at high temperatures (oil-continuous). Dynamic conductivity is the emulsion conductivity measured under a stirring condition where the system is considered not in equilibrium state when the measurement is taken (Anton *et al.*, 2008). PIT temperature is taken as an average temperature between the temperatures at highest and lowest conductivity (Izquierdo *et al.*, 2002). It has been found that the PIT temperature is larger than the real PIT temperature in a heating process but smaller in a cooling process. Furthermore, the delay is larger when the heating rate is faster or the cooling rate is slower. However, the hysteresis zone was found to be disappeared with the addition of an alcohol, and so the real PIT temperature can be determined (Marquez *et al.*, 2003). The PIT temperature and initial droplet size are nearly independent in surfactant concentration when the initial phase equilibria is either D or D+W (Morales *et al.*, 2003).

The knowledge from the PIT method enables a user to choose a proper surfactant if a storage temperature is known, and to determine an optimum storage temperature if a certain surfactant needs to be used in the production of nanoemulsions. Most of the research has been done to investigate the formation of aqueous-continuous nanoemulsions, while oil-continuous nanoemulsions are not focused on. The method, which has been developed by Shinoda and Saito (1969), where the emulsion system is heated to a temperature close to PIT temperature followed by a quenching process and stored at a temperature far away from the PIT temperature, is widely used in the research, as well as in industries.

2.2 Emulsion stability

The stability of an emulsion system has been discussed by researchers (Binks, 1998; Tadros *et al.*, 2004; Solans *et al.*, 2005) in terms of creaming, sedimentation, flocculation, coalescence, Ostwald ripening and inversion, which are shown in Figure 2.4.

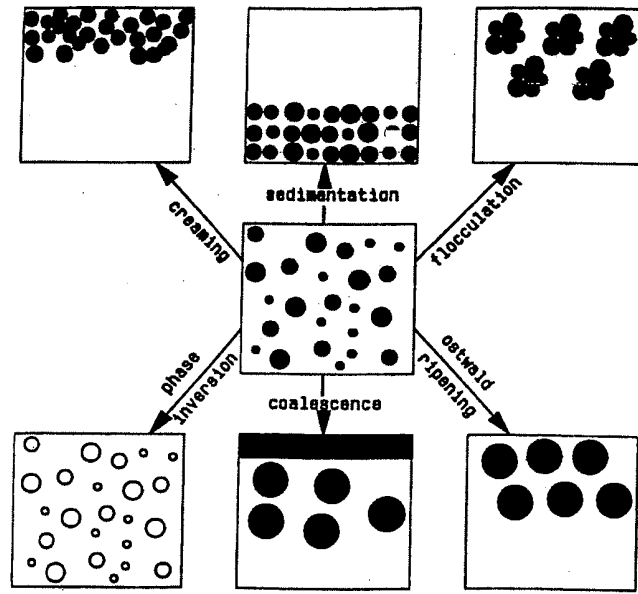


Figure 2.4: Schematic representation of the break-down processes in emulsions (Tadros, 2004).

Sedimentation and creaming are due to the gravity effect and density difference between continuous and dispersed phases. These two mechanisms are the most possible reasons to cause phase separation as the distance between droplets is closer and so the possibility of collision is increased.

Flocculation may occur due to the van der Waals attractive energy exceeding the repulsive energy. Flocculation will cause droplets to “stick together” without any droplet growth. Flocculation can be effectively reduced by providing energy barriers on the surface of the droplets. The energy barriers can be provided by either an ionic surfactant (repulsive force) or a non-ionic surfactant (steric mechanism).

Ostwald ripening occurs because the Laplace pressure of droplets is different with different droplet sizes. Small droplets (high Laplace pressure) have higher solubility than the larger ones (low Laplace pressure). This causes the large droplets to become bigger while small droplets to become smaller and shrink. The droplet growth rate, ω , also referred as ripening rate, can be estimated by the Lifshitz-Slezov and Wagner (LSW) theory (Taylor and Ottewill, 1994):

$$\omega = \frac{dr_n^3}{dt} = \frac{8}{9} \frac{C_\infty \gamma V_m D}{RT} \quad (1)$$

where r_n is the number average radius, t the storage time, D the diffusivity of oil phase in continuous phase, C_∞ the bulk solubility of oil phase, γ the interfacial tension, V_m the molar volume of the oil, R the gas constant and T the absolute temperature. By plotting r_n^3 versus the storage time t , a linear graph can be obtained with the slope equal to the Ostwald ripening rate, if Ostwald ripening is dominant in the instability of emulsions.

When the energy of adhesion between two droplets is larger than the turbulent energy causing dispersion, coalescence between droplets will occur. Furthermore, coalescence is a result from the thinning and disruption of the liquid film between the droplets and the joining of these droplets. The droplet size will become bigger and bigger by combining different droplets, and cause phase separation in the end of the process. The growth rate of the droplets due to coalescence is shown as follow (Uson *et al.*, 2004):

$$\frac{1}{r^2} = \frac{2}{r_0^2} - \left(\frac{8\pi}{3}\right)\omega t \quad (2)$$

Where r is average droplet radius after t , r_0 is the radius at $t=0$, and ω is the frequency of rupture per unit of surface of the film. A straight line can be plotted between $\frac{1}{r^2}$ and t , when coalescence is the main factor to destabilise a emulsion system.

Due to the large reduction in the gravity force by the ultra small-sized droplets in nanoemulsions, Brownian motion becomes significant and thus sedimentation and creaming are impossible. Therefore, Ostwald ripening and coalescence are the main destabilising factor in nanoemulsions. Those two factors are hard to directly differentiate with any equipment, thus equation (1) and (2) play an important role to determine the dominant destabilising factor. The stability of nanoemulsions produced by low energy method is discussed in details in Section 2.2.1.

2.2.1 Instability in Nanoemulsions

The O/W nanoemulsions produced by the EIP method are mainly destabilised by Ostwald ripening, where the rate is increased by increasing oil concentration (Sole *et al.*, 2006) and by decreasing surfactant concentration (Liu *et al.*, 2006). However, the instability of the W/O nanoemulsions produced by the EIP method can be attributed to Ostwald ripening at short storage time and at low concentration of dispersed phase; while Ostwald ripening and coalescence destabilise the nanoemulsions at longer storage time and at high concentration of dispersed phase (Porras *et al.*, 2004; Uson *et al.*, 2004). Moreover, Ostwald ripening has also been found to be the main destabilising factor in O/W nanoemulsions produced by the PIT method (Morales *et al.*, 2003; Tadros *et al.*, 2004; Izquierdo *et al.*, 2005; Ee *et al.*, 2007).

At low surfactant concentration, surfactants exist in monodisperse form and are concentrated at the interfaces by hydrophilic-hydrophobic oriented adsorption. With the increase of surfactant concentrations, the surface is completely covered by the surfactant molecules and the excess surfactant particles cannot accommodate at the surface and so the surfactant molecules start to form larger aggregates (micelles). The micelles are oriented with the hydrophilic groups pointing towards the aqueous volume phase and the hydrophobic groups towards the interior of the micelles. The concentration at which the aggregation of surfactant starts to be formed is known as critical micelles concentration (CMC) (Kosswig, 2000; Tadros, 2006). CMC plays an important role in the Ostwald ripening rate and therefore the stability of O/W nanoemulsions produced by PIT method.

The interfacial area and interfacial tension are determined by the amount of surfactant, where smaller droplets and more stable emulsions can be produced by using larger amount of surfactant and the surface area is larger while interfacial tension is lower. The interfacial tension decreases with surfactant concentration but becomes constant after reaching the CMC (Tadros, 2006). At surfactant concentration lower than CMC, the Ostwald ripening rate decreases with the surfactant concentration, due to the decrease of the interfacial tension which agrees with the LSW theory (Equation 1) (Taylor and Ottewill, 1994). However, nanoemulsions with a concentration below CMC are normally unstable against coalescence due to the insufficient coverage of surfactant on the interface. At concentration above CMC, it has been found that the Ostwald ripening rate increases with the surfactant concentration (Taylor and Ottewill, 1994; Izquierdo *et al.*, 2002; Izquierdo *et al.*, 2004). The higher Ostwald ripening rate has been discussed because of the formation of micelles which enhance the oil to be diffused from small to large droplets, and the relationship between the rate the diffusion rate (D) is shown in Equation 1 (Taylor and Ottewill, 1994; Tadros *et al.*, 2004). Furthermore, the solubility of n-dodecane, which is also directly proportional to the rate (Equation 1), increases with surfactant concentration (Taylor and Ottewill, 1994).

However, there are some works showing that Ostwald ripening rate is not always increased with surfactant concentration. When surfactant concentration is increased, the resistance of mass transfer of oil increases and leads to a decrease in the ripening rate. The change of ripening rate with surfactant concentration was suggested to be insignificant (Kabalnov, 1994; Taylor and Ottewill, 1994) while it decreases with surfactant concentration (Liu *et al.*, 2006). From these works, it is suggested that the formed micelles does not mediate a mass transfer of oil from smaller to larger droplet, but only absorbs the oil (Kabalnov, 1994; Liu *et al.*, 2006). Furthermore, it is also suggested that the phenomenon of the mass transfer by micelles is different in different surfactant systems. Thus, there is still not a clear relationship between the ripening rate and surfactant concentration.

According to Equation 1, the Ostwald ripening rate is decreased with the increase of the temperature, however, this is not consistent with the results obtained by Taylor (2003). The increase of the rate is because of the oil solubility in aqueous phase is increased (which increases the Ostwald ripening rate) when temperature is increased. Therefore, it has been found that the increasing ripening rate with the oil solubility is more significant than the decreasing rate with the increase of temperature. There are several methods available to reduce the ripening rate: (1) by an addition of a second less soluble oil (Binks *et al.*, 2000; Izquierdo *et al.*, 2002) or (2) by using surfactant monolayers that are less permeable to the oil (an ionic surfactant) (Taisne and Cabane, 1998).

The theoretical Ostwald ripening rate (predicted from Equation 1) has been compared with the experimental value, and the findings indicate agreement with the theoretical one when surfactant concentration is below CMC. Above the CMC, the experimental rate is found to be larger than the theoretical value and the order of the difference increases with the increase of surfactant concentration (Kabalnov *et al.*, 1990; Taylor and Ottewill, 1994). A two or three order difference was found in a dodecane-SDS emulsion system with the surfactant concentration was higher than CMC (Taylor and Ottewill, 1994). This difference is because Equation 1 only considers the molecular

solubility of hydrocarbon; however, micellar solubilities of hydrocarbon in surfactant concentration higher than CMC are higher than molecular solubilities by several orders of magnitudes.

A more appropriate formula to determine the Ostwald ripening in different emulsion system was proposed in Equation 3 (Taylor, 2003).

$$\omega = \frac{dr_n^3}{dt} = k \frac{C_\infty \mathcal{N}_m D}{RT} \quad (3)$$

where k is a constant which is 0.89 in LSW theory. However, the constant is 2.23 in Taylor (2003) and 1.46 in Kabalnov *et al* (1990) in order to fit the experimental data. Generally, LSW theory is valid in the case of immobile particles when the molecular diffusion is the only mechanism of mass transfer (Kabalnov *et al.*, 1990). Therefore, LSW theory is not suitable to predict the Ostwald ripening rate for a known emulsion system but is a good tool to determine the Ostwald ripening as a main destabilising factor in nanoemulsions (by plotting a linear graph in r_n^3 - t plot).

Although it has been found that the nanoemulsions produced by high pressure methods have lower Ostwald ripening rates than those produced by the PIT method (Tadros *et al.*, 2004), the consumption of the energy by the PIT method has made the method more suitable to be used for industrial purposes. Furthermore, it has been shown that the PIT method is a better method for production of smaller emulsions than the EIP method (Shinoda and Saito, 1969). Therefore, the PIT method is widely used in emulsions industry and also is chosen to be used in this research.

2.3 Electrolyte effect

The stability of emulsions is not only controlled by the interfacial tension, but also the properties of the continuous solution close to the interface. Emulsions stabilised with non-ionic surfactant are mainly via steric stabilization, where the emulsions are coated with an absorbed layer of some material which to prevent a close approach between droplets. An electrostatic mechanism can stabilise the emulsions by providing same electrical charged (either positive or negative) to all droplets and so they will repel one another (Hunter, 2001; Morais *et al.*, 2006). In an electrolyte solution, the distribution of the ions around the charged emulsions is not uniform, and the arrangement of the electric charge on the oil droplet associated with the charge balance in solution is called the electrical double layer of the droplet. The layer is made up of two parts, which are the inner region (Stern layer) and the outer region (diffuse layer). Stern layer is where the ions are stronger bonded (counterions) while diffuse layer is where the ions are distributed broadly and fewer firms associated (co-ions). The interaction between charged droplets is mainly governed by the overlap of diffuse layer and thus the potential between droplets is depend on the boundary of the Stern and diffuse layers, rather than the potential at the droplet surface. Therefore, there are two forces acting between droplets, which are repulsive (positive) force from the double layer and attractive (negative) force from Van der Waals potential (Dukhin and Sjoblom, 1996; Morais *et al.*, 2006). As the surface potential cannot be directly determined, the electrostatic interaction between droplets is replaced by zeta potential. Zeta potential is a difference of potential between a point situated at a sufficiently large distance from the droplet surface and a point situated on the surface of the shear,

ie., the plane in which the relative movement of the phases occurs (Morais *et al.*, 2006). Stable emulsions are expected to have positive zeta potential (repulsive) and are more stable with larger zeta potential.

With the addition of an electrolyte and the increase of concentration, the ionic strength of continuous phase increases and the electrical double layer is being compressed (reduction in its thickness), thus the repulsion force between droplets is reduced (Baloch and Hameed, 2005; Morais *et al.*, 2006). Comparing the repulsive force with the London-Van der Waals force (attractive), the net force is more attractive in a highly electrolyte concentration. Therefore, the instability of emulsions will be governed predominantly by flocculation. However, some research has shown flocculated colloids can be redispersed by washing away the electrolyte, which clearly indicates that the aggregation is caused by the presented hydrated ions and not by the primary forces (Van del Waals force) in a highly electrolyte concentration solution (Dukhin and Sjoblom, 1996). Moreover, the change of zeta potential with salt concentration is more pronounced in ionic surfactant system compared to non-ionic surfactant system (Binks *et al.*, 2000; Morais *et al.*, 2006).

Cloud point (CP) is the temperature at which the non-ionic surfactant becomes insoluble in water and forms two phases, where one is surfactant rich phase and the other is pure water phase. CP is highly affected by the addition of an electrolyte by salting-in or salting-out mechanism. The raise of the cloud point to extend the solubility temperature of a non-ionic surfactant is called salting-in; while the opposite effect from an electrolyte is called salting-out. For a polyoxyethylated surfactant, Na^+ and Cl^- salt out while Ca^{2+} salts in the surfactant. Comparing NaCl and CaCl_2 , NaCl is a stronger salting-out agent and has higher effect in reducing the interaction of the oxyethylene chains (EO group) with the aqueous phase. Furthermore, the reduction in CP is more profound in the NaCl system with electrolyte concentration (Shinoda and Takeda, 1970; Morini *et al.*, 2005). In an aqueous C_{12}EO_7 system, the formation of micelles is affected by the addition of an electrolyte where salting-out salts increases their compactness while salting-in salts increases their looseness (Morini *et al.*, 2005). However, the effect of the addition of electrolytes is not absolute and may change with concentration (Morini *et al.*, 2005).

The phase diagrams of an ionic surfactant (SDS) emulsion system with the addition of sodium chloride (NaCl) have been reported by Bellocq (1996). The main effect from the addition of NaCl in an ionic surfactant emulsion system is to produce very swollen lamellar phases and promote the formation of microemulsions. In an anionic surfactant emulsion system, WIII is only obtained in the presence of salt. In Figure 2.5 and Figure 2.6, WIII region exists in the intermediate salt concentrations and in a narrow range. The self-diffusion coefficient (D) of water, toluene, butanol and SDS with salt concentrations is clearly shown in Figure 2.5. At low salinity, the diffusivity of water is higher than toluene and so toluene will solubilise in the water phase to produce O/W emulsions; however the D value decreases with the salinity, the formation of W/O being more preferable at high salinity. At the intermediate electrolyte concentration, the self-diffusion of water and toluene are in the same order and both oil and water are able to move freely and form a bicontinuous phase (WIII region). Furthermore, the diffusivity of toluene increases with salinity, thus a higher Ostwald ripening rate is expected in a higher electrolyte concentration ($\omega \propto D$ in Equation 1).

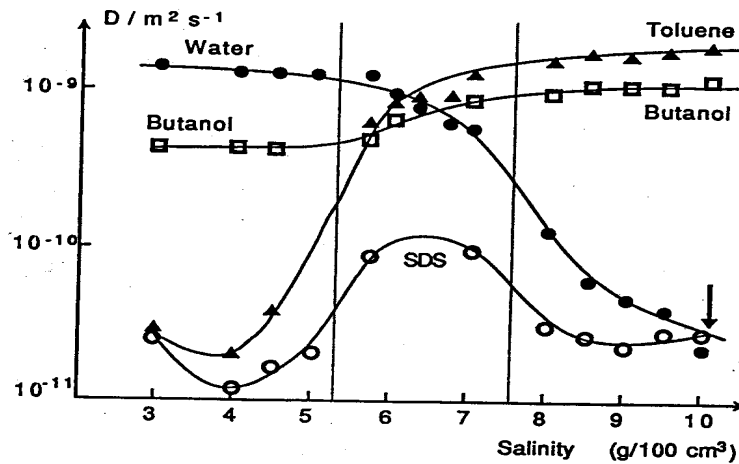


Figure 2.5: Self-diffusion coefficient of the constitutes of Winsor microemulsions as a function of the salt concentration (Bellocq, 1996).

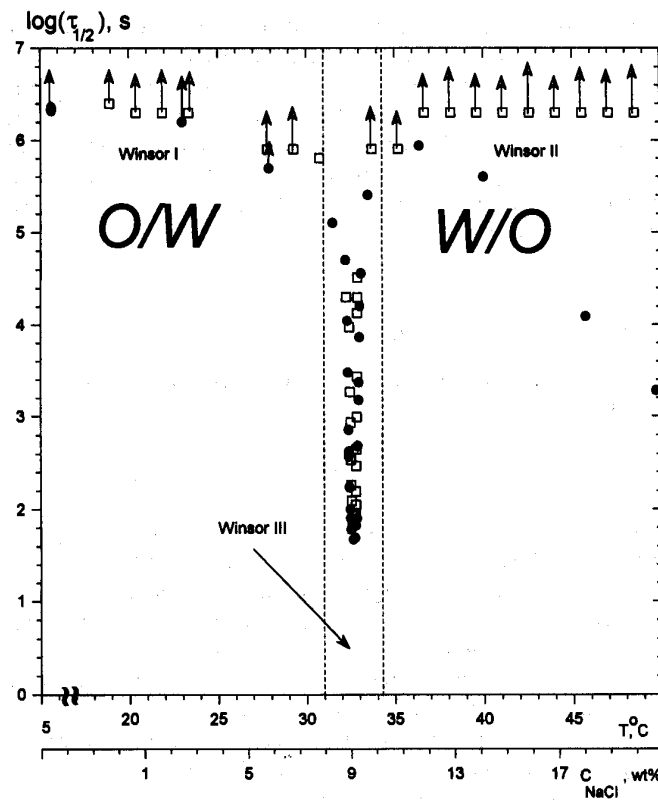


Figure 2.6: The stability of an emulsion system as a function of salinity and temperature, where $\log(\tau_{1/2})$ is the logarithm of macroemulsion lifetime.

Type of emulsions, which will be formed regarding to the electrolyte concentration is clearly shown in Figure 2.6. That phase inversion will occur at a crucial salt concentration has been reported by many researchers (Wasan *et al.*, 1988; Kabalnov, 1998; Binks *et al.*, 2000; Salager *et al.*, 2002; Srivastava *et al.*, 2006). Comparing the phase inversion from the change of salinity and temperature, at low salinity (or temperature), O/W emulsions (WI) are produced while W/O emulsions (WII) are produced at high salinity (or temperature). At intermediate salinity (or temperature), WIII is formed. The stability of the emulsions with salinity and temperature is also

shown in Figure 2.6. The stability of emulsions with salinity behaves the same way with the stability with temperature, where the stability of emulsions starts to be decreased when they are close to the WIII region. Very unstable emulsions or no emulsions are produced in the WIII region. The intermediate salinity which forms WIII region can be treated as the PIT temperature in the phase inversion by temperature.

The phase inversion process in the change of salinity is the same with the process with temperature which has been shown in Figure 2.6. Furthermore, the phase inversion caused by an electrolyte is a TPI process which is the same with PIT process, thus O/W and W/O emulsions can be interchangeable by concentrating (from O/W to W/O) or diluting (from W/O to O/W) the system (Dukhin and Sjoblom, 1996).

In addition to the effects mentioned above, electrolytes also affect the process during the phase inversion, especially the PIT temperature. It has been known that the PIT temperature is highly dependent on the HLB number (type of non-ionic surfactant). Although the HLB number is assigned to a definite surfactant, the real HLB of an emulsion system, which determine the formation of emulsions, is dependent on some additives (eg. salt and alcohol) as well as on the type of oil. Shinoda and Saito (1969) have investigated the relationship between the PIT temperature and the electrolytes and the electrolyte concentrations. In their work, with a sodium chloride concentration up to 6wt% in a non-ionic surfactant emulsion system, the reduction of HLB was 1.2 but it was 14°C in PIT temperature. The decrease of the PIT temperature is due to the increase of the internal pressure of solution which results in the interaction between water and non-ionic surfactant being weakened. Moreover, the nature of a non-ionic surfactant becomes more lipophilic with the addition of electrolyte by salting-out effect (Anton *et al.*, 2007). Different electrolytes behave differently in the change of PIT temperature. Between NaCl and CaCl₂ emulsion systems, NaCl system always has lower PIT temperature and the reduction in PIT temperature with electrolyte concentration is larger in NaCl system (Shinoda and Takeda, 1970), due to the stronger salting-out effect (Morini *et al.*, 2005).

During the formation of O/W emulsions, the time required for oil, water and surfactant to contact and form emulsions is called apparent equilibration time (t_{APE}) (Salager *et al.*, 2002). When the sodium chloride concentration increases but below the concentration of the inversion point, t_{APE} is decreased. The time is nearly zero at the optimum salt concentration, where the emulsions are in WIII region (Salager *et al.*, 2002). This optimum salt concentration is useful to prepare emulsions with a very short time, where a quick production is needed.

There are arguments about the stability of the addition of electrolyte in an emulsion system. The addition of sodium chloride in an emulsion system has been proved to increase the interdroplet interactions and viscosity of the continuous medium (Salager *et al.*, 2002; Baloch and Hameed, 2005; Martinez *et al.*, 2007) and to improve the stability against creaming but to enhance the coalescence (Baloch and Hameed, 2005). The droplet size increases with salt concentration at low surfactant concentration (Baloch and Hameed, 2005). When the surfactant concentration is high but below CMC, the interfacial tension decreases but oil solubility increases with salt concentration (Taylor and Ottewill, 1994; Binks *et al.*, 2000), thus the ripening rate can be either increased or decreased by the addition of salt (Equation 4) and so to affect the emulsion stability. When the emulsions are prepared above the CMC,

droplet size decreases with salt concentration (Binks *et al.*, 2000) due to the lower interfacial tension.

2.3.1 The Effect of Electrolytes on Macroemulsions

The droplet sizes of emulsions mainly depend on the type of oil, type of surfactant (ionic or non-ionic), surfactant concentration (CMC), type of electrolytes, and electrolyte concentration. In macroemulsion system, the droplet size can be increased (Baloch and Hameed, 2005; Binks *et al.*, 2006) or decreased (Binks *et al.*, 2000; Binks *et al.*, 2006) by increasing the NaCl concentration, which is clearly shown in Figure 2.7. The droplet median diameter was around 100 μm at very low NaCl concentrations (less than 0.01mol/kg) and decreased to around 25 μm at 0.05mol/kg. Above 0.05M, the size was larger than 50 μm and increased with increasing the concentration.

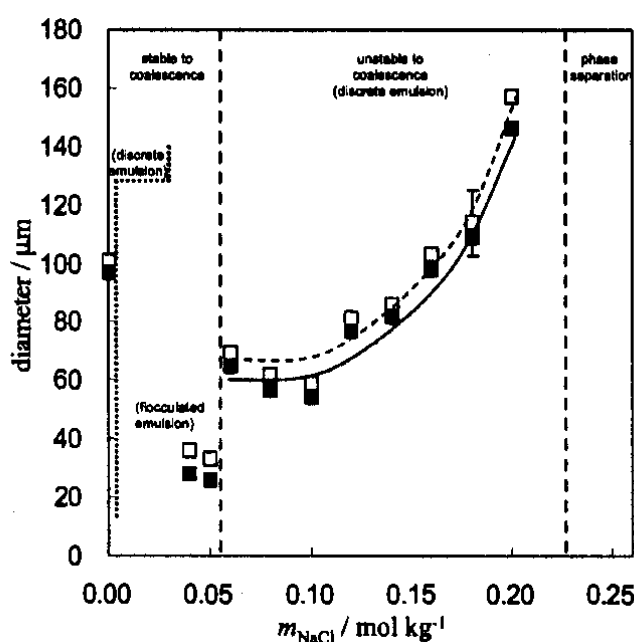


Figure 2.7: Droplet size and the stability of O/W emulsions versus NaCl concentration in a aqueous dispersion for batch of equal volumes of methyl myristate and a 1.0wt% aqueous dispersion of P4VP/SiO₂ microgel particles at pH 4 (Binks *et al.*, 2006). (\square , arithmetic mean diameter; \blacksquare , median diameter)

Figure 2.7 also shows the stability of macroemulsions with the addition of sodium chloride. At low salt concentrations (below 0.055mol/kg), the emulsions are simple but unstable against flocculation (creaming), however the flocculation decreases with decreasing salt concentration and reaches a minimum, where the flocculation and coalescence are suppressed by the electrostatic repulsion (Binks *et al.*, 2000); at intermediate concentration (0.055 to 0.23 mol/kg), the emulsions are unstable against coalescence and the rate increases with the concentration; at high concentration (above 0.23mol/kg), there is phase separation where the emulsions are close to WIII region. In a n-Heptane-in-water (O/W) emulsions stabilised by an anionic surfactant sodium bis(2-ethylhexylsulfosuccinate) (AOT), the most stable emulsions exist at 0.035M NaCl (Binks *et al.*, 2000).

At low salt concentrations, there is almost no droplet deformation or film formation, but with increasing the concentration, the deformation and the interdrop attraction increase (double layer being compressed) and thus facilitate coalescence. As the interfacial tension decreases with increasing the NaCl concentration (Taylor and Ottewill, 1994), the droplet deformation is promoted and thus coalescence is enhanced (Binks *et al.*, 2000). The minimum creaming rate suggested in Bink *et al* (2000), which existed at 0.035M NaCl, was because of the decrease of the electrostatic repulsion between the charged emulsion interfaces when the salt concentration was increased. The lower electrostatic repulsion caused the droplets to start attracting each other and led to droplets flocculation resulting in gel formation. With the formation of a gel network, the viscous resistance upon buoyancy compression of the emulsion could be due mainly to energy dissipation within the thin liquid films formed between flocculated droplets. Therefore, the network could be much greater than the resistance experienced by a single droplet when moving upward (Binks *et al.*, 2000). At low electrolyte concentrations, the electrostatic repulsion makes the coalescence impossible, thus Ostwald ripening mainly destabilises macroemulsion system (Binks *et al.*, 2000). Furthermore, the Ostwald ripening rate decreases with increasing NaCl concentrations due to the decreasing of interfacial tension (Taylor and Ottewill, 1994). Coalescence is more profound at high electrolyte concentrations as the electrical double layer is compressed and the distance between charged droplets is closer (Binks *et al.*, 2000).

It is evident from the previous works, the addition of electrolytes in macroemulsions generally enhances the instability of the system, unless it is done in an optimum salt concentration, where the creaming rate is minimised by a formation of gel network (Binks *et al.*, 2000; Binks *et al.*, 2006).

2.3.2 The Effect of Electrolytes on Nanoemulsions

The droplet size of light liquid paraffin oil nanoemulsions is found unchanged (Srivastava *et al.*, 2006) but slightly increased in canola oil nanoemulsions (Morais *et al.*, 2006) with increasing sodium chloride concentration. However, there is a sudden increase in droplet size at the inversion point, where the system is close to WII-WIII phase transition region (Srivastava *et al.*, 2006). The interfacial tension is in its minimum at WI-WIII phase transition region, and it is increased by either decreasing or increasing the salt concentration (Srivastava *et al.*, 2006). For the stability of O/brine nanoemulsions, Ostwald ripening was found to be the most dominant destabilising factor (Taylor and Ottewill, 1994). Taylor and Ottewill (1994) have found that the ripening rate decreases with sodium chloride concentration but not as significant as the decrease in interfacial tension. The lower increase in Ostwald ripening is because of the increase in the concentration also increases in the micellar size and results in an increase of solubility of oil in aqueous phase (Equation 1).

A detailed study of the effect of electrolyte on the formation and stability of nanoemulsions has been done by Morais and co-workers (2006), where the nanoemulsions were produced by EIP method. In their work, trivalent cations (Al^{3+} in AlCl_3) were found to be more readily adsorbed on the O/W interface and thus the effect on zeta potential of nanoemulsions is more significant than monovalent (Na^+ in NaCl) and divalent cations (Ca^{2+} in CaCl_2) systems. However, the addition of salt (even Al^{3+}) has shown no effect on initial droplet size, the growth of droplet size and the stability of nanoemulsions. The unchanged droplet size and stability are because

of the electrolytes could not be absorbed on the surface of the droplet; therefore a decrease in double layer thickness is not enough to decrease the system stability. In a non-ionic surfactant emulsion system, H^+ and OH^- ions are much more easily adsorbed on the O/W interface, and obstruct the bonding between droplet interface and additives (Na^+ and Cl^-). Nevertheless, it has been suggested that the instability of nanoemulsions will only exist in a very narrow electrolyte concentration (Morais *et al.*, 2006). The increase or decrease of zeta potential in an electrolyte emulsion system (with non-ionic surfactant) is not always related to the stability or droplet sizes, especially when steric mechanism (by non-ionic surfactant monolayer) is dominant in the stability (Morais *et al.*, 2006).

To date, only O/W nanoemulsions produced by EIP method (Morais *et al.*, 2006) or by diluting microemulsions (Taylor and Ottewill, 1994) have been investigated with the effect of the addition of electrolytes. The effect on the formation and stability of nanoemulsions produced by PIT method is yet unknown or less-understood. Thus, the investigation of the effect of electrolytes on the O/W nanoemulsions produced by PIT method in this research will provide a preliminary study for nanoemulsion industries, where the PIT method is becoming a more effective way to produce nanoemulsions.

2.4 Reversibility

PIT is a TPI process where the O/W and W/O emulsions can be interchangeable by tailoring the emulsion temperature. However, the droplet size and size distribution of an emulsion system, before and after the reversibility process (from WI to WII then back to WI or vice versa), have not been well identified. If an ageing or destabilized aqueous-continuous nanoemulsion system can be reverted back to its fresh prepared state in terms of the droplet size and the size distribution, it may be said that the nanoemulsions have the reversibility ability. The reversibility ability plays an important role in nanoemulsion industries as it will allow no restriction to store and deliver nanoemulsions.

It has been found that the droplet size and stability of O/W nanoemulsions produced by the PIT method are sensitive to temperature (Tadros *et al.*, 2004; Ee *et al.*, 2007), which the droplet size increases and stability decreases with increasing the temperature close to PIT temperature. They need to be handled at their optimum temperatures (which was suggested to be 20-65°C below PIT temperature in Shinoda and Saito (1969)) in order to prolong the shelving life. However, in some system where the PIT temperature is below room temperature (20°C), it is not cost effective to store and deliver the nanoemulsions. Furthermore, the optimum temperature is difficult to be maintain during the packing and handling process in emulsion industries. Therefore, the ability to allow the ageing or destabilized O/W nanoemulsions to reverse their properties is important in emulsion industries especially nanoemulsion industries.

By initially preparing emulsions with larger droplet sizes, followed by a quenching process to bring the emulsions to lower temperatures (WI region), the redissolution of the oil drops from big droplet to small droplet has been shown in the cooling process (Evilevitch *et al.*, 2000). Less time is needed for a complete resolubilization if the quenching temperature is lower. Furthermore, the resolubilisation rate is faster if the process involves a structural change (phase change), where a direct transfer of oil is

possible (Evilevitch *et al.*, 2000). This redissolution mechanism has provided a basic concept for developing the reversibility ability in this research.

This research also arises from our previous work which was done in decane nanoemulsions system. The droplet sizes of decane-water nanoemulsions which were stored at their optimum temperature, were reversible if the system was heated to a temperature higher than the optimum temperature and cooled back to the optimum temperature (Ee *et al.*, 2007). However, the droplet size and size distribution of O/W or O/brine nanoemulsions, before and after the reversibility process, are not well investigated. Furthermore, the complete reversibility processes, such as the heating temperature and procedures, are not well developed. Additionally, the effect of the addition of electrolytes on reversibility ability has not been studied. In this research, all the unsolved issues in the reversibility ability of n-dodecane nanoemulsions produced by the PIT method are addressed.

3.0 Approach and Methods

The materials which are used in this research and their specifications are listed in this section. Additionally, the reasons why the materials are chosen are discussed. After the materials have been chosen, this section also includes the list of the investigated systems and the description of why the systems are chosen. Furthermore, the methods to produce and analyse the nanoemulsions system are described.

3.1 Materials

Table 3.1 lists the materials used in this research as well as their chemical formula, purity, purpose in this research and the sources. All materials were used as they were received. There was no further purification for all materials.

Table 3.1: List of materials.

Material	Chemical Formula	Purity	Purpose	Provider
n-Dodecane	$\text{CH}_3(\text{CH}_2)_{10}\text{CH}_3$	99%	Disperse phase	Merck, Australia
Poly(oxyethylene)(4) lauryl ether/ Brij30	$\text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{-CH}_2)_4\text{OH}$	Technical grade	Surfactant	Sigma-Aldrich
Sorbitan monooleate / Span 80 (S80)	$\text{C}_{24}\text{H}_{44}\text{O}_6$	Technical grade	Surfactant	Sigma
Sodium Chloride	NaCl	99%	Additive	Chem-Supply
Calcium Chloride	CaCl_2	99%	Additive	Chem-Supply
Tap water	H_2O	618 $\mu\text{S/cm}$	Continuous phase	The University of Adelaide
Reverse Osmosis (RO) water	H_2O	94.6 $\mu\text{S/cm}$	Continuous phase	The University of Adelaide
Ultra-pure Millipore (MilliQ) water	H_2O	1.42 $\mu\text{S/cm}$	Continuous phase	The University of Adelaide

The effect of electrolytes on the formation of n-dodecane nanoemulsions has been investigated by Taylor and Ottewill (1994) but the nanoemulsions were not produced by the PIT method, and thus n-dodecane was chosen as dispersed phase in this research. The main surfactant used in this research was polyoxyethylene (4) lauryl ether (Brij30), as it is the surfactant mostly used by researchers (Forgiarini *et al.*, 2001; Izquierdo *et al.*, 2002; Izquierdo *et al.*, 2004) to produce O/W nanoemulsions by the

PIT method, and has also been used in previous work (Ee *et al.*, 2007). Brij30 is a surfactant with high HLB (O/W emulsions is preferable to be produced), in order to investigate a system with different surfactant system, a surfactant with low HLB and to produce W/O emulsions was chosen to be mixed with Brij30. Sorbitan monooleate (Span80) was chosen as it has been used to blend with other high HLB surfactant to produce O/W nanoemulsions by low energy method (Liu *et al.*, 2006). Sodium chloride (NaCl) and calcium chloride (CaCl₂) are the common electrolytes to add into the production of emulsions. Furthermore, much investigation has been done in the effect of NaCl and CaCl₂ on the formation of emulsions (Shinoda and Takeda, 1970; Taylor and Ottewill, 1994; Binks *et al.*, 2006; Morais *et al.*, 2006), and provided a fundamental study in this research. For continuous phase, milliQ water (with conductivity is 1.42 μS/cm) was used in most of the experiment to produce O/W nanoemulsions or to prepare brine to produce O/brine nanoemulsions. However, tap water (with conductivity is 618 μS/cm) and reverse osmosis (RO) water (with conductivity is 94.6 μS/cm) were also used to investigate the effect of quality of the continuous phase. As the tap water and RO water were taken straight from the tap, particles/contaminants were expected in emulsion system.

3.2 Investigated system

Table 3.2, lists the oil concentrations (R), type of surfactant, surfactant concentrations, type of electrolyte and electrolyte concentrations, which are the conditions/factors to produce n-dodecane nanoemulsions by the PIT method.

R, an oil percentage in a mixture of oil and aqueous phases $\left(R = \frac{O}{O + W} \right)$, was mostly investigated at 0.2, which has been a common oil concentration to be investigated in the production of nanoemulsions produced by the PIT method (Izquierdo *et al.*, 2002; Morales *et al.*, 2003). R was also increased to 0.3 and 0.4 to investigate the systems with different oil concentrations. In the first set of experiments, R was 0.2, only Brij30 and NaCl were used as surfactant and an electrolyte, surfactant concentration was 4wt% and the electrolyte concentration was started from 0.01M and with an increment of 0.02M to 0.05M. 0.1M NaCl as an additional experiment to investigate the effect of high salt concentration. After the first set of experiments, all the experiments were investigated in no electrolyte, low electrolyte concentration (0.001M), intermediate concentration (0.01M) and high concentration (0.1M), for both NaCl and CaCl₂ system.

Surfactant concentration varied from 4 to 8wt% and only with Brij30 was carried out in one of the experiment sets to find out the optimum surfactant concentration to produce the most stable nanoemulsions (without phase separation for at least 30 days), within systems with different eNaCl concentrations. After the optimum surfactant concentration was determined (which was 7wt% in pure milliQ water system and 6wt% in NaCl systems), only the optimum concentration was used to investigate the effect of NaCl with different surfactant (Brij30 + Span80) and effect of CaCl₂ in R=0.2 system. In the systems with different oil concentrations, the surfactant-to-oil ratio (R_{so}) was kept constant, which was 0.3191 (in R=0.2 and 6wt%[S]) and 0.3763 (in R=0.2 and 7wt%[S]). The fixed surfactant-to-oil ratio was to ensure there were no

other mechanisms (eg. insufficient surfactant concentration) to cause the formation and stability of nanoemulsions other than the effect of sodium chloride on the number of droplet formed.

Table 3.2: List of experiments.

$R\left(\frac{O}{O+W}\right)$	Surfactant	[Surfactant] (wt%)	Salt	[Salt] (M)
0.2	Brij30	4	NaCl	0, 0.01, 0.03, 0.05, 0.1
0.2	Brij30	4-8	NaCl	0, 0.001, 0.01, 0.1
0.3	Brij30	$R_{so}=0.3191$; 0.3763	NaCl	0, 0.001, 0.01, 0.1
0.4	Brij30	$R_{so}=0.3191$, 0.3763	NaCl	0, 0.001, 0.01, 0.1
0.2	Brij30 + S80	6 & 7	NaCl	0, 0.001, 0.01, 0.1
0.2	Brij30	6	CaCl ₂	0, 0.001, 0.01, 0.1

3.3 Methods

3.3.1 Phase Behaviour

When temperature increases, emulsions are changed from WI region (O/W) to WII region (O/W) by passing through WIII region. In WIII region, it has been suggested that it is a complicated phases and consisting of lamellar liquid crystal phase (LLC) (anisotropic phase) and bicontinuous phase (L₃) (Kunieda *et al.*, 1996; Kabalnov, 1998; Anton *et al.*, 2008). However, the sequence of the formation of LLC and L₃ phases in WIII region was suggested differently in Anton *et al* (2008) and Kuneida *et al* (1996). Kuneida and co-workers (1996) found that LLC was formed before L₃ in the phase inversion process while the result was totally opposite in Anton *et al* (2008). The phases involved in the phase inversion process have been well identified by a high-magnification optical microscope whereby the anisotropic phase has been identified under polarizing light (Sole *et al.*, 2006; Anton *et al.*, 2008).

In this research, only one system with R=0.2, 0.01M NaCl, 6wt% Brij30 was chosen to determine the phases involved during the phase inversion process and to verify the argument the sequence of the formation of LLC and L₃ in WIII region.

n-Dodecane, 0.01M NaCl and Brij30 were well-mixed by a magnetic stirrer, then small amount of samples were put on glass lens then covered by cover slips, which was similar with the method undertaken in Anton *et al* (2008). In order to keep the samples in within the cover slips, the edges of the cover slips were sealed with nail varnish. Then, the lens were put into sealable bags and stored in different temperatures at least one day, in order to well-equilibrate the systems, before observing under optical microscope. The anisotropic phase was identified under polarized light.

The optical microscope used was Leica DM LA which is mounted within the Leica As LMD, a third generation of laser microdissection system from Leica Microsystems, Germany. This machine was provided by Adelaide Microscopy, Adelaide, Australia. The magnification used in this research was either 63x or 150x. A polarizer filter was inserted when a total black image was obtained from normal bright field setting and to expect the formation of anisotropic phase (LLC).

3.3.2 PIT Temperature Determination

The setting for the PIT point determination is shown in Figure 3.1. The conductivity of emulsion system was kept monitoring through a digital conductivity meter purchased from Jenway (model 4510) equipped with a platinised-platinum cell with a cell constant equal to one. The conductivity meter was connected to a computerized data acquisition system while the data was logging by serial communication software (version 1.1, Jenway, UK). The conductivity probe was submerged in an emulsion system, which the system was place in a water bath. The water bath was positioned on a heat plate to ensure the heat was evenly distributed to the system and decrease the rate of heat loss. A mercury (Hg) thermometer was also used to measure the temperature of the system. When the temperature was increased, concentrated W/O emulsions were produced from dilute O/W emulsions by phase inversion process, therefore the viscosity of the system was increased. The viscous fluid was hard to be well-mixed with the same stirring rate and also it was “stick” on the platinised cell of conductivity probe so that an incorrect temperature reading was obtained from the conductivity meter. Therefore, a mercury (Hg) thermometer was also used to measure the temperature of the system. As the logged data was from Jenway 4510, a linear relationship would be determined between the temperatures from probe and thermometer, then the data would be converted and plot against the conductivity value.

According to Kuneida et al (1996), PIT temperature can be determined by monitoring the changes of the conductivity values with temperatures in a non-ionic surfactant emulsion system. There will be a sudden drop in the conductivity curve when the aqueous-continuous emulsion system becomes an oil-continuous emulsion system, when temperature is increased. A typical conductivity curve for an emulsion system experiencing phase inversion is shown in Figure 3.2. The conductivity value was increased slowly initially with temperatures and reached a maximum (1st max) before it started to drop. However, it was not continuously decreased and a well-defined second peak (2nd max) could be observed after it reached a minimum value (1st min). After the second peak, the conductivity dropped to zero (2nd min). PIT temperature was taken as an average temperature between the temperatures at the 1st max and 2nd min conductivity values (Izquierdo *et al.*, 2002) as shown in Figure 3.2.

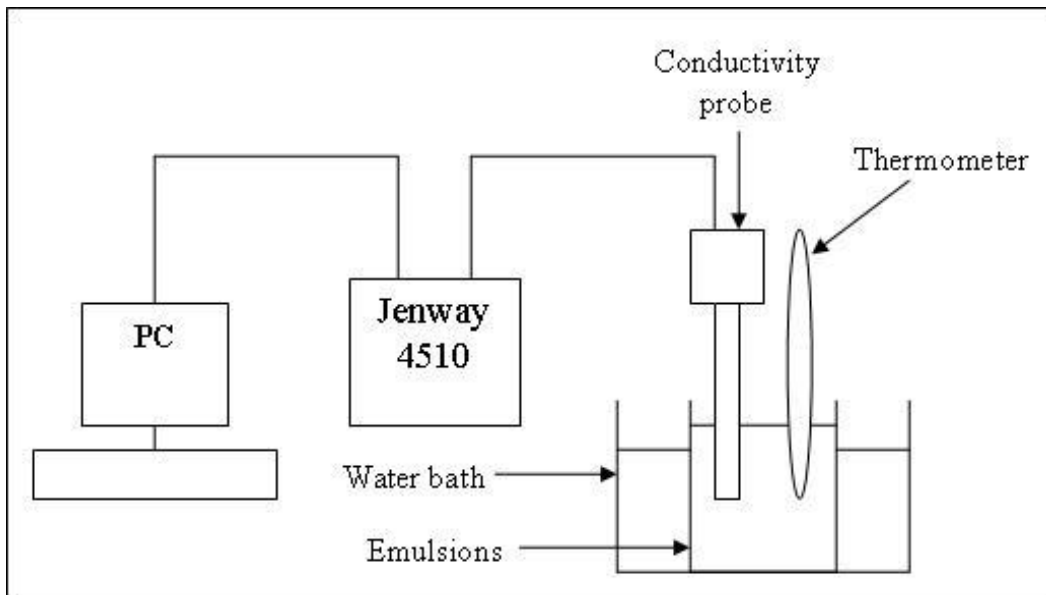


Figure 3.1: Schematic diagram for the setting of PIT determination.

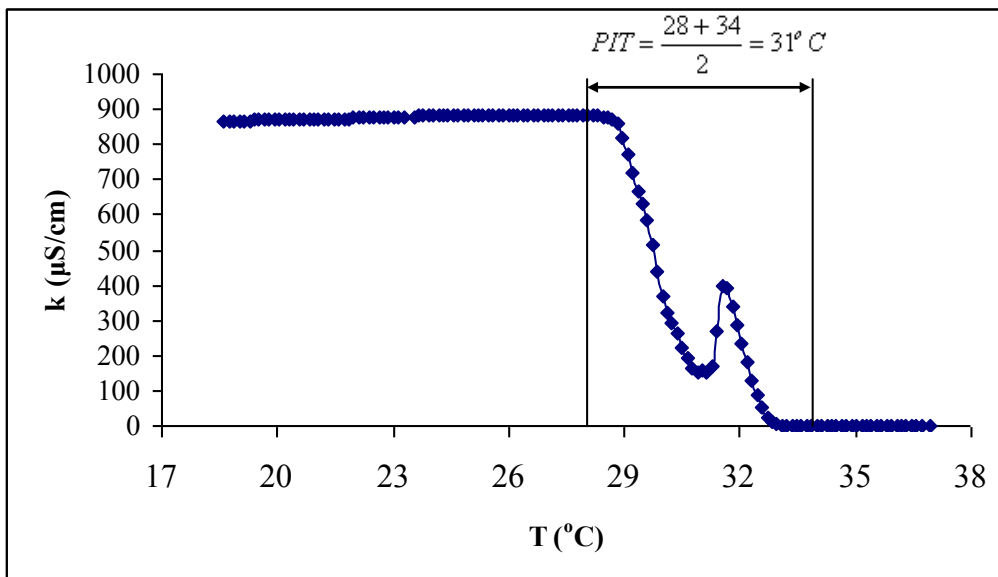


Figure 3.2: A typical conductivity curve for an emulsion system experiencing phase inversion process. (R=0.2, 0.01M NaCl, 4wt% Brij30).

3.3.3 Formation of Nanoemulsions

The method to produce n-dodecane nanoemulsions by the PIT method was developed based on the work done by Shinoda and Saito (1968). The nanoemulsions were produced by a two-step procedure. First, continuous phase (water or brine) was added into a mixture of oil and surfactant and left for stirring for at least five minutes to ensure they were well-mixed. Then by using a hot plate, the temperature was gradually increased to a temperature, which was 4-5°C higher than the temperature at zero-conductivity, to form W/O emulsions. After the W/O emulsions were formed, the system underwent a quenching process by immediately being placed into an ice

bath and rapidly cooled to 15°C (measured by Hg thermometer), to produce O/W nanoemulsions. The cooled emulsions were stored at 20±1°C. However, for some systems in which PIT temperatures were lower than 20°C (eg. mixed surfactant system), they were cooled to 5°C and stored at 10°C. The emulsions were continuously stirred by a magnetic stirrer during the heating and cooling process. However, for those systems which were chosen to be investigated at 10°C, the storage temperature was 10°C instead of 20°C.

3.3.4 Stability and Reversibility Study

To effectively assess the stability of an emulsion, zeta potential and size measurement should be performed (Morais *et al.*, 2006). The stability of n-dodecane nanoemulsions were determined by measuring the changes of droplet size and polydispersity index (PdI) over a period of storage time. PdI ranging from 0 to 1.0 is an indication for quality of dispersion. PdI is strictly below 0.2 in order to be considered as a monodispersed system and suitable for measurement (Izquierdo *et al.*, 2005; Sadurni *et al.*, 2005). Furthermore, the days taken for the emulsions to have phase separation were determined by visual observation. The measurement of droplet size and PdI was stopped after emulsions were phase separated.

All droplet size and PdI were determined by dynamic light scattering (DLS) by using Malvern Zetasizer Nano, Series 3600 (Malvern, UK), with a 532nm green laser and a scattering angle of 173°. All measurements were duplicated three times and an average value was reported. All samples were diluted with continuous phase of the parent emulsions (Binks *et al.*, 2000) and the thermal equilibrium time for each sample (around 1ml) was 5 minutes prior to beginning the measurements. The droplet size determined from the DLS technique was cumulants based average radius (r_z), which has a relationship of $r_z=1.27r_n$ with the number average radius (r_n) (Taylor, 2003). This relationship needed to be applied to determine the Ostwald ripening rate as Ostwald ripening is suggested to be the main the destabilisation factor in nanoemulsions produced by the PIT method.

Zeta potential was measured by the Malvern Zetasizer Nano, which is the same machine used to measure the droplet size and PdI. Before the sample was filled into a capillary cell, the capillary cell was rinsed with alcohol and milliQ water (high quality water). The sample was first prepared by diluting the emulsions with its continuous phase, and then the diluted sample was taken by a syringe with at least 1ml capacity. When injecting the sample, the capillary cell was inverted and slowly injected into one of the sample ports with the diluted sample. When the sample was filled just over half-way of the cell, the cell was turned back and injecting the sample continued until it started to emerge from the second sample port. The syringe was kept at the first port, and then the stopper was inserted in the second port. After that, the syringe was removed and the stopper was inserted in the first port. The filling process was to remove all the air bubbles out from the cell. All measurements were done in 20°C or 10°C and were repeated five times then an average value was reported in this thesis.

The Malvern Zetasizer Nano systematically and automatically sets the intensity of the laser and the attenuator (diaphragm) of the photomultiplier to adapt the sample. Therefore, the reproducibility of the experimental measurement conditions is insured.

Reversibility testing was carried out after emulsions had been stored for three days. The droplet size and PDI were measured in different temperatures while the test was done in two processes:

1. Cooling process: emulsions were cooled to 10°C lower than the storage temperature then back to the storage temperature;
2. Heating process: emulsions were heated to 10°C higher than the storage temperature then back to the storage temperature.

3.3.4.1 Study on Different Dilution Agent

In this study, the sample was not prepared by diluting the nanoemulsions by their parent continuous phase. The nanoemulsions were diluted by a continuous phase, which has higher or lower electrolyte concentration than the parent continuous phase. For example, 0.01M NaCl nanoemulsions were diluted by milliQ water, 0.001M NaCl and 0.1M NaCl solution. Other than the difference in the dilution agent, all other pre-treatments (eg. thermal equilibrium time) or conditions (eg. measured temperature) for measuring the droplet size and PDI remained unchanged.

3.3.5 Cryo-Scanning Electron Microscope (cryo-SEM)

Cryo-SEM was carried out to confirm the result measured from DLS and also to have a visualised result of the structures of the nanoemulsions.

Samples were examined in a Philips XL30 Field Emission Scanning Electron Microscope, equipped with an Oxford Instruments CT1500 HF Cryotransfer Stage.

A small amount of sample was transferred to a rivet using a syringe, leaving a drop of sample sitting on the top of the rivet. It was then rapidly frozen in nitrogen slush (-210°C) in a vacuum system, and transferred under vacuum to the preparation chamber. The frozen sample was fractured using a scalpel blade in the preparation chamber. The temperature of the sample was raised to -92°C, and held there for approximately two minutes, to allow small amount of surface ice to sublime away, leaving the features of interest in relief. The temperature was then lowered to -110°C (at which sublimation has stopped), and the sample coated with platinum (approximately 2nm) to make it electrically conductive. It was then loaded onto the microscope stage (held at a temperature lower than -150°C) and examined at accelerating voltage of 10-15kV.

4.0 Results and Discussions

The results obtained from this research are presented and are discussed in this section. The results are presented in graphically, as figures or in a table form. There are eleven sub-sections starting from system consistency (section 4.1) to the effect of storage temperature (section 4.11). The sub-sections are started from the basic and evolved into more depth to investigate the effects of sodium chloride and calcium chloride in the formation and stability of n-dodecane nanoemulsions produced by the PIT method.

4.1 System consistency

An emulsion system was heated to W/O emulsions and cooled to O/W emulsions and the dynamic conductivity of the emulsions was monitored. The conductivity curves for heating and cooling were used to determine the consistency of the emulsion system, if those curves were overlapped.

As shown in Figure 4.1 and Table 4.1, in the heating process, the conductivity was slightly increased from 863 μ S/cm to 926 μ S/cm (first maximum) when temperature was increased from starting temperature (16 $^{\circ}$ C) to 22.7 $^{\circ}$ C. After 22.7 $^{\circ}$ C, the conductivity was dropped to 83.85 μ S/cm (first minimum) at 27 $^{\circ}$ C before it reached the second maximum conductivity of 313 μ S/cm at 27.8 $^{\circ}$ C. After the second maximum, it started to drop again and reached zero at 30.7 $^{\circ}$ C. The PIT temperature in the heating process was an average temperature between the temperatures at first maximum conductivity and at zero conductivity, which was 26.7 $^{\circ}$ C (average of 22.7 $^{\circ}$ C and 30.7 $^{\circ}$ C).

In the cooling process, the system was cooled from 31.7 $^{\circ}$ C (final temperature in heating process) to 16 $^{\circ}$ C (starting temperature in heating process). The zero conductivity was existed from 31.7 $^{\circ}$ C to 31.1 $^{\circ}$ C, where the conductivity started to increase. At 28.2 $^{\circ}$ C, it reached the second maximum conductivity, which was 323 μ S/cm. After the second maximum, it dropped to the first minimum, which was 82.15 μ S/cm at 27.4 $^{\circ}$ C. The conductivity was then increased after 27.4 $^{\circ}$ C when the system was continuously cooled and reached a maximum (first maximum) at 24 $^{\circ}$ C with conductivity was 936 μ S/cm. From 24 $^{\circ}$ C to 16 $^{\circ}$ C, the conductivity was decreased to 874 μ S/cm from 936 μ S/cm. The PIT temperature in cooling process was 27.5 $^{\circ}$ C (average of 31.1 $^{\circ}$ C and 24 $^{\circ}$ C).

The both curves were almost overlapped with the cooling process slightly moved to the right, in the conductivity measurement (Figure 4.1). The heating and cooling rate were same (0.24 $^{\circ}$ C/min) to minimise the effects in the process, as the rate has been suggested to cause the variation in PIT temperature (Marquez *et al.*, 2003). The difference in the PIT temperatures between the heating and cooling processes is 0.8 $^{\circ}$ C, which was considered negligible if it was compared to previous published work (Marquez *et al.*, 2003), which there was a PIT temperature of 9 $^{\circ}$ C between heating and cooling process in kerosene-brine-Tween systems. As shown in Figure 4.1, the differences in the conductivity curve mostly existed in WIII (where k was from first maximum to first minimum) and WII region (where k was from second maximum to second minimum). It was because of the conductivity insensitivity in LLC (or L₃) phases and in concentrated W/O emulsions. This insensitivity would cause the delay in the conductivity reading. High viscosity was expected in LLC (or L₃) and in

concentrated W/O emulsions (Sole *et al.*, 2006). The high viscous fluid would increase the dragging force of the emulsions due to the magnetic stirrer and therefore increasing difficulty to well-stir the system. Furthermore, when diluted O/W emulsions was changed to concentrated W/O emulsions (by increasing system temperature), the number of droplets was increased (Esquena *et al.*, 2003), and thus the heating lag time was expected (Marquez *et al.*, 2003).

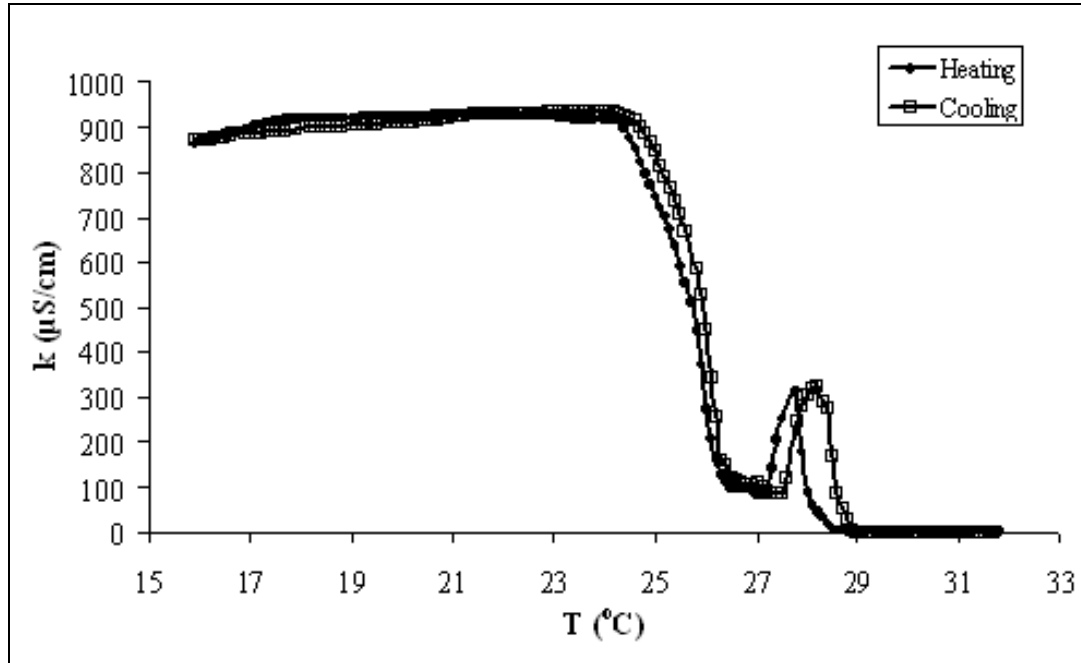


Figure 4.1: Dynamic conductivity curves for system with R=0.2, 0.01M NaCl, 4wt% Brij30 in the heating and cooling processes.

Table 4.1: Important data extracted from Figure 4.1 to determine PIT temperatures for heating and cooling processes.

	1 st maximum		1 st minimum		2 nd maximum		Zero k	PIT
	k (μS/cm)	T (°C)	k (μS/cm)	T (°C)	k (μS/cm)	T (°C)	T (°C)	(°C)
Heating	926	22.7	83.85	27	313	27.8	30.7	26.7
Cooling	936	24	82.15	27.4	323	28.2	31.1	27.5

Since the PIT temperatures were found to be considered consistent in the heating and cooling processes from this work, one of the processes (heating process) would only be carried out to determine the PIT temperature in the following studies.

4.2 Effect of heating rate

The PIT method is a method involving the change in temperatures (energy) of an emulsion system, therefore incurring different energy rate will alter the phase

inversion process and the phases involved in the process. It has been found that a hysteresis zone (a formation of LLC or L_3) is presented in a kerosene-brine-Tween system during the phase inversion process (in either heating or cooling process), and the standard PIT temperature for the emulsion system is affected by the zone (Marquez *et al.*, 2003).

In this study, only heating process was investigated as it was the only process to be carried out to determine the PIT temperature after the study from Section 4.1 and to produce O/W nanoemulsions. Figure 4.2 indicates the temperatures for second maximum and second minimum were higher when the heating rate was increased. For example, the temperature at which the second maximum appeared was increased to 26.2°C and 27.0°C from 25.3°C, when the heating rate was increased to 0.006°C/s and 0.007°C/s from 0.005°C/s. The phase inversion process was shifted to right (higher temperature) when heating rate increased thus the PIT temperature was increased when heating rate was increased. The difference between the PIT temperatures was 1.5°C with a difference of 0.002°C/s in heating rate. The changes in the phase inversion process and PIT temperature with the heating rate have been suggested in Marquez *et al* (2003) and found behaving similarly with current results; although different non-ionic surfactants was used in their works. However, the shapes of the conductivity curves with the existence of first maximum, first minimum, second maximum and second minimum remained unchanged. It was expected there were same phases produced during the phase inversion process while the heating rate was increased.

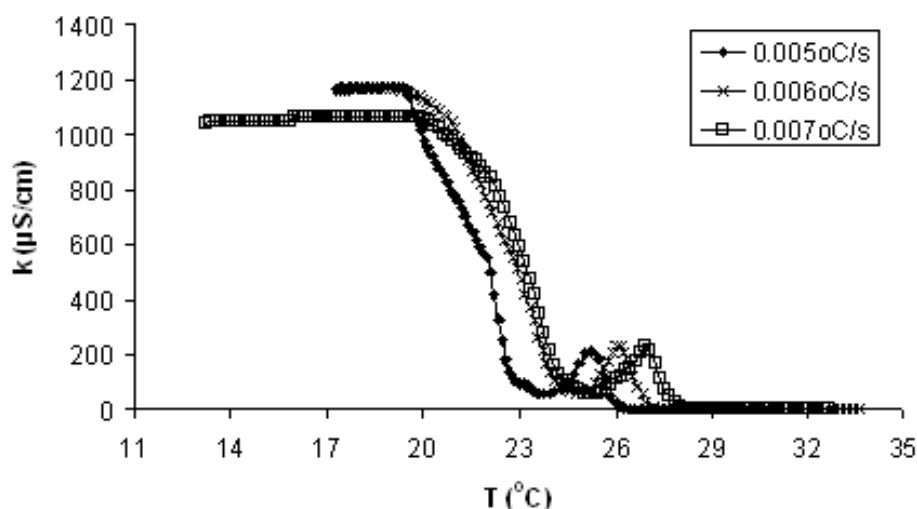


Figure 4.2: Dynamic conductivity value as a function of temperatures for an emulsion system containing 0.01M CaCl_2 and 6wt% Brij30 in different heating rate. Oil/aqueous=20/80 w/w.

A temperature transition, ΔT_{trans} , is defined as a temperature difference between temperatures at highest and lowest conductivity value. ΔT_{trans} can be determined from the same conductivity curve which is used to determine PIT temperature by subtracting the temperature of second minimum by the temperature of first minimum, which is shown in Figure 4.3. ΔT_{trans} represents the width of the transitional zone between O/W and W/O emulsions and is linked with the surfactant concentration at water/oil interface. It has been found that a larger and more stable transitional microemulsions zone is held at a higher surfactant concentration (higher ΔT_{trans})

(Anton *et al.*, 2007). The PIT temperature and ΔT_{trans} were higher when the heating rate was higher (Figure 4.2 and Table 4.2). Although there was a small decrease in ΔT_{trans} when the heating rate was from 0.006°C/s to 0.007°C/s , the decrease (0.3°C) was relatively small compared to the increase from 0.005°C/s ($0.9\text{-}1.2^\circ\text{C}$). The increase in ΔT_{trans} showed that more n-dodecane was solubilised and the transitional zone was more stable, when the energy rate was higher and faster. Furthermore, faster energy rate also prevented the breakdown of the structures in the transitional zone. As there were no first minimum and second maximum formed in previous work (Marquez *et al.*, 2003), no comparison in ΔT_{trans} can be done.

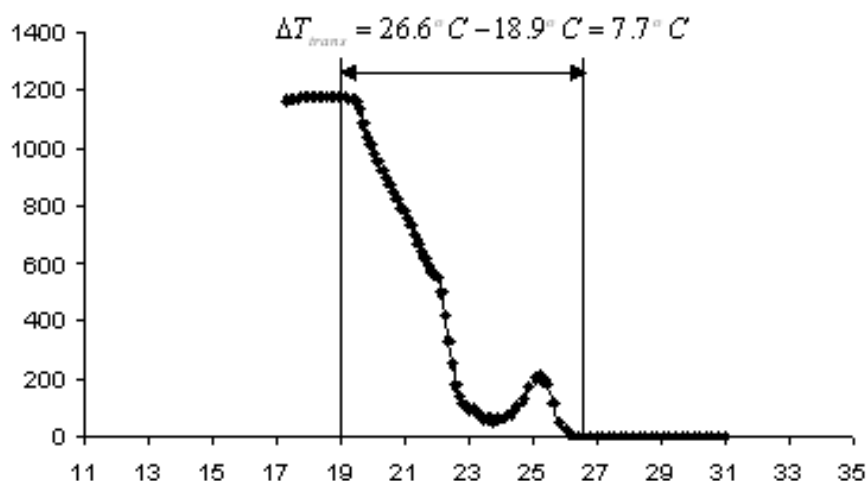


Figure 4.3: Dynamic conductivity value as a function of temperatures for an emulsion system containing 0.01M CaCl_2 and $6\text{wt}\%$ Brij30 with heating rate is 0.005°C/s . Oil/aqueous= $20/80$ w/w.

After the effects of heating rate on phase inversion process had been investigated, an alcohol was added into the system. It has been reported that the hysteresis zone (LLC or L_3) could be removed and standard PIT temperature could be determined by the addition of alcohol (Marquez *et al.*, 2003). Ethanol (EtOH - $\text{C}_2\text{H}_5\text{OH}$) was chosen in current research as it has been suggested that the effect on the physical chemistry of an emulsion system is essentially nothing with any alcohol (Marquez *et al.*, 2003). In this research, 1.2 vol% of EtOH was added into an emulsion system with $7\text{wt}\%$ Brij30. A pure milliQ water system instead of brine system was used as continuous phase, while as the heating rate was remained the same in the systems with and without EtOH, to prevent any other factors (salting-out by NaCl etc) to affect the phase inversion process.

As shown in Figure 4.4, the conductivity curve was shifted to left (lower temperature) when 1.2 vol% of EtOH was added into an emulsion system with $7\text{wt}\%$ Brij30 and R was 0.2 . The heating rate for the systems with and without EtOH was 0.0065°C/s . Although the curve was moved, the shape of the curves (existence of the maximum and minimum) was the same in the systems with and without EtOH. Thus, the existence of WI, WII and WIII during the phase inversion process was involved in both systems. Furthermore, the PIT temperatures for both systems are shown in Table 4.2, which they were 21°C and 21.1°C , in system with and without EtOH, respectively. The phases involved in phase inversion process and PIT temperatures are almost unchanged and it may be because of the low alcohol (EtOH) concentration (1.2 vol% in $7\text{wt}\%$ Brij30) while the sec-butanol concentration was relatively higher in Marques

et al (2003) (5 vol% in 5wt% of Tween) to eliminate the hysteresis zone. As the surfactant concentration was comparative higher than the EtOH concentration in this study, the effect from surfactant on phase inversion process and PIT temperature was more significant. From the obtained results, the hysteresis zone could not be removed by 1.2 vol% EtOH in an emulsion system with 7wt% Brij30 and R was 0.2, in the heating process.

Table 4.2: PIT temperature and width of transitional zone for systems with different heating rate or for systems with and without ethanol.

	PIT (°C)	ΔT_{trans} (°C)
0.01M CaCl ₂ , 6wt% Brij30, R=0.2		
+0.005°C/s	22.7	7.7
+0.006°C/s	23.4	8.9
+0.007°C/s	24.2	8.6
Pure miliQ water, 7wt% Brij30, R=0.2 (+0.0065°C/s)		
No EtOH	21.0	9.8
With EtOH (1.2vol%)	21.1	8.1

Although the hysteresis zone could not be removed by the addition of 1.2 vol% EtOH, the ΔT_{trans} was decreased to 8.1°C from 9.8°C, when EtOH was added. It may be because the solubilisation of n-dodecane was higher in the absence of ethanol. Furthermore, it also suggested that the hysteresis zone was reduced when EtOH was added (lower ΔT_{trans} in Table 4.2), which agreed with the fact that the hysteresis zone could be removed at higher EtOH concentration. However, the effect of higher EtOH concentration was not investigated in this research and would be left as a recommendation for future research. As the system without EtOH has higher ΔT_{trans} and the PIT temperature was almost the same with the one in system with EtOH, systems without ethanol were used to determine the formation and stability of nanoemulsions in following studies. Furthermore, the LLC phase (hysteresis zone) has been suggested as an essential process to produce stable emulsions (Marquez *et al.*, 2003; Morales *et al.*, 2003).

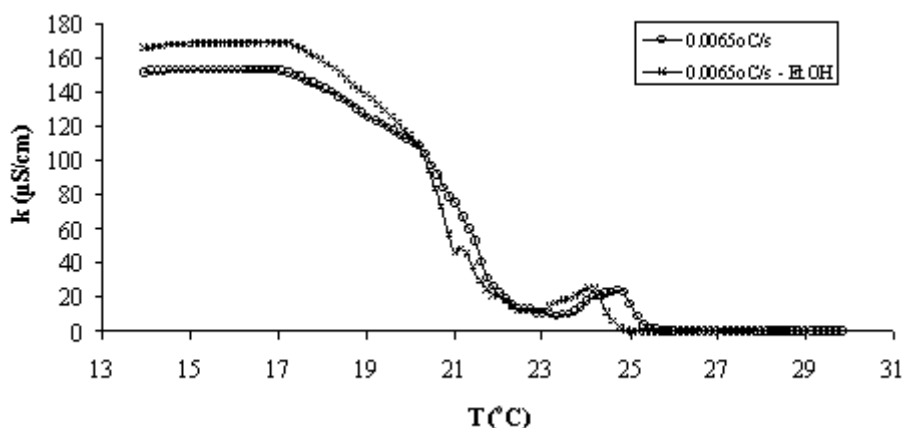


Figure 4.4: Dynamic conductivity value as a function of temperatures for systems with and without ethanol (1.2 vol%). Continuous phase is pure milliQ water and with 7wt% Brij30. Oil/aqueous=20/80 w/w.

4.3 Phase behaviour

In order to utilise the PIT method to produce aqueous-continuous nanoemulsions, the phase equilibria of the emulsion system with temperatures needs to be determined. The determination of phases involved during the phase inversion process can be done either by measuring emulsions conductivity with temperatures or by a detailed study from a microscopy method.

The explanation about the continuous change of conductivity of emulsion with temperature (dynamic conductivity) during the phase inversion process has been discussed in Kunieda *et al* (1996) and Anton *et al* (2008). However, Kuneida and co-workers (1996) pointed out that the first drop in the curve was due to the formation of lamellar liquid crystal phase (LLC) and the second peak was due to the formation of bicontinuous phase (L_3); while Anton *et al* (2008) suggested that the first drop was due to the formation of L_3 and the second peak was due to the formation of LLC phase. Therefore, it is necessary to find out the real mechanism for the phases involved in the phase inversion process for present study.

System with $R=0.2$, 0.01M NaCl, 6wt% Brij30 was chosen to identify the phases involved in the phase inversion process. The dynamic conductivity with temperatures is shown in Figure 4.5 and was found to have similar trend with reported works (Kunieda *et al.*, 1996; Izquierdo *et al.*, 2002; Izquierdo *et al.*, 2004) (Anton *et al.*, 2008). The 1st maximum conductivity appeared at 17.9°C and the 2nd maximum one appeared at 25.9°C. In between the 1st and 2nd maximum conductivity, the 1st minimum appeared at 24.5°C. The conductivity of the system reached zero at 27.8°C. The high-conductivity system at temperatures lower than 17.9°C is due to the formation of O/W emulsions (WI type) and with Brij30 is more hydrophilic; while the zero-conductivity system at temperatures higher than 27.8°C is due to the formation of W/O emulsions (WIII type), where oil acts as a continuous phase. During the phase inversion process (WIII type), there were some structural changes in the emulsion system and the arrangement of the surfactant on the oil-water interface was changed

from 17.9°C to 27.8°C; and the processes in WIII region have caused the increase and decrease in the conductivity.

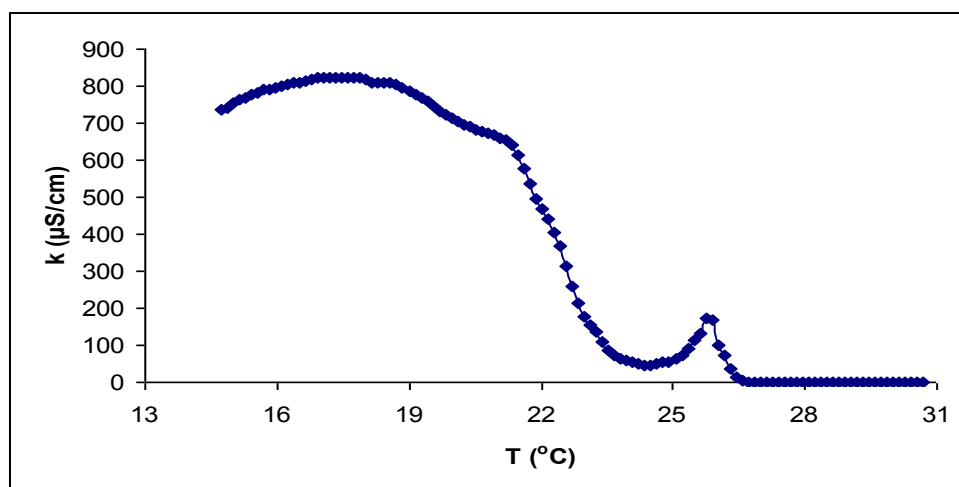


Figure 4.5: Dynamic conductivity behaviour as a function of temperatures for emulsion system with R=0.2, 0.01M NaCl, 6wt% Brij30.

Some important properties from Figure 4.5 were extracted and listed in Table 4.3. A phase equilibrium experiment was carried out at different temperatures to study in more details on the phases produced during the inversion process. The chosen temperatures for the phase equilibrium experiment are shown in Table 4.3, which were based on the dynamic conductivity behaviour of the emulsion system in Figure 4.5. Moreover, the reasons to choose the temperatures are shown in Table 4.3, which are written beside the chosen temperatures. The phases involved during the phase inversion process are shown in Figure 4.6. There were O/W emulsions with droplet size was 2μm in diameter at 14°C (Figure 4.6(a)). As no structural images were shown in Figure 4.6(b) & (c), they were microemulsions (due to the limited magnification of Leica As LMD) as they were appeared to have high conductivity values at 18°C to 22°C. When the conductivity was close to the first minimum value, LLC appeared at 24°C (Figure 4.6(d)) under polarising light, where the structure was the same with previous work (Anton *et al.*, 2008). At 25°C, where the conductivity was close to the second peak, there were polydispersed emulsions with low stability produced, which is clearly shown in Figure 4.6(e). Figure 4.6(f) showed no structural image at 26°C. With the low conductivity value, it was expected to be bicontinuous phase or the emulsions produced at 25°C were “solubilised” at 26°C before a W/O emulsions were formed at 30°C (Figure 4.6(g)). A “concrete” liked structure was shown in Figure 4.6(g) as concentrated W/O emulsions were produced from dilute O/W emulsions, when temperature was increased.

However, the determined phases from phase equilibrium experiments were not comparable to the phases involved in the dynamic conductivity behaviour, as the LLC or L₃ phase formed at equilibrium appeared after a few minutes, thus those phases may be formed after a delay or may not be formed under continuous stirring (Anton *et al.*, 2008). Additionally, a homogeneous (isotropic) system is needed in order to measure its conductivity; the anisotropic phase in LLC is expected to give an incorrect conductivity in the dynamic condition.

Table 4.3: Some important properties in dynamic conductivity behaviour of system with R=0.2, 0.01M NaCl, 6wt% Brij30 and their corresponding temperatures. The third column shows the chosen temperatures to investigate the phase inversion under optical microscopy.

Important Property	Temperature (°C)	Chosen Temperature for Optical Microscope (°C)
1 st maximum	17.9	14 (o/w emulsions)
1 st minimum	24.5	18 (1 st max.)
2 nd maximum	25.9	22 (1 st falling)
2 nd minimum	27.8	24 (near 1 st min.)
		25 (near 2 nd max.)
		26.5 (2 nd falling)
		30 (w/o emulsions)

Although a direct linkage between those two experiments (dynamic and static state) could not be drawn, the basic idea for causing the decrease and increase of conductivity in dynamic behaviour during phase inversion process could be understood. As the affinity of a non-ionic surfactant changes with temperatures, there are water-continuous emulsions at low temperatures to create a high-conductivity system (14°C in Figure 4.6(a)). When the temperature increases, the curvature of the surfactant monolayer is gradually changes from positive to negative. Before the system was totally converted to oil-continuous emulsions (30°C in Figure 4.6(g)), there were two or more phases appear to create the first-falling and second-peak in the conductivity curve. From the phase equilibrium images, there were microemulsions, LLC and L₃ phases between the O/W and W/O emulsions, and they were expected to be formed as a single phase or multiphase in dynamic behaviour. However, equilibrium time was necessary to form the transitional phases; some similar structures were expected to be formed in the dynamic behaviour. As the LLC has a sandwich-like structure (Figure 2.3) and water is mostly trapped and so to obstruct the conducting potential (Kunieda *et al.*, 1996), a LLC-liked structure was expected to be formed at the first-falling. L₃ phase always has higher conductivity value than LLC phase (Forgiarini *et al.*, 2001) but lower than O/W emulsions due to its flexible surfactant bilayer (Figure 2.3), thus the existence of second peak may be because of a L₃-liked structure. Furthermore, results from phase equilibrium are well agreed with dynamic behaviour at low and high temperatures, where there were O/W emulsions before 17.9°C and W/O emulsions after 27.8°C.

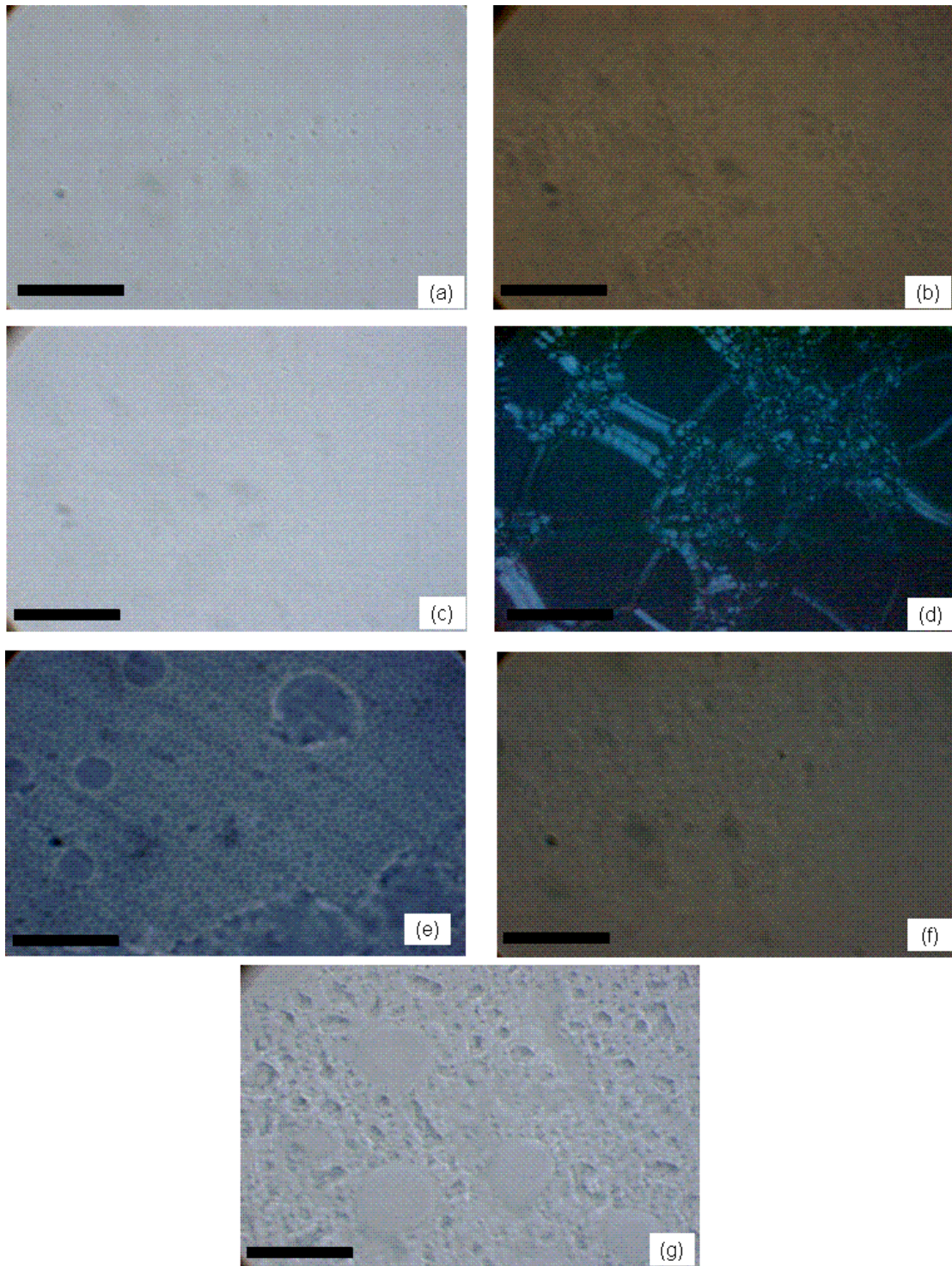


Figure 4.6: Optical micrograph (Leica As LMD) of emulsions undergoing a phase inversion, between crossed polarizer and analyser. The system contains 0.01M NaCl, 6wt% Brij30, 18.8wt% n-Dodecane. Temperatures are (a) 14°C, o/w emulsions, (b) 18°C, close to 1st maximum, (c) 22°C, 1st falling, (d) 24°C (polarised), close to 1st minimum, (e) 25°C, close to 2nd maximum, (f) 26°C, 2nd falling, and (g) 30°C, concentrated w/o emulsions. The scale bars indicate 50µm except in (a) is 20µm.

4.4 Effect of quality of the continuous phase

Tap water ($k=618 \mu\text{S}/\text{cm}$), reverse osmosis (RO) water ($k=94.6 \mu\text{S}/\text{cm}$) and milliQ water ($k=1.42 \mu\text{S}/\text{cm}$) were used to prepare a continuous phase with 0.01M sodium chloride (NaCl), for producing n-dodecane emulsions with 19.2wt% oil and 4wt% Brij30. The objective of this experiment was to investigate the effect of the quality of continuous phase on the production of O/brine (nano)emulsions.

Figure 4.7 shows the phase inversion process (dynamic conductivity behaviour) for n-dodecane emulsions in 0.01M NaCl with different quality of water but fixed oil and surfactant concentrations. Tap water has the highest initial conductivity value as it contains more contaminants or salts than RO and milliQ water, the common salts existed in tap water are Ca^{+2} and Mg^{+3} . The figure shows the temperature to start the phase inversion process (temperature at 1st maximum) was increased from 22.7°C to 28°C when the continuous phase was changed from tap water to milliQ water. However, the phases involved in the phase inversion process were the same with different continuous phase as the trend of curves were same. The conductivity of brine made from milliQ water was higher than the one from RO water at all temperatures, as shown in Figure 4.7. It may be attributed to the higher purity of milliQ water and therefore ions of Na^+ and Cl^- is easier to bond with water molecule in milliQ than RO causing higher effect in conductive potential in milliQ.

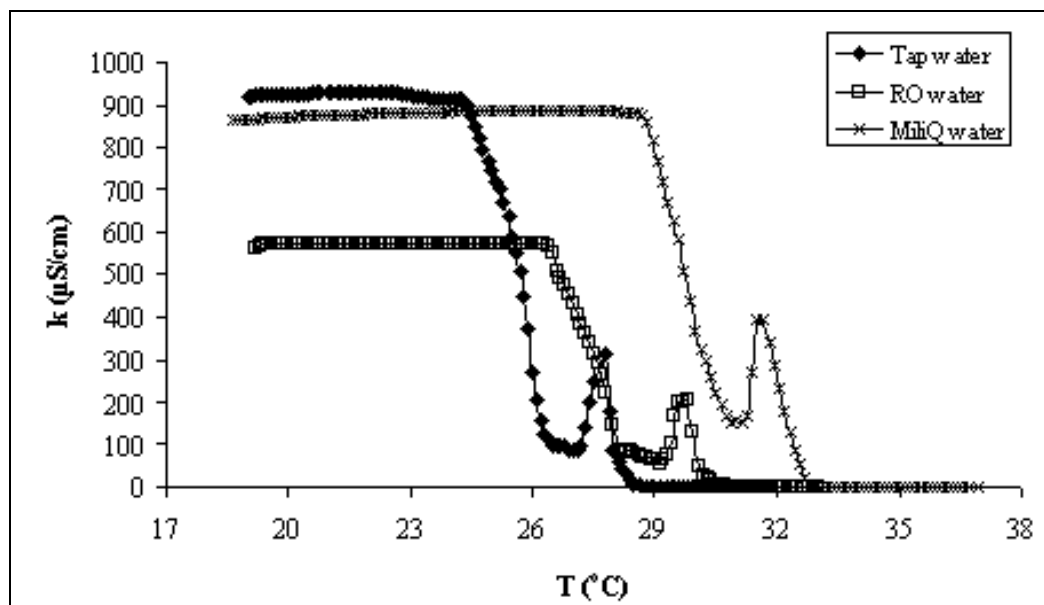


Figure 4.7: Dynamic conductivity as a function of temperatures in n-dodecane/brine emulsions with different quality of water at 19.2wt% of oil, 4wt% of Brij30 and 0.01M NaCl.

The conductivity curve was shifted to the right (higher temperature) (Figure 4.7) when water quality is higher (from more contaminants to less contaminant) showing that the formation of WIII region was delayed. Due to the salt (electrolyte) concentration was increased from milliQ water to tap water, the hydrophilic tail of the non-ionic surfactant was dehydrated and so the formation of W/O was more preferable, thus the formation of W/O from O/W emulsions was hastened (Shinoda and Takeda, 1970; Kunieda *et al.*, 1989; Anton *et al.*, 2007). Furthermore, the PIT temperature was decreased around 4°C from milliQ water to tap water, where the PIT temperatures for different continuous phase are shown in Table 4.4. Table 4.4 also

shows ΔT_{trans} and the conductivity ratio between 1st maximum and 2nd maximum, which indicates the completeness of the formation of L₃ phase. The reduction of PIT temperature was attributed to the dehydrated non-ionic surfactant and the reduced solubility of the surfactant in aqueous phase (Shinoda and Takeda, 1970). The reduction was considered small if it was compared to the 14°C drop in an emulsion system with a sodium chloride concentration was 6wt% in Shinoda and Takeda (1970). In current study, the lower total concentration of salt (0.02wt% NaCl equivalent) has caused the smaller reduction in the PIT temperature. Moreover, the different kinds of salt (more than Na⁺ and Cl⁻) and other contaminant (particles) presented in tap water also affected the smaller reduction in the PIT temperature.

In Kunieda *et al* (1996), the conductivity ratio between 1st maximum and 2nd maximum in a decane was reported as 1.67, which was smaller than the ratio obtained in current study. However, the results were not comparable due to the different investigated system which was n-dodecane system in current study. As the value of 2nd maximum represented the formation of L₃-liked structure in WIII region, the lower ratio showed that the formation of the structure was depressed, by either salts or contaminants in tap water. The ratio decreased when the quality of water increased (Table 4.4), and thus the formation of transitional phase (L₃ phase) was more significant in the system with salt/salts (Bellocq, 1996). As the transitional phase (WIII region) was more significant in tap water, the transitional temperature (ΔT_{trans}) was the highest among the three systems (Table 4.4). Furthermore, the contaminants (particles) in tap water might obstruct the heat absorption of emulsions to proceed the inversion and thus causing a longer ΔT_{trans} .

Table 4.4: Some significant characteristics from phase inversion process in emulsions with different water quality as continuous phase. (19.2wt% n-dodecane, 4wt% Brij30 and 0.01M NaCl)

Water Quality	PIT (°C)	$\frac{1^{st} \text{ max}}{2^{nd} \text{ max}}$	ΔT_{trans} (°C)
Tap water	26.7	2.96	8.0
RO water	28.8	2.76	5.0
miliQ water	30.9	2.23	5.7

From the results above, they have shown that salt (in tap water) does have effect on the formation of emulsions (phase inversion process, PIT, ΔT_{trans} , etc). This leads to a further investigation in the effect of salt on the production of nanoemulsions. As in most scientific fields, water with high quality is used to produce nanoemulsions to prevent any contamination; milliQ was chosen to be used in the following experiments.

4.5 Effect of NaCl concentration at fixed oil and surfactant concentrations

The aim of this experiment was to investigate the effect of the concentration of sodium chloride in an emulsion system containing 19.2wt% of n-dodecane and 4wt% of non-ionic surfactant (Brij30). This experiment was initially done by an increment of 0.02M in sodium chloride concentration from 0.01 to 0.05M, and then followed by an extra experiment to investigate the effect of the 0.1M NaCl system.

4.5.1 Phase Inversion Process

The dynamic conductivity curves for systems with different sodium chloride concentrations from zero to 0.1M are shown in Figure 4.8. In all systems, the trend of the conductivity curves was the same with the existence of 1st maximum, 1st minimum, 2nd maximum and zero conductivity. Therefore, from zero to 0.1M NaCl system, the phases involved in the phase inversion process did not change with the addition of sodium chloride or concentration. Although the 0.1M NaCl was doubled the concentration than those systems with systematic change in concentration (0.01-0.05M NaCl), the 0.1M NaCl was still considered as low concentration. It was because it has been suggested that the change of the phase behaviour is insignificant in a low concentration of electrolyte (Eicke *et al.*, 1994). The initial conductivity value was increased with sodium chloride concentration due to the increase of ions in the solution. When sodium chloride was firstly introduced into a pure milliQ water system, the temperature of 1st maximum conductivity was decreased to 27°C from 29.2°C (Figure 4.8). Therefore, a lower PIT temperature was expected in brine system as PIT temperature is determined by the temperatures at 1st maximum and zero conductivity. However, when the concentration of sodium chloride was increased, the temperature of 1st maximum conductivity was increased to 27.3°C at 0.1M NaCl from 27°C at 0.01M NaCl (Figure 4.8), indicating the PIT temperature was expected to be increased with salt concentration. From this experiment, system without sodium chloride was found to have conductive potential. It was because the non-ionic surfactant, Brij30, was in technical grade (Morales *et al.*, 2003). The sodium chloride, which has been widely used by researchers in an oil/non-ionic surfactant/water emulsion system, is unnecessary if only the PIT temperature is desired to be determined.

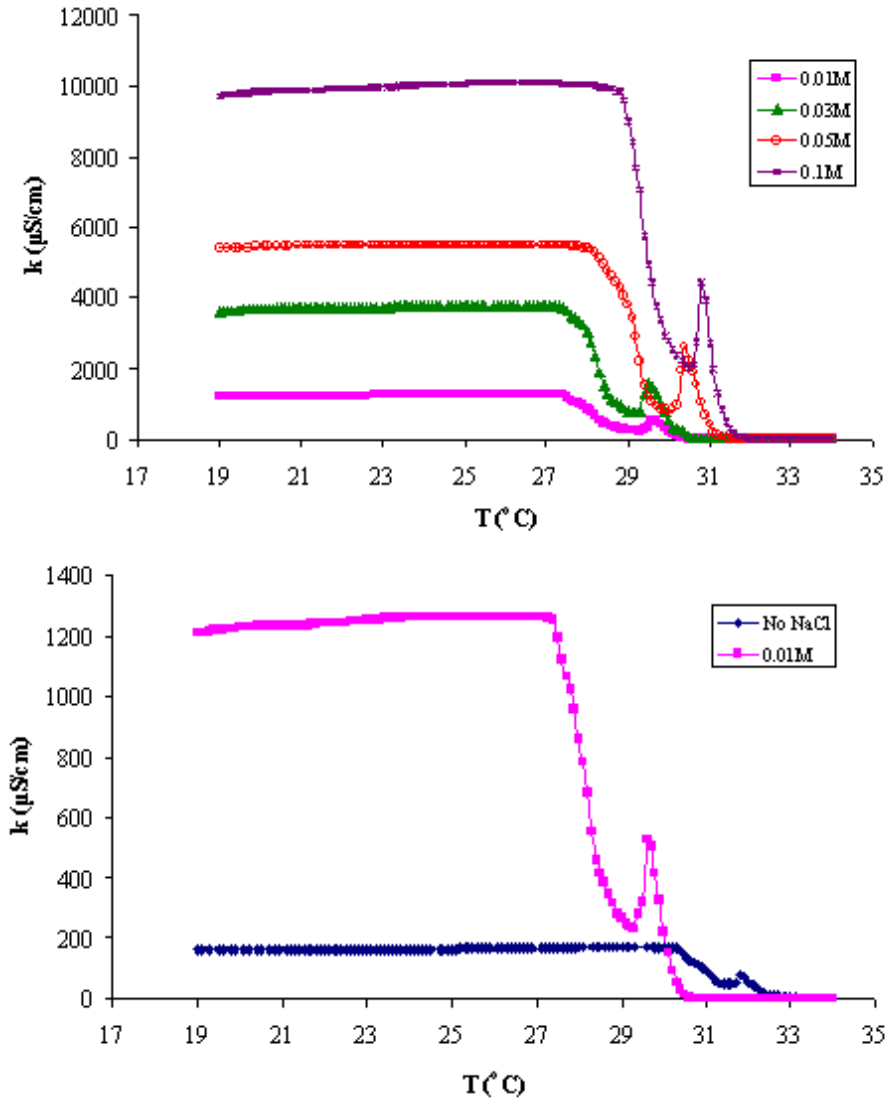


Figure 4.8: Dynamic conductivity as a function of temperatures in n-dodecane emulsion system with 19.2wt% of oil, 4wt% of Brij30 and different salt concentrations.

Figure 4.9 shows the PIT temperature and ΔT_{trans} as a function of the concentration of NaCl for the emulsions with 19.2wt% n-dodecane and 4wt% Brij30. The figure indicates the PIT temperature was decreased 2.3°C and ΔT_{trans} were decreased 0.3°C when sodium chloride was initially added. However, the PIT temperature remained constant while ΔT_{trans} was increased from 4.4°C to 5.6°C when the NaCl concentration was increased from 0.01M to 0.1M. The PIT temperature for 0.01M NaCl in this experiment was 29.2°C, which was different with the 45.5°C published in Izquierdo *et al* (2002), however, the result showed similarity with other researchers (Pourjavaheri-Jad *et al.*, 2009). This difference may be attributed to the different quality of n-dodecane, non-ionic surfactant and sodium chloride as well as the sensitivity of the conductivity meter. In present experiment, the average temperature for PIT was 30.1°C with a standard deviation was 0.9°C. The PIT was generally lower in a brine system than in a pure milliQ water system, which agreed with the fact suggested by Shinoda and Takeda (1970). The change in the PIT temperature with NaCl concentration was different with the work done by Shinoda and Takeda (1970), where the PIT temperature was continuously decreased while increasing the NaCl

concentration. Furthermore, the maximum reduction of PIT temperature between the pure milliQ water and brine systems was 1.8°C in current study, which was considerable small compared to the 14°C published by Shinoda & Takeda (1970). In their works, the sodium chloride concentration was up to 6wt% (1M NaCl) while only concentration up to 0.15wt% (0.1M NaCl) was conducted in present study. Moreover, n-dodecane was used in present study while cyclohexane was used in their work. The chemical structures of those oils are different where n-dodecane is a straight-chain alkane while cyclohexane consists of a 6 vertexed ring. The results from both works were incomparable. The reduction in the PIT temperature has been suggested as being caused by the dehydration of hydrophilic tail in a non-ionic surfactant (Shinoda and Takeda, 1970; Anton *et al.*, 2007). The interaction between water and the non-ionic surfactant is weakened after the surfactant has been dehydrated.

From the systems studied here, the transitional temperature (ΔT_{trans}) was between 4.4°C and 5.6°C (with a standard deviation 0.5°C) in the systems containing pure milliQ water or sodium chloride (Figure 4.9). As shown in Figure 4.9, ΔT_{trans} decreased 0.3°C from pure milliQ water system to 0.01M NaCl but it increased with NaCl concentration after that. From 0.03M to 0.1M NaCl, the ΔT_{trans} was higher than the one in pure milliQ water system. As the reduction from zero to 0.001M NaCl was small (with a 0.5°C standard deviation), the ΔT_{trans} was almost the same or higher in brine system than in pure milliQ water system. It has been suggested that the transitional zone only appeared in a system containing sodium chloride (Bellocq, 1996). Therefore, from present study, it was found that transitional zone was wider with the formation of phases in the zone was more complex, when the sodium chloride was added.

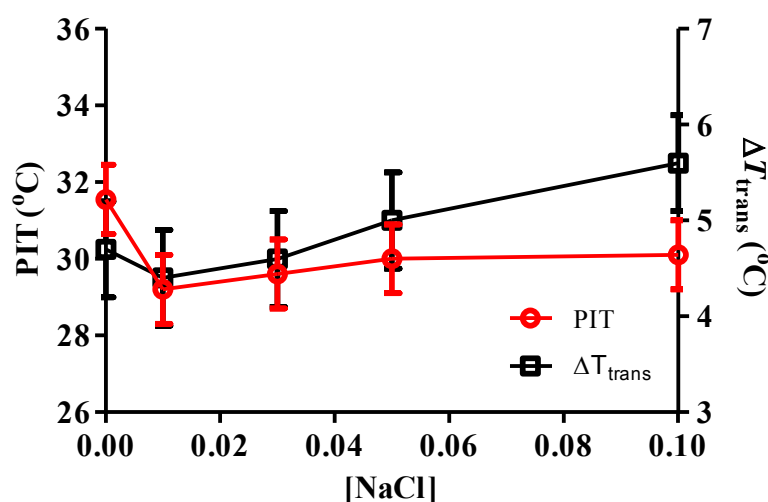


Figure 4.9: PIT and transitional temperatures as a function of salt concentrations of an emulsion system with 19.2wt% n-dodecane and 4wt% Brij30.

4.5.2 Formation and Stability of Nanoemulsions

n-Dodecane nanoemulsions were produced by gradually increase the temperature of n-Dodecane/Brij30/aqueous mixture from 19°C to 34°C (where the PIT temperature

was taken as 30.1°C) followed by a quenching process. The effect of sodium chloride on the formation of the nanoemulsions is shown in Figure 4.10 and Figure 4.11 for their initial size distribution (PSD) and initial number average droplet diameter and polydispersity indices (PdI). The size distribution is shown in logarithm scale as used in Malvern Zetasizer (Malvern, UK) and so this standard is followed up. Furthermore, logarithm scale has been used to discuss the formation of nanoemulsions by researchers (Fernandez *et al.*, 2004).

In Figure 4.10, the initial size distributions of n-dodecane nanoemulsions with 19.2wt% disperse phase and 4wt% Brij30 were monodisperse (single-peak curve). A higher and narrower distribution curve was found at the systems with NaCl concentration being 0.01M and 0.03M; while in pure milliQ water and 0.1M NaCl system, the distribution curves were lower and wider. The trends in Figure 4.10 are well agreed with the numerical results showed in Figure 4.11. Figure 4.11 shows the initial PdI and number average diameter of 19.2wt% n-dodecane nanoemulsions were lower in brine systems than in pure milliQ water system. The droplet size and PdI were dramatically decreased from 126nm and 0.074 in pure milliQ water system to 106nm and 0.043 in 0.01M NaCl system (Figure 4.10). From 0.01-0.1M NaCl, the droplet size and PdI were increased to 122nm and 0.068, where the 0.1M NaCl system produced the biggest droplets with highest PdI, which the same result was shown in Figure 4.10 with the widest PSD curve among the brine systems. In present study, from 0.03M to 0.1M NaCl, the initial droplet size and PdI remained almost constant, which were similar with reported work (Srivastava *et al.*, 2006).

The number average diameter for the systems with and without sodium chloride was ranged from 106-126nm, with the pure milliQ water system contained the largest droplets. The droplet size for the pure milliQ water system agreed with the result from Izquierdo *et al* (2002) although it was measured at different temperature, showing that the initial droplet size was independent on temperature as long as the nanoemulsions was produced by the PIT method (Shinoda and Saito, 1969). The smaller droplet size (lower PdI) (Figure 4.11) and better size distribution (Figure 4.10) in brine system were due to the lower interfacial tension caused by sodium chloride (Taylor & Ottewill, 1994). Theoretically, the interfacial tension decreases with the NaCl concentration and therefore smaller droplets are expected at higher concentration, however, it does not agree with the results from present study. It was because the addition of sodium chloride dehydrated Brij30 and the surfactant became less hydrophilic to produce stable aqueous-continuous nanoemulsion, thus promoted the increase of the droplet size and also the PdI, at higher salt concentration. Furthermore, Figure 4.10 clearly indicates the widest PSD appeared in 0.1M NaCl among brine systems as the concentration was too high to produce very fine aqueous-continuous nanoemulsions and started to produce oil-continuous emulsions. Although the addition of NaCl could improve the quality of m-dodecane nanoemulsions in terms of the droplet size and size distribution, it is needed to be done in moderate concentration, which has also been suggested by other researchers (Bink et al, 2006).

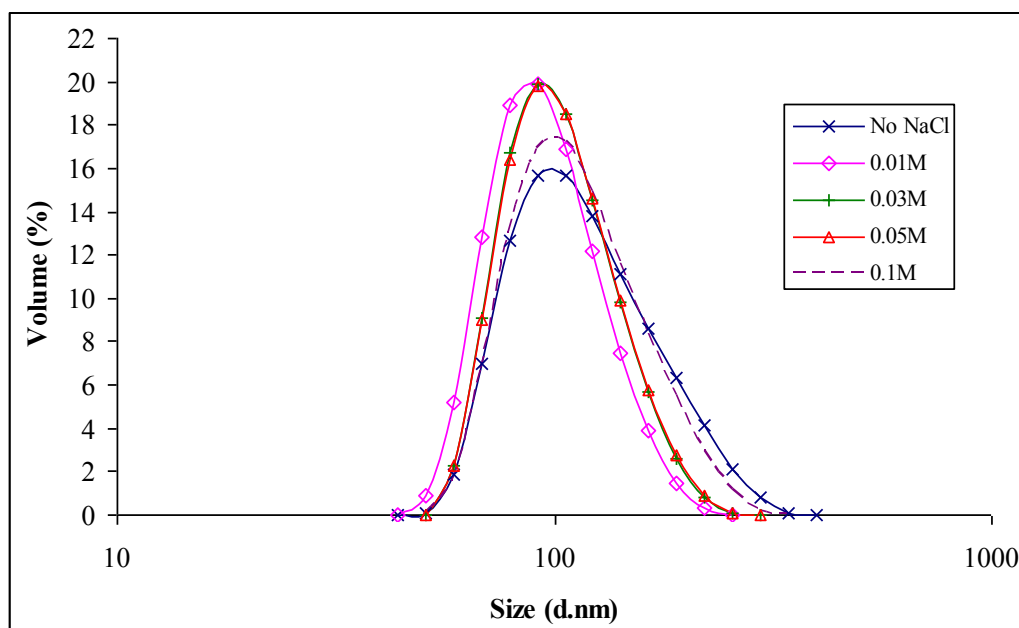


Figure 4.10: Initial size distribution (PSD) curve for emulsions with 19.2wt% n-dodecane, 4wt% Brij30 and from 0 to 0.1M NaCl. The measurement was done at 20°C.

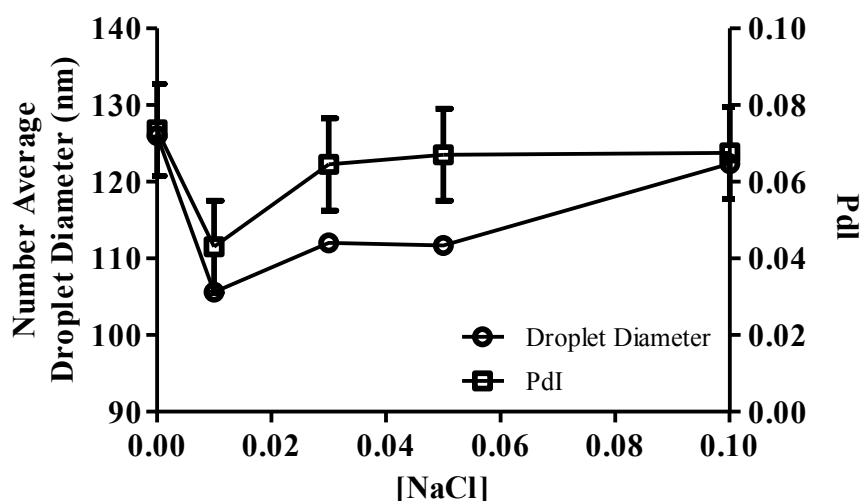


Figure 4.11: Initial number average droplet diameter and polydispersity index (Pdl) for emulsions with 19.2wt% n-dodecane, 4wt% Brij30 and from 0 to 0.1M NaCl. The measurement was done at 20°C.

In this study, stable nanoemulsions were expected to be remained in one phase and with no changes in droplet size and Pdl, for a period which was as long as possible. The droplet size and Pdl for nanoemulsions without phase separation were measured by the DLS technique while the phase separated nanoemulsions were considered as the droplets have been broken down and lost their stability. In present study, the growth of the droplet size and Pdl as a function of time, which was used as a tool to determine the stability of nanoemulsion, were done in a storage period of three days. It was because the systems containing sodium chloride with concentration at and more than 0.05M were phase separated in three days, after they had been produced (Table 4.5).

The growth of the droplet size and Pdl for the n-dodecane nanoemulsions as a function of sodium chloride concentration is shown in Table 4.5, with a 3-day storage period. From the table, the droplet diameter was 106-126nm with the largest droplet produced in the pure milliQ water system. After a three-day storage period, their droplet sizes were almost doubled (eg. from 106nm to 216nm in 0.01M NaCl system) and with the 0.1M NaCl system consisted of the largest droplets. In the system containing pure milliQ water as continuous phase, the Pdl was almost the same (lower than 0.1) for both freshly prepared and ageing (3 days) nanoemulsions (Table 4.5). However, in brine systems (0.01-0.1M NaCl), the Pdl was increased in five to thirteen folds from freshly prepared to 3-day ageing nanoemulsions (eg. from 0.043 to 0.551 (13 folds) in 0.01M NaCl system). Furthermore, the polydispersity (high Pdl) in brine systems for the 3-day ageing nanoemulsions is also shown in Figure 4.12, where there were double-peak PSD. For pure milliQ water system, the PSD curve for 3-day ageing nanoemulsions remained single peak (Figure 4.10).

From Table 4.5 and Figure 4.12, nanoemulsions with 19.2wt% n-dodecane and 4wt% Brij30 were stable (in a period of three days) when there was no sodium chloride added into the system, where the pure milliQ water system had the longest period to stay in one phase (10 days) and no changes in Pdl and PSD curve after the sample had been stored for three days. In systems containing sodium chloride, 0.03M NaCl system had the ability to maintain in one phase for 10 days which was the longest period among the systems from 0.01M to 0.1M NaCl. The Pdl was increased five folds from 0.065 to 0.305 in 0.03M NaCl, which was the lowest growth rate in three-days ageing O/brine nanoemulsions. Furthermore, in the PSD curve (Figure 4.12), the 0.03M NaCl had the highest first peak and the second peak was more left (smaller droplet size) among the brine systems. Therefore, according to the results from the growth rate in droplet size and Pdl and the PSD, the most stable nanoemulsions with 19.2wt% n-dodecane and 4wt% Brij30 was the system with continuous phase being pure milliQ water and 0.03M NaCl among the systems containing 0.01M to 0.1M NaCl.

Table 4.5: Stability of nanoemulsions with 19.2wt% n-Dodecane, 4wt% Brij30 and with different NaCl concentrations. The measurement was done and the samples were stored at 20°C.

[NaCl] (M)	Days to Phase Separation	d (nm)		Pdl	
		0 day	3 days	0 day	3 days
0	10	126	192	0.074	0.059
0.01	7	106	216	0.043	0.551
0.03	10	112	194	0.065	0.305
0.05	3	112	194	0.067	0.611
0.1	3	122	224	0.068	0.546

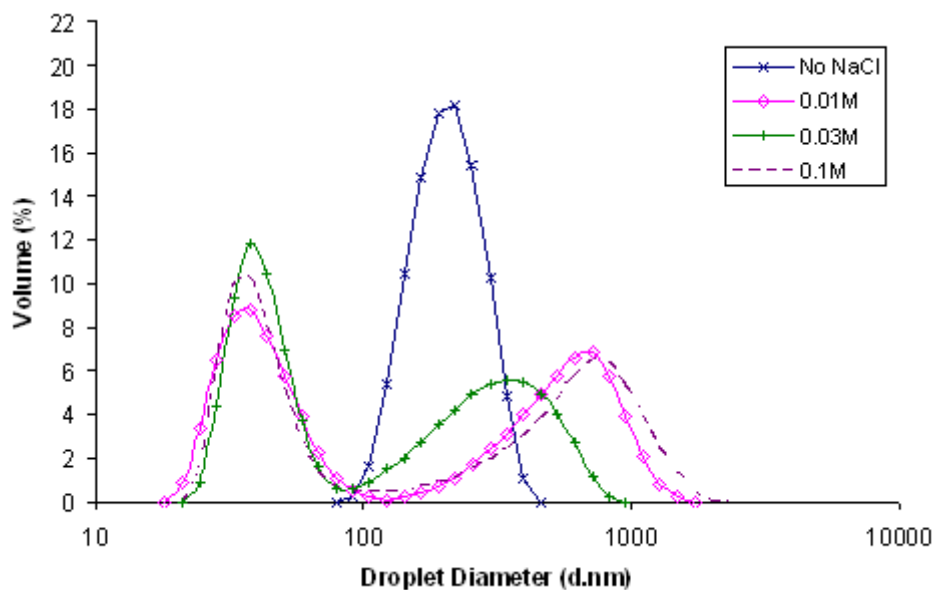


Figure 4.12: Size distribution (PSD) curve for 3-day ageing emulsions with 19.2wt% n-dodecane, 4wt% Brij30 and from 0 to 0.1M NaCl. The measurement was done at 20°C.

It has been well established by researchers that Ostwald ripening is the main mechanism for the instability of nanoemulsions (Taylor and Ottewill, 1994; Izquierdo *et al.*, 2002; Fernandez *et al.*, 2004; Izquierdo *et al.*, 2004). A straight line can be drawn in r_n^3 -t plot if Ostwald ripening is the major instability factor (Equation 1). Due to the fast phase separation in systems containing 0.05M and 0.1M NaCl (in 3 days), no ripening rate could be calculated. Straight lines were plotted in Figure 4.13 for nanoemulsions with and without sodium chloride, where the sodium chloride concentrations were 0.01M and 0.03M. In Figure 4.13, the data from pure milliQ water system fitted the straight line better than brine systems with the highest regression value (R^2) which is shown in Table 4.6. The slope of the straight line, which was also the Ostwald ripening rate according to Equation 1, was also calculated and shown in Table 4.6. The data started to scatter more (lower R^2) when sodium chloride was added or when the NaCl concentration was increased. Therefore, Ostwald ripening was the main destabilising factor in O/W nanoemulsions while other factors like flocculation or/and coalescence were part of the destabilising factors in O/brine nanoemulsions with 0.01M or 0.03M NaCl. Moreover, by analysing the results in Table 4.5, the PDI of n-dodecane/pure milliQ water nanoemulsions was independent of time, which also showed that Ostwald ripening was the main instability factor (Taylor and Ottewill, 1994; Taisne and Cabane, 1998; Porras *et al.*, 2004; Uson *et al.*, 2004). The increase ripening rate from pure milliQ water to 0.01M NaCl system (Table 4.6) may be because increase in bulk solubility of n-dodecane, which is higher than the decrease of interfacial tension in low electrolyte concentration; however, the increment of osmotic pressure retards the ripening rate in 0.03M NaCl system (Walstra, 1996). An Ostwald ripening rate of $9.3 \times 10^{-27} \text{ m}^3\text{s}^{-1}$ was reported at 25°C for water/Brij30/n-dodecane nanoemulsions by Izquierdo *et al.* (2002). This value, which is consistent with our result, represents only 1 order of magnitude difference due to the different storage and measured temperatures.

From Figure 4.14, where the $1/r^2$ was plotted as a function of time, there was no straight line fitted with the data. According to Equation (2), if coalescence is the main

destabilising factor in emulsions, a straight can be obtained from the $1/r^2$ -t plot, thus the instability of 0.01M and 0.03M NaCl may be attributed to flocculation as well as the Ostwald ripening. The droplets tended to approach each others as the electrical double layer is compressed by the addition of sodium chloride and so the repulsion force between droplets is reduced (Baloch and Hameed, 2005; Morais *et al.*, 2006). For pure milliQ water, the regression value in $1/r^2$ -t was smaller than the one in Ostwald ripening (Table 4.6), therefore the effect of coalescence in pure water system was insignificant.

n-Dodecane nanoemulsions with 0.21 vol% SDS and 0.3vol% pentanol have been reported to have ripening rate was $7.3 \times 10^{-27} \text{ m}^3\text{s}^{-1}$ in 0.01M NaCl system and $6 \times 10^{-27} \text{ m}^3\text{s}^{-1}$ in 0.03M NaCl system (Taylor and Ottewill, 1994). In present experiment, they were $807.9 \times 10^{-27} \text{ m}^3\text{s}^{-1}$ and $7.6 \times 10^{-27} \text{ m}^3\text{s}^{-1}$. The difference may due to the usage of different surfactant and surfactant concentration as well as the effect from the pentanol. The lower ripening rate in 0.03M NaCl system in current study was agreed with results from previous work (Taylor and Ottewill, 1994), although the data was more scattered in 0.03M than 0.01M NaCl system. If the results were only compared between 0.01M and 0.03M NaCl, their data fitted into straight line in a similar way (Figure 4.13), where the R^2 values were about the same (Table 4.6). For a nanoemulsion system with 19.2wt% n-dodecane and 4wt% Brij30, if the sodium chloride concentration was varied from 0.01M to 0.1M, 0.03M NaCl is a better NaCl concentration to produce good quality nanoemulsions (smaller size and lower PdI) and higher stability (lower growth rate in PdI and lower ripening rate).

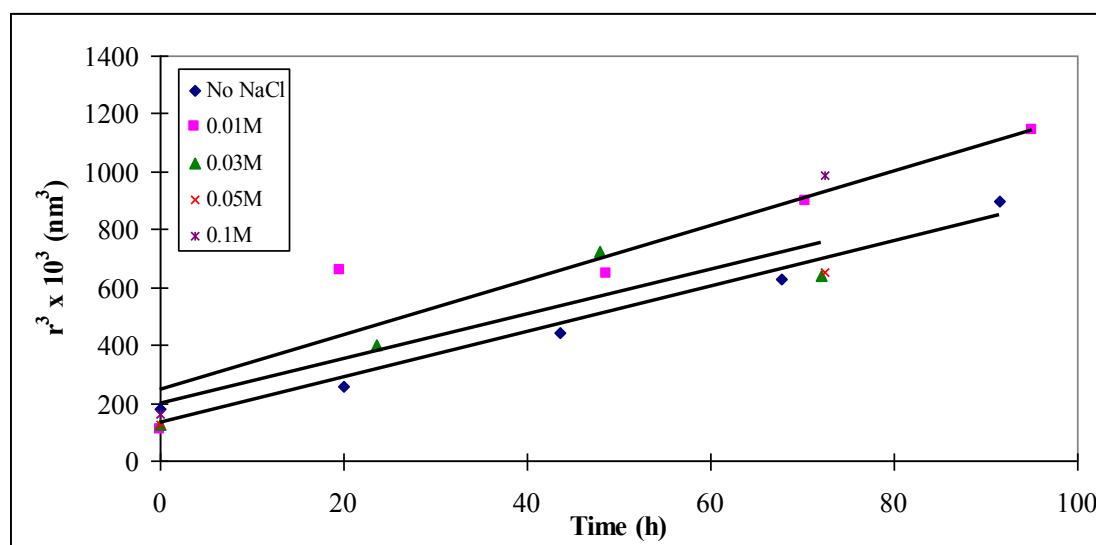


Figure 4.13: Plot of r_n^3 as a function of storage time (20°C) in aqueous-continuous nanoemulsions system with 19.2wt% n-Dodecane, 4wt% Brij30 and different NaCl concentrations.

Table 4.6: The regression and the Ostwald Ripening rate of nanoemulsions with 19.2wt% dispersed phase (n-dodecane) and 4wt% Brij30, as a function of sodium chloride concentrations. The measurement was done at 20°C.

NaCl (M)	ω ($\times 10^{27}$) ($\text{m}^3 \text{s}^{-1}$)	R^2 (r^3-t)	R^2 ($1/r^2-t$)
0	53.6	0.9781	0.9505
0.01	807.9	0.8743	0.5766
0.03	7.6	0.8019	0.7281
0.05	N/A	-	-
0.1	N/A	-	-

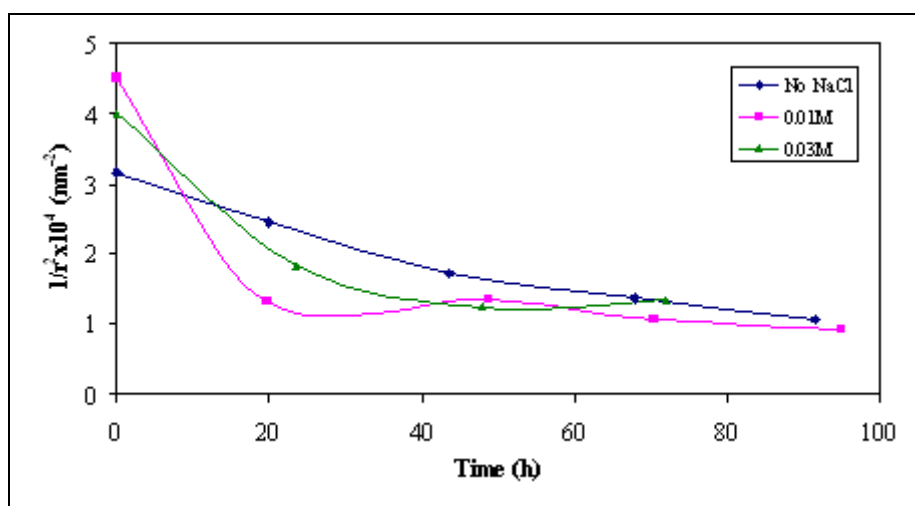


Figure 4.14: Plot of $1/r^2$ as a function of time in aqueous-continuous nanoemulsions with 19.2wt% n-dodecane, 4wt% Brij30 and different NaCl concentrations. The samples were stored and measured at 20°C.

The instability of 0.05M and 0.1M NaCl nanoemulsions (fastest growth rate in PdI and quickest to have phase separation) was due to the dehydrated non-ionic surfactant and so unstable O/W nanoemulsions were produced or W/O emulsions were started to be produced. The surfactant became more hydrophobic when the amount of Na^+ was increased in the continuous phase (the NaCl concentration was increased). The present study has shown that there was an optimum sodium chloride concentration (0.03M in 0.01-0.1M NaCl) to produce O/brine nanoemulsions with smaller droplet sizes, finer size distribution and higher stability, but restricted to a system with the ratio of oil to liquid mixture was 0.2 ($R=0.2$) and 4wt% Brij30. A similar optimum sodium chloride concentration to produce good quality (small size and high stability) methyl myristate-in-water macroemulsions has been reported (Binks *et al.*, 2006). The present result provided a preliminary investigation in the effect of sodium chloride and the optimum NaCl concentration in the production of n-dodecane nanoemulsions by the PIT method, since the effect has not been well investigated.

4.5.3 Reversibility of Nanoemulsions

By utilising the PIT method, O/W and W/O emulsions regardless of their characteristics can be interchanged by tailoring the affinity of non-ionic surfactant with temperatures (Shinoda and Saito, 1969; Izquierdo *et al.*, 2002; Fernandez *et al.*, 2004; Izquierdo *et al.*, 2004). However, the characteristics such as their droplet sizes and size distribution after the conditions have been reversed by the PIT method have not been well investigated. Ee *et al.* (2007) have shown that ageing decane-water nanoemulsions could be reproduced into their initial droplet sizes when they were stored and measured at their optimum temperatures. In present study, the reversibility ability of nanoemulsions was due to the ability of ageing nanoemulsions to revert back to the freshly prepared state in terms of their droplet sizes and PDI.

Nanoemulsions have been widely produced by the PIT method nowadays due to low energy and surfactant usage. However, the stability of the nanoemulsions is very sensitive to temperatures, so that they are easily destabilised (growth in droplet size and phase separation) if they are not stored at their optimum temperatures (Shinoda and Saito, 1969; Ee *et al.*, 2007). In most industries, the nanoemulsions are unable to be stored or delivered at their optimum temperatures; therefore, the reversibility ability will play an important role in emulsion industries such as pharmaceuticals.

Due to the fast destabilisation in some of the nanoemulsion systems (0.05M and 0.01M NaCl were only stable for three days without phase separation), the reversibility testing was done in a period of three days. The reversibility testing was done in two ways: cooling and heating processes. In the cooling process, the sample was cooled from a storage temperature (20°C) to 10°C and returned to 20°C; in heating process, the sample was heated from a storage temperature (20°C) to 30°C (higher than the PIT temperature in brine systems) and returned to 20°C. Figure 4.15 shows the reversibility ability of 3-day ageing n-dodecane nanoemulsions after the heating or cooling process. All the results presented in Figure 4.15 were measured at 20°C which was the final temperature in cooling and heating processes, and also the storage temperature.

From Figure 4.15(a), a PSD curve with single-peak and larger droplet size (curve was shifted to more right) appeared in the pure milliQ water system when the system was analysed after it had been stored for three days. After the cooling process, at 20°C, the curve was overlapped with the 3-day ageing nanoemulsions. However, when the system was treated by the heating process, system became polydispersed (wider and lower PSD curve). In brine systems (only 0.03M and 0.1M are shown in Figure 4.15(b) and (c)), the 3-day ageing nanoemulsions were polydispersed with two-peak in the PSD curves, while the curves were single-peak in freshly prepared samples. Similar to the pure milliQ water system, the PSD curve for the system after being treated by the cooling process, the PSD curve for brine systems was overlapped with the 3-days ageing nanoemulsions. In the systems containing 0.01-0.05M NaCl (only 0.03M NaCl is shown in Figure 4.15(b)), their PSD curves at 20°C were overlapped with those in freshly prepared sample after the heating process. For 0.1M NaCl system (Figure 4.15 (c)), the 3-day ageing nanoemulsions were still polydispersed with two peaks in the PSD curve, after the heating process. Therefore, within the studied systems, the size distribution of the droplets in the 3-day ageing nanoemulsions was able to be

reproduced in systems containing 0.01-0.05M NaCl and also by the heating process. A more detailed study in the reversibility ability is shown in Figure 4.16, where the droplet size and PDI of ageing nanoemulsions was measured at different temperatures during the two processes. The investigation in droplet size and PDI was done in pure milliQ water system and sodium chloride concentration from 0.01M-0.1M (only 0.03M and 0.1M are shown), and with the storage periods were one and three days.

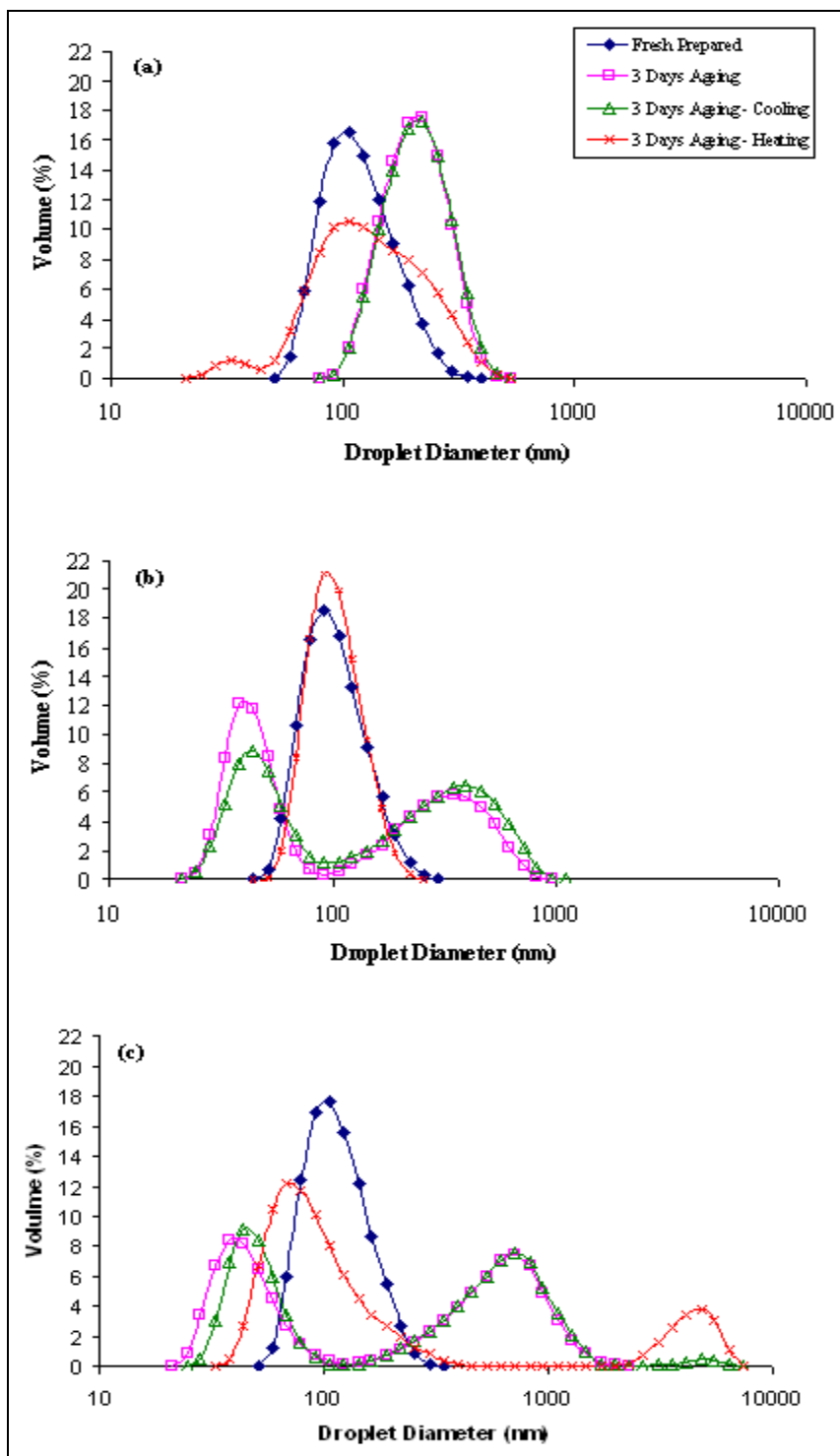


Figure 4.15: The reversibility testing on PSD in n-dodecane nanoemulsions with (a) No NaCl, (b) 0.03M NaCl and (c) 0.1M NaCl. The systems contain 19.2wt% n-dodecane and 4wt% Brij30. All presented results were measured at 20°C.

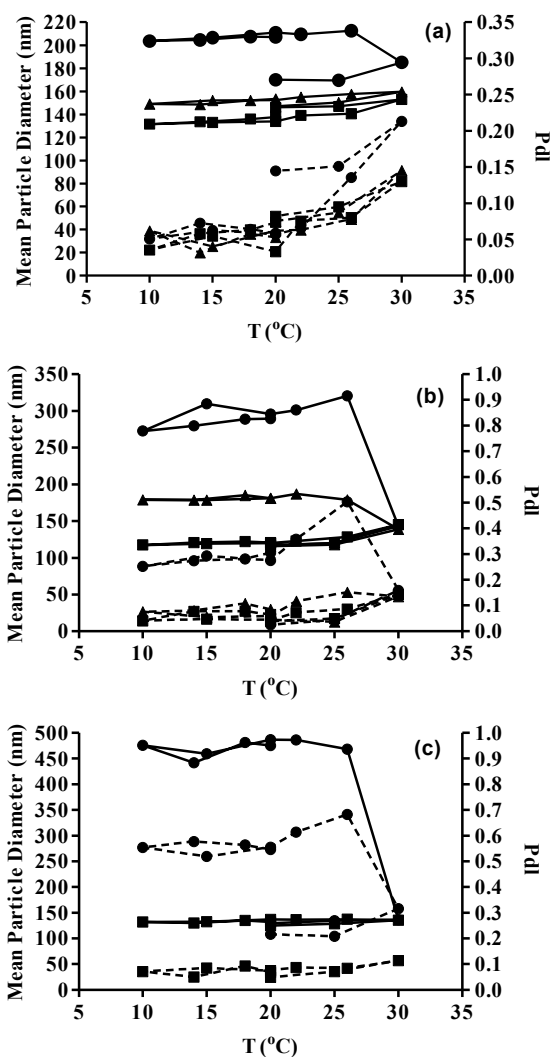


Figure 4.16: The reversibility testing on droplet size (—) and polydispersity indices (PDI) (---) at (a) 0M, (b) 0.03M and (c) 0.1M NaCl nanoemulsions with 19.2wt% n-dodecane and 4wt% Brij30. The storage periods are: freshly prepared (■), 1 day (▲) and 3 days (●). The measured temperature sequence is 20°C→10°C→20°C→30°C→20°C.

The droplet size and PDI remained almost constant when temperature decreased; however, they behaved differently in freshly-prepared and ageing nanoemulsions when the temperature was increased (Figure 4.16). For a freshly-prepared sample, the droplet diameter and PDI increased with temperatures, where they were 120nm and 0.06 at 20°C but 145nm and 0.145 at 30°C, in a 0.03M NaCl system. For ageing nanoemulsions (3 days), the droplet diameter and PDI were 290nm and 0.305 at 20°C but 143nm and 0.159 at 30°C, in a 0.03M NaCl system. The droplet size and PDI increased with temperatures in a freshly-prepared sample, but with a sharp decrease appeared at 30°C in ageing nanoemulsions. When temperature increased, emulsion system changed from WI to WIII region, and the interfacial tensions reached minimum to form small sized droplets (Shinoda and Saito, 1968). However, if the temperature is higher than the PIT temperature, the emulsions exist in WII region and therefore incorrect DLS was obtained as the setting has been set for O/W emulsions. If a nanoemulsion system with very small droplet size (lowest interfacial tension) is

produced, the system will appear as transparent; however, in present study, the sample appeared as concentrated milky when it was in 30°C, therefore, W/O emulsions were formed at 30°C and caused an invalid result, which was the sharp decrease in the DLS measurement.

From the reversibility testing and according to the results from Figure 4.15 and Figure 4.16, there were three different results in the systems containing sodium chloride with concentration from zero to 0.1M. First, in a pure milliQ water system at 20°C, the initial droplet size and Pdl were 138nm and 0.073, however, for three-day ageing nanoemulsions, they were 211nm and 0.057 after the cooling process and 170nm and 0.145 after the heating process. Second, in the 0.03M NaCl system, the droplet size and Pdl for three-day ageing nanoemulsions were 296nm and 0.275 after the cooling process and 117nm and 0.024 after the heating process while they were 120nm and 0.064 in the freshly-prepared sample, where all measurements were done at 20°C. Finally, for the 0.1M NaCl ageing nanoemulsions, the droplet size and Pdl were 487nm and 0.555 after the cooling process and 130nm and 0.216 after the heating process, whereby the initial droplet size and Pdl were 132nm and 0.068, respectively, at 20°C.

There was an irreversible system for a pure milliQ water system, where the PSD curve was not overlapped between the 3-day ageing and freshly prepared nanoemulsions (Figure 4.15(a)) at 20°C. Furthermore, the system did not have the ability to revert the droplet size and Pdl from ageing to freshly-prepared nanoemulsions, in both cooling and heating processes (Figure 4.16(a)). However, there were reversible systems for 0.01M, 0.03M and 0.05M NaCl system (only 0.03M is shown in Figure 4.16(b)), where their droplet sizes and Pdl of ageing nanoemulsions were overlapped with the freshly-prepared samples after the heating process (from 30°C to 20°C). Moreover, as shown in Figure 4.15(b), the PSD for 3-day ageing 0.03M NaCl nanoemulsions (after the heating process) at 20°C was the same with the one which was freshly prepared. Finally, 0.1M NaCl nanoemulsions was partial reversible system, where only the droplet size of ageing nanoemulsions could be reverted after the heating process (Figure 4.16(c)).

From the results above, they clearly showed that for the system studied, the reversibility ability only existed in the heating process and in those systems containing sodium chloride (with either fully or partially reversible). As in the heating process, the systems were heated to 30°C, a temperature which was higher than or close to the PIT temperatures, the systems were expected to experience phase inversion during the process. Therefore, the reversibility ability of nanoemulsions produced by the PIT method was governed by the phase inversion process. It was also because the redissolution of oil drops from big droplet to small droplet is encouraged when the process involves a structural change (or a phase change) (Evilevitch *et al.*, 2000).

There have been not much literatures available discussing about the reversibility ability of nanoemulsions produced by the PIT method. The exact mechanism to cause the behaviour has not been clearly identified and would require further detailed study. However, some possible reasons could be drawn for the n-dodecane nanoemulsions with 19.2wt% oil and 4wt% Brij30. First, the reversibility ability would only appear if the properties of the surfactant (eg. affinity at different temperatures, minimizing interfacial tension) were maintained. It has been well-known that sodium chloride is

good to use as a preservative agent (Feldman, 2005), and to preserve the properties of the surfactant to its original state during the storage periods and during the repeated heating/cooling process. As long as the surfactant retained its original properties, it would have the same level of hydration and dehydration properties during the phase inversion process and enabled the rejuvenation of O/brine nanoemulsions.

Secondly, with the addition of electrolyte, a non-ionic surfactant becomes more active and its affinity to oil and water interface is more sensitive to temperature (Shinoda and Takeda, 1970). A particular affinity of surfactant might exist at a certain temperature. This particular affinity would cause a certain degree of curvature of surfactant monolayer and hence droplets with fixed sizes could be formed at the certain temperature.

As discussed in previous sections, 0.1M NaCl system was very unstable as the surfactant was highly dehydrated and became more lipophilic. Since the nature (affinity) of the surfactant was changed permanently by the high amount of sodium chloride, the 0.1M NaCl system only had the ability to revert the droplet size but not the PDI. Additionally, the partial reversibility ability may also attribute to the instability of the system and the rate of coalescence was faster than the rate of droplet rejuvenation.

4.6 Effect of NaCl concentration at different surfactant concentrations

After investigating the effect of sodium chloride in n-dodecane nanoemulsions ($R=0.2$, produced by the PIT method) with 4wt% Brij30 in Section 4.5, a wider concentration of Brij30 was carried out. In this section, the increment of the sodium chloride concentration was by a factor of 10 starting from 0.001M to 0.1M. The oil concentration was kept at $R=0.2$ while the surfactant concentration was from 4wt% to 8wt% with an increment of 1wt%.

4.6.1 Phase Inversion Process

The dynamic conductivity curve of n-dodecane emulsions with 4-8wt% Brij30 and 0-0.1M NaCl was determined but only pure milliQ water and 0.001M NaCl systems are shown in Figure 4.17. For a pure milliQ water system, the shape of conductivity curve was similar in the systems with surfactant concentrations from 4 to 8wt%, which consisted of 1st maximum, 1st minimum, 2nd maximum and 2nd minimum. However, in the sodium chloride system (0.001M NaCl), a conductivity curve without 1st minimum and 2nd maximum appeared at 8wt% Brij30. Therefore, the phases involved during the phase inversion process were different in a sodium chloride system at 8wt% Brij30, compared to the pure milliQ water system, when the oil concentration was $R=0.2$.

The initial conductivity value increased with surfactant concentrations in a pure milliQ water system but it was independent of surfactant concentration in a brine system (Figure 4.17). It was because the conductivity potential of sodium chloride was much stronger than a technical grade non-ionic surfactant. The change of conductivity curve with surfactant concentrations at a fixed sodium chloride

concentration were the same with published works (Izquierdo *et al.*, 2002; Izquierdo *et al.*, 2004; Anton *et al.*, 2007; Anton *et al.*, 2008), where the transitional phase occurred at lower temperature when the surfactant concentration was increased. The 2nd maximum only appears at high surfactant concentration but will be depressed if the surfactant concentration is too high (Izquierdo *et al.*, 2002). In present study, the ratio between 2nd maximum and 1st maximum decreased to 0.15 from 0.44, when surfactant concentration was increased from 4 to 8wt% in no NaCl system. The ratios were similar to the work done by Anton and co-workers (2008); however, the reason for causing it is yet unclear and remained an unsolved issue. According to a ternary phase diagram in a decane system (Forgiarini *et al.*, 2001), the formation of LLC is more extended than bicontinuous phase at high surfactant concentrations. This provides a lower 1st minimum and therefore lowered the ratios.

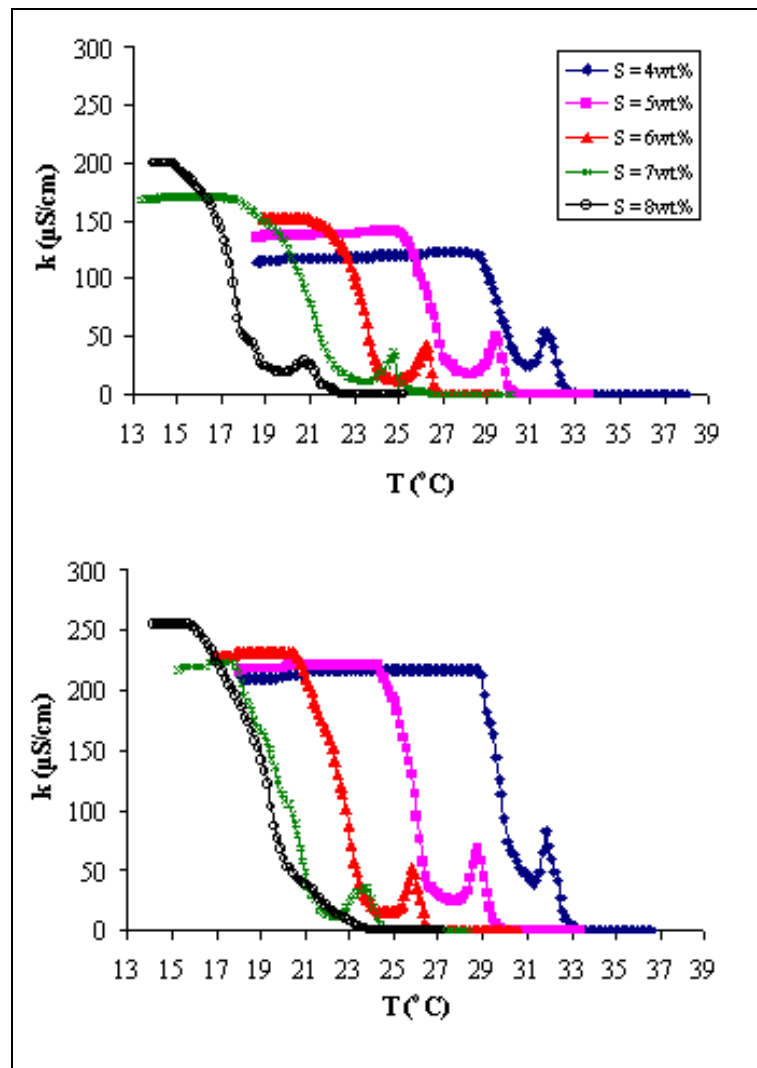


Figure 4.17: Dynamic conductivity values as a function of temperatures for emulsion systems (R=0.2) with pure milliQ water (top) and 0.001M NaCl (bottom) and with 4-8wt% of Brij30.

At a fixed surfactant concentration, the first maximum appeared further to the left (lower temperature) when sodium chloride was added, where there was 24.7°C in no NaCl system and there was 24.3°C in 0.001M NaCl system (Figure 4.17). The decrement of temperature became more significant at higher surfactant concentration. As the conductivity curve has shifted to lower temperature in salt system, the phase

inversion occurred at lower temperature and the PIT temperature was lowered, which were similar with previous works (Shinoda and Takeda, 1970; Anton *et al.*, 2007). The depression of the PIT temperature is shown in detail in Figure 4.18, indicating a decrease of 0.5°C from pure milliQ water system to 0.001M NaCl system and a decrease of 2°C from pure milliQ water system to 0.01M and 0.1M NaCl systems. These reductions were quite significant in present study since the result of the PIT temperature was within standard deviation as low as 0.63. The depression was because of dehydration of non-ionic surfactant (more hydrophobic), thus causing the decrease of its solubility in aqueous phase (Shinoda and Takeda, 1970; Kunieda *et al.*, 1989; Anton *et al.*, 2007). Furthermore, the depression was more significant at higher surfactant concentrations due to the larger amount of surfactant, which is shown in Figure 4.18. However, the reductions in present study were considered small if they were compared to published work (Shinoda and Takeda, 1970) as the investigated systems were being differently (same discussion had been done in Section 4.5). The decrease in the PIT temperature with surfactant concentrations agreed with published work, due to the accumulation of short ethylene oxide chain at oil-water interface (Izquierdo *et al.*, 2002).

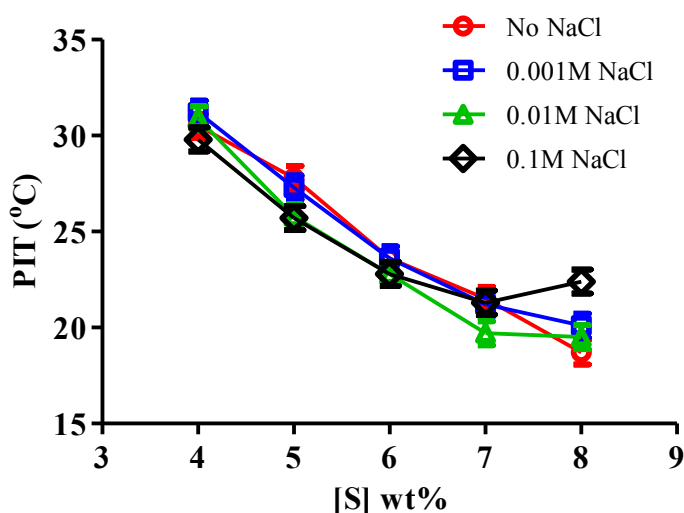


Figure 4.18: PIT temperature as a function of surfactant concentrations in different sodium chloride concentrations. Oil/aqueous=20/80 w/w.

Figure 4.19 shows the ΔT_{trans} as a function of surfactant concentration in systems with different NaCl concentration. It shows that ΔT_{trans} continuously increased with surfactant concentrations in 0.001M and in 0.1M NaCl systems but a maximum ΔT_{trans} existed in pure milliQ water and 0.01M NaCl systems. The maximum ΔT_{trans} occurred at 7wt% Brij30 in pure milliQ water system but at 6wt% Brij30 in 0.01M NaCl system. At fixed surfactant concentration (except the 7wt% in pure milliQ water and 6wt% in 0.01M NaCl), the ΔT_{trans} remained almost the same with sodium chloride concentration. This result confirmed that ΔT_{trans} is more dependent on surfactant concentration than salt concentration, which has been described by Anton and co-workers (2007). As the transitional zone has been suggested to be linked with the completeness of solubilisation of the oil component to produce nanoemulsions (Sole *et al.*, 2006) and the stability of nanoemulsions (Morales *et al.*, 2003), the maximum ΔT_{trans} in pure milliQ water and 0.01M NaCl system showed that n-dodecane was more soluble in those surfactant concentrations and therefore the stability of the

nanoemulsions produced in those conditions was expected to be higher than other surfactant concentrations.

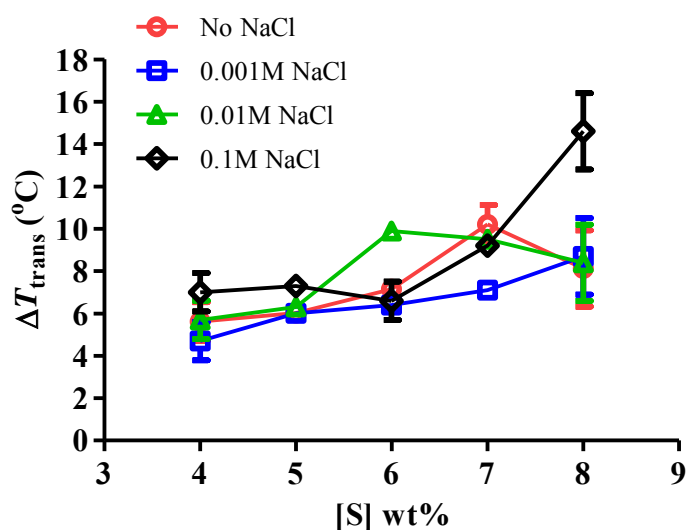


Figure 4.19: Width of transitional zone as a function of surfactant concentrations for system containing different sodium chloride concentrations. Oil/aqueous=20/80 w/w.

4.6.2 Formation and Stability of Nanoemulsions

As shown in Figure 4.18, all PIT temperatures were higher than 15°C, thus aqueous-continuous nanoemulsions were produced when an emulsion system was heated to a temperature with zero-conductivity (WII region) followed by a rapid cooling process. The procedures were similar with the previous experiment in Section 4.4 & 4.5. The cooled system was stored at 20°C to simulate in a normal storage temperature in emulsion industries.

Some selected emulsion systems with their initial size distribution (PSD) at 20°C are presented in Figure 4.20. In pure milliQ water system, when the surfactant concentration was lower than 8wt% (only 7wt% Brij30 is shown in Figure 4.20), the size distribution was monodispersed with single peak in the PSD curve. However, the emulsions started to be polydispersed (more than two peaks in the graph) from 8wt% Brij30. For a brine system, the system started to be polydispersed from 7wt% Brij30 as shown in Figure 4.20. Other than in these surfactant concentrations (with or without sodium chloride), the nanoemulsions were monodispersely distributed, where the PSD curves were in the same shape with those presented in Figure 4.10. The polydispersity at 7wt% Brij30 was because the storage (analysed) temperature (20°C) was close to the corresponding PIT temperature (20-21°C from 0.001M to 0.1M NaCl) and so nanoemulsions was being unstable (Shinoda and Saito, 1969). In addition, the PIT temperature for 8wt% Brij30 was lower than 20°C (Figure 4.18) and so no nanoemulsions, but only WIII region (LLC or bicontinuous phase) was formed at the storage temperature. According to the results from Figure 4.20, in the studied systems, the range of surfactant concentration to produce monodispersed nanoemulsions (with single-peak PSD curve) was 4-7wt% in a pure milliQ water system and was 4-6wt%

in a brine system. The surfactant range became narrower when sodium chloride was added into the system.

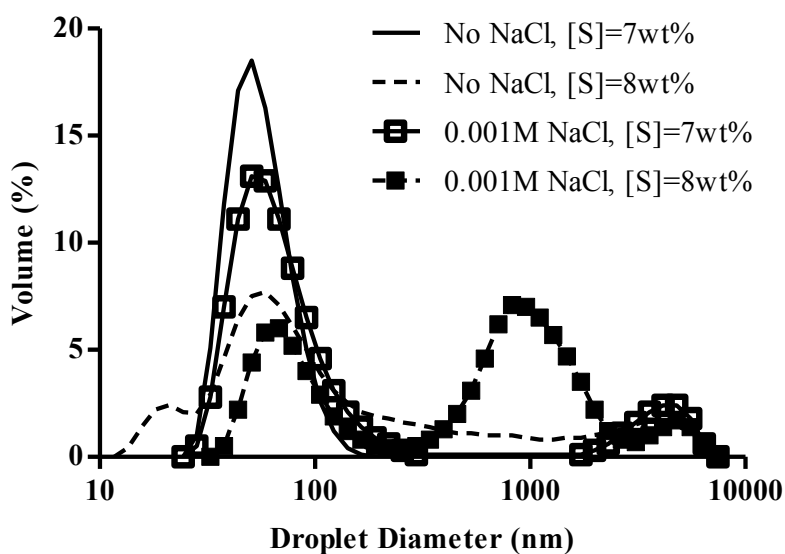


Figure 4.20: Initial size distribution at 20°C for n-dodecane nanoemulsions with some selected systems. Oil/aqueous=20/80 w/w.

The initial number average droplet diameter and PDI as a function of surfactant concentrations, for systems containing sodium chloride concentration from zero to 0.1M are shown in Figure 4.21. Nanoemulsions with PDI smaller than 0.2 and droplet sizes smaller than 120nm could be produced at 4-7wt% Brij30 in a pure milliQ water system but at 4-6wt% Brij30 in a brine system. When surfactant concentration was below monodispersed nanoemulsions production concentrations, the droplet size was decreased when the concentration of either NaCl or Brij30 was increased (Figure 4.21). However, the PDI was almost unchanged with both concentrations before the system became polydispersed.

Within the surfactant concentration which monodispersed nanoemulsions were produced, nanoemulsions with mean droplet sizes ranging from 77 to 110nm were produced in a pure milliQ water system while 65 to 104nm were produced in brine systems (Figure 4.21) with a standard deviation as low as 0.73nm. The smaller droplets were formed due to the low interfacial tension caused by sodium chloride (Taylor & Ottewill, 1994). However, the addition of sodium chloride did not affect the fineness of nanoemulsions produced by the PIT method (no changes in PDI). Among the brine systems, 0.1M NaCl appeared to have the largest droplet size, PDI (Figure 4.21) and wider size distribution (same behaviour in Figure 4.10). It was because the non-ionic surfactant was highly dehydrated as discussed in Section 4.5. As the usage of surfactant determine the surface area (interfacial tension), lower interfacial tension is achieved at higher surfactant concentrations (Izquierdo *et al.*, 2002; Tadros *et al.*, 2004). Therefore, within the range of surfactant concentration to produce monodispersed nanoemulsions, smaller droplets have been produced in higher surfactant concentrations (Figure 4.21). In present study, nanoemulsions with smallest droplet size and lowest PDI appeared at 7wt% of Brij30 in a pure milliQ

water system and at 6wt% in brine systems (Figure 4.21), have also appeared as the most stable nanoemulsions (Table 4.7 and Figure 4.22(b)).

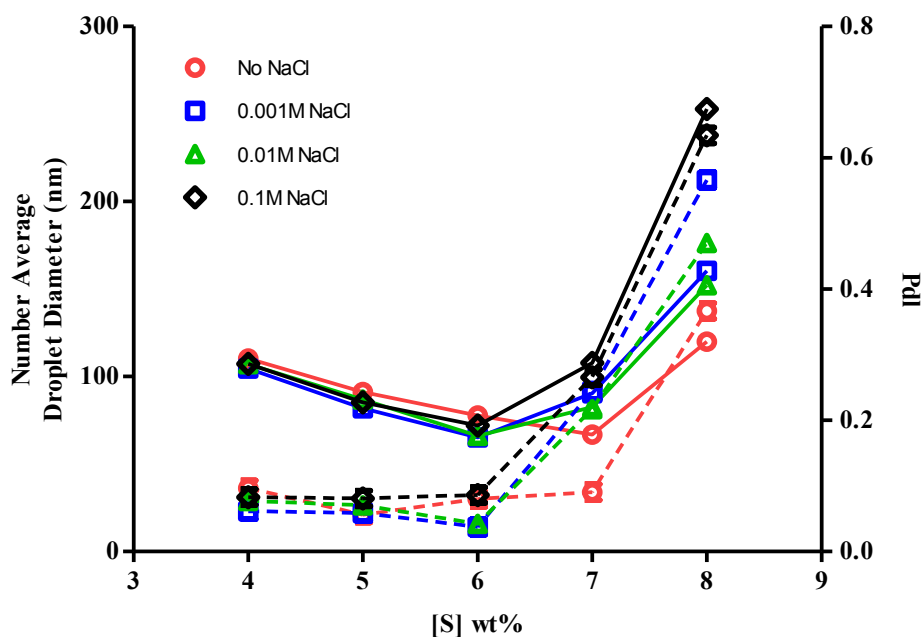


Figure 4.21: Initial droplet size and polydispersity index (PDI) as a function of surfactant concentrations at 20°C for n-dodecane nanoemulsions with different sodium chloride concentrations. Oil/aqueous=20/80 w/w. Solid line: Droplet Diameter; Dashed Line: PDI.

The stability of the nanoemulsions with different sodium chloride and surfactant concentrations were investigated and the results are shown in Table 4.7, Figure 4.22 and Figure 4.23. According to Table 4.7, nanoemulsions with 7wt% Brij30 in pure milliQ water system and 6wt% Brij30 in systems with sodium chloride were the most stable n-dodecane nanoemulsions in present study, as they could remain as one-phase transparent for more than 30 days. The days taken for phase separation were decreased from 4wt% to 6wt% Brij30 in pure milliQ water system while were decreased from 4wt% to 5wt% Brij30 in brine systems.

Table 4.7: Days taken for phase separation for n-dodecane nanoemulsions (oil/aqueous=20/80 w/w) stored at 20°C with different sodium chloride and surfactant concentrations.

[S] (wt%)	No NaCl	0.001M NaCl	0.01M NaCl	0.1M NaCl
4	10	9	7	3
5	4	5	5	2
6	3	>30	>30	>30
7	>30	-	-	-
8	-	-	-	-

A detailed study in the stability of nanoemulsions was done by measuring the growth of the droplet size and PDI as a function of time before the phase separation, which is shown in Figure 4.22. From Figure 4.22(a), the growth of the droplet size and PDI for nanoemulsions containing 4wt% Brij30 but different sodium chloride concentration was increased with the storage period. For example, in a pure milliQ water system, the particle size was almost doubled (110 to 290nm) while PDI was almost increased six times (0.1 to 0.6) after the system has been stored for a week. However, for the stable nanoemulsions, which were stable against phase separation more than 30 days (as shown in Table 4.7), there were no changes on their droplet sizes and PDI during the studied period (Figure 4.22(b)).

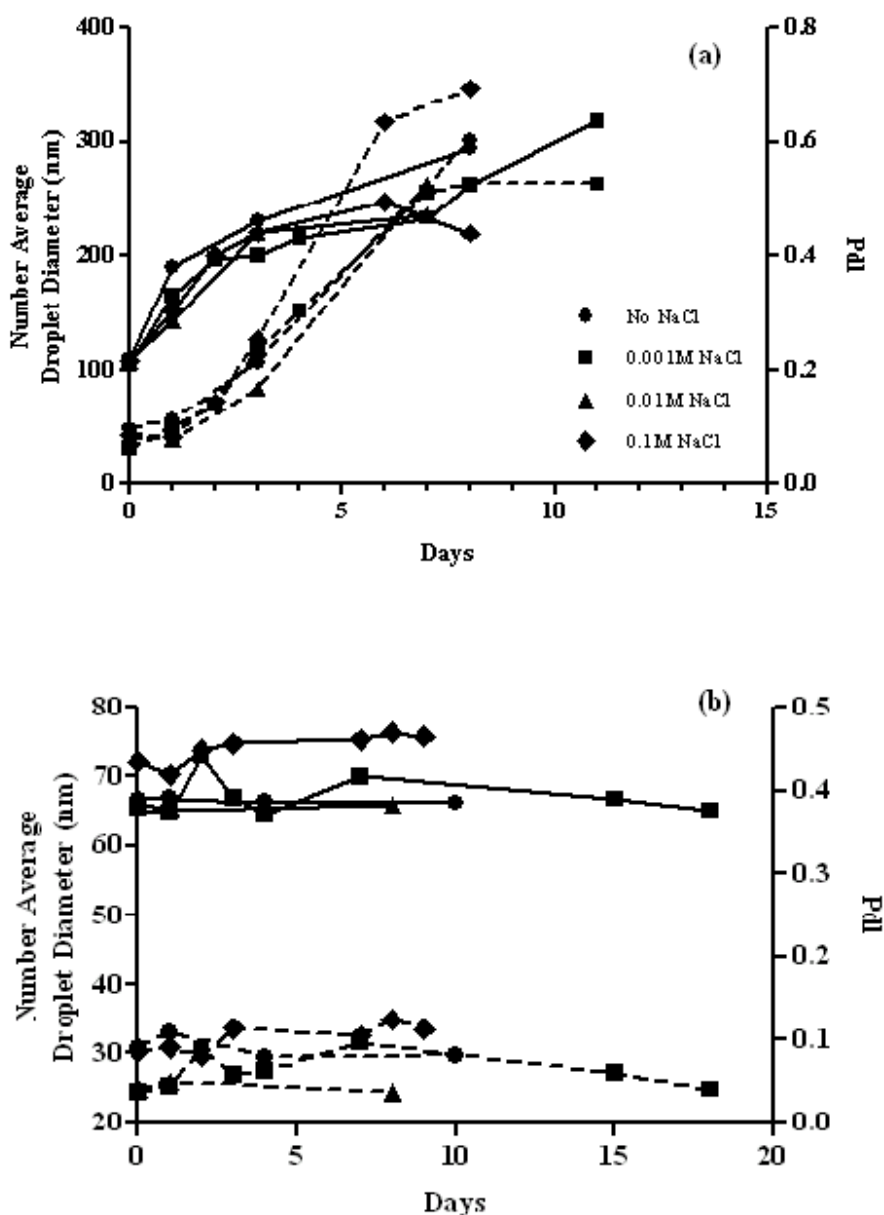


Figure 4.22: (a) The stability for n-dodecane nanoemulsions containing 4wt% of Brij30 with different sodium chloride concentrations; (b) the most stable nanoemulsions within the investigated systems (Circle: No NaCl, 7wt% of Brij30; Square: 0.001M NaCl, 6wt% of Brij30; Triangle: 0.01M NaCl, 6wt% of Brij30; Diamond: 0.1M NaCl, 6wt% of Brij30). (Solid line: mean droplet diameter; Dashed line: PDI)

The instability factors of system with $R=0.2$ and 4wt% Brij30 at 20°C are shown in Figure 4.23(a) and (b) by plotting r^3 (Ostwald ripening) or $1/r^2$ (coalescence) as a function of storage time. It has been found that Ostwald ripening was the main factor causing instability in the nanoemulsions with pure milliQ water and other factors (eg. coalescence, flocculation) were also involved in sodium chloride system from Section 4.5. From Figure 4.23(a), the regression value for system with 0.001M NaCl was 0.9768 and was in between 0.9781 in pure milliQ water system and 0.8743 in 0.01M NaCl, which have been shown in Table 4.6. Ostwald ripening was still dominant in the instability in 0.001M NaCl since there was no straight line could be drawn in the $1/r^2$ -t plot. It was because the 0.001M NaCl concentration was low and the effect of electrolyte was insignificant.

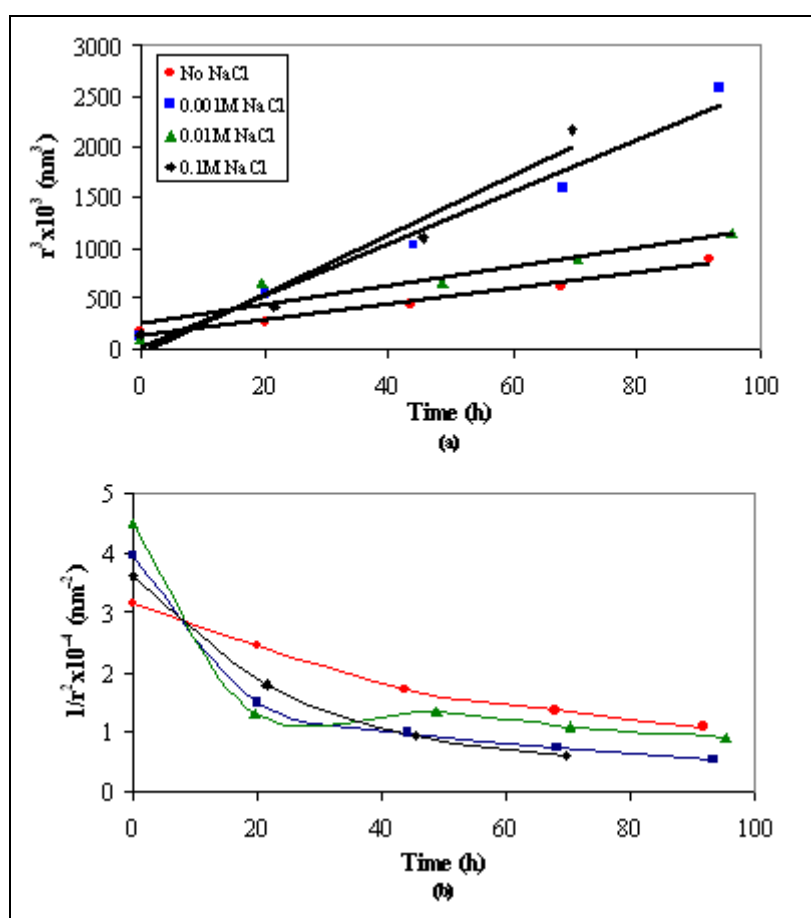


Figure 4.23: Plot of (a) r^3 and (b) $1/r^2$ as a function of storage time in aqueous-continuous nanoemulsions with $R=0.2$ and 4wt% Brij30. Storage and measured temperature was 20°C.

For system containing 0.1M NaCl, the droplet size and PDI were always the largest in n-dodecane nanoemulsions ($R=0.2$), during the studied period (Figure 4.22). Furthermore, 0.1M NaCl nanoemulsions with either 4wt% or 5wt% of Brij30 were the most unstable system in present study as they were the quickest systems to have phase separation (Table 4.7). The main mechanism to cause the instability was expected to be flocculation as there was no straight line could be obtained from the data in r^3 -t (Figure 4.23 (a)) and $1/r^2$ -t (Figure 4.23 (b)). It was because the thickness of the double layer is decreased when the concentration of sodium chloride is increased, thus the flocculation was more significant (Morais *et al.*, 2006). Moreover, the non-ionic

surfactant was more dehydrated and produced unstable O/brine nanoemulsions when the NaCl concentration was higher (Anton *et al.*, 2007).

Other than studying the phase separation and the growth of droplet size and PDI, Scanning Electron Microscopy images, which are shown in Figure 4.24, were used to show detailed structures of n-dodecane/water nanoemulsions with 6wt% and 7wt% Brij30. Due to the crystallization of sodium chloride during the freeze-fracture process in Cryo-SEM, only systems with pure milliQ water were chosen to be studied. From the images, there were elongated- or ellipsoid-shaped “holes” existed in the system with 6wt% Brij30, after the system has been stored for two days. Additionally, the droplet size was around 1 μ m and polydisperse distributed. When the surfactant concentration was increased to 7wt% Brij30, the 9-day nanoemulsions in a pure milliQ water system showed a monodispersed system with an average droplet diameter was 200nm. The larger droplet size in SEM may be due to the freeze-fracture process, which the sample experienced some thermal pre-treatment (rapidly frozen in nitrogen slush and sublimation) causing the growth of the droplets. Furthermore, the larger droplet size was also expected by the error from the transmission light and microscopy images. Although there were some ellipsoid-shaped hole appeared in the 7wt% Brij30 system, the amount was less than 6wt% Brij30 nanoemulsion system. In present study, the destabilising factor to cause the growth of the droplets (Figure 4.22) and phase separation (Table 4.7) in system with 6wt% Brij30 and pure milliQ water was flocculation as the droplets were stuck together and showed ellipsoid-shaped “holes” in SEM images. The flocculation was because of the van del Waals force (Robins and Hibberd, 1998). By considering the amount of flocculation and storage period (Figure 4.24), the most stable n-dodecane nanoemulsions with R=0.2 and pure millilQ water system was the one with 7wt% Brij30, which the stability was also shown in Table 4.7 and Figure 4.22(b).

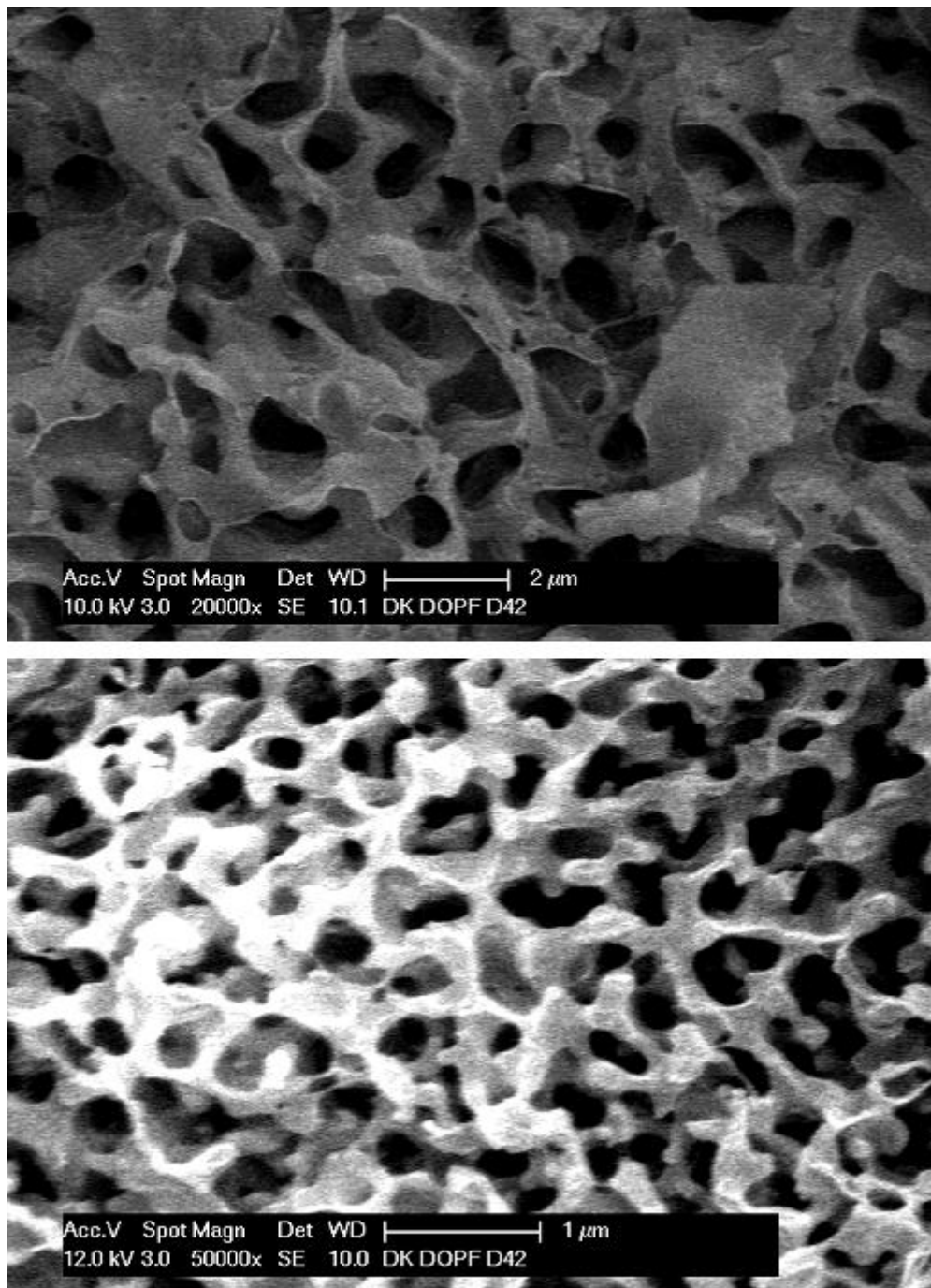


Figure 4.24: SEM images: (top) aged nanoemulsions (2 days) with R=0.2, 6wt% Brij30, No NaCl, (bottom) aged nanoemulsions (9 days) with R=0.2, 7wt% Brij30, No NaCl. Storage temperature is 20°C.

As shown in Table 4.8, the zeta potential of pure milliQ water and sodium chloride systems were close to zero or being negative. At fixed surfactant concentration (6wt% Brij30), the potential was changed from -1.112mV to -0.2336mV (less negative) when sodium chloride was added and when the salt concentration was up to 0.1M. At fixed NaCl concentration (0.01M NaCl), the attractive force between droplets was increased when the surfactant concentration was increased. The negative charged non-ionic surfactant system (Morais *et al.*, 2006) or the unchanged zeta potential with surfactant (Liu *et al.*, 2006) and electrolyte concentrations (Morais *et al.*, 2006) have been

reported by researchers for aqueous-continuous nanoemulsions. From present study, the results were similar with the results produced by those researchers, where the charged electron of the interface of the nanoemulsions was not significantly changed by the addition of sodium chloride and by increasing surfactant concentration. However, the slight increase in attractive force between droplets indicate higher tendency for flocculation when sodium chloride was added and the force increased when NaCl concentration was increased. The increasing attractive force showed the instability of nanoemulsions in brine system. It has been reported that a higher electrostatic repulsion is existed when more non-ionic surfactant is used (Dukhin and Sjoblom, 1996). However, in present study, the attractive force between droplets was slightly increased in 0.01M NaCl nanoemulsions system when the surfactant concentration was increased. This might due to the compression effect of the electrical double layer, by the addition sodium chloride, was more pronounced than the increase of electrostatic repulsion. Furthermore, the non-ionic surfactant was dehydrated by the sodium chloride and so the electrostatic repulsion ability was also affected. Within the studied systems, the change of zeta potential as a function of NaCl was insignificant (not dominant to the instability of nanoemulsions). Therefore, the stability of nanoemulsions with and without sodium chloride in present systems was mostly dependent on the salting and dehydrating effect.

Table 4.8: Zeta potential of nanoemulsions with pure milliQ water, 0.01M and 0.1M NaCl and with different surfactant concentrations. Oil/aqueous=20/80 w/w. Measurement temperature is 20°C.

[NaCl] (M)	[Brij30] (wt%)	Zeta Potential (mV)
0	6	-1.112
0.01	4	-1.128
	5	-1.035
	6	-0.950
0.1	6	-0.2336

In this study, it has been found that there was an optimum surfactant concentration to produce the most stable n-dodecane nanoemulsions (with R=0.2) at 20°C in either a pure milliQ water or a brine system. The optimum surfactant concentration appeared in between two unstable regions, which has been found to exist in a sudden change manner (Tadros *et al.*, 2004). For nanoemulsions with R=0.2, the optimum surfactant concentration was 7wt% in a pure milliQ water system and was 6wt% in a brine system. The most stable nanoemulsions at those surfactant concentrations might be due to the complete coverage of surfactant on oil-water interface. For surfactant concentration lower than that, there were not enough surfactants to cover the interface and so the coalescence was more likely to occur. Furthermore, at higher surfactant concentrations, the PIT temperature was close to or lower than 20°C and so no stable nanoemulsions could be produced (Shinoda and Saito, 1968).

There was a reduction of 1wt% in the optimum surfactant concentration to produce the most stable nanoemulsions from a pure milliQ water system to a brine system. This may be due to the compression effect (double layer being compressed) from the addition of sodium chloride on the surfactant monolayer. Therefore, the interfacial concentration of surfactant would be increased. Additionally, the salting out effect would decrease the solubility of surfactant in the aqueous phase and increase the interfacial concentration of Brij30 on the oil phase. Although the 1wt% reduction was insignificant for practical purposes (large-scale production), it was quite a big achievement in small-scale or laboratory-scale production; and this finding has shown the effect of sodium chloride in the formation and stability of nanoemulsions produced by the PIT method and led to a further investigation. However, the addition of electrolyte in the production of aqueous-continuous nanoemulsions need to be done carefully as there was a maximum concentration to prevent the non-ionic surfactant to be highly dehydrated and become hydrophobic and produce oil-continuous emulsions instead of producing aqueous-continuous nanoemulsions.

4.6.3 Reversibility of Nanoemulsions

Previously, Ee *et al* (2007) have shown that decane nanoemulsions at their optimum temperatures have the ability to revert their droplet sizes, from ageing to fresh-prepared nanoemulsions. Then, in nanoemulsions with 4wt% Brij30 and $R=0.2$, it has been shown that only O/brine nanoemulsions have the reversibility ability and the ability was governed by the phase inversion process (Section 4.5). As only one surfactant concentration was investigated in Section 4.5.3, a wider surfactant concentration range with only four sodium chloride concentrations was carried out in this experiment. The reversibility testing in this experiment was done in a period of three days, to make the result to be consistent and comparable with Section 4.5.3, although most of the nanoemulsions were stable against phase separation more than three days.

The results of the reversibility testing for most ageing nanoemulsions in this study were similar to Section 4.5.3 (only 5wt% Brij30 with pure milliQ water and 0.01M NaCl systems are shown in Table 4.9): the droplet size and PdI for a 3-day ageing O/brine nanoemulsions (0.01M NaCl in Table 4.9) were same with those in freshly prepared sample, only after a heating process. For 0.01M NaCl system, the droplet size and PdI were 86.9nm and 0.07 in freshly-prepared sample but became 102.2nm and 0.527 after it has been stored for three days. Meanwhile, the size and PdI were 101.6nm and 0.547 after the cooling process and were 82.3nm and 0.048 after the heating process. As the PIT temperatures for NaCl systems containing 5wt% Brij30 and $R=0.2$ were lower than 30°C, the reversibility ability was governed by the phase inversion process as there was no reversibility behavior in cooling process. The ability to reproduce the droplet size and PdI was also restricted to the system containing sodium chloride, whilst the droplet size and PdI of the 3-days ageing O/W nanoemulsions in 5wt% Brij30 were not the same with the freshly prepared sample after the cooling or heating process (as shown in Table 4.9).

Table 4.9: Reversibility testing for nanoemulsions with oil/aqueous=20/80 w/w after three days storage time. The test was done in a temperature sequence: 20°C→10°C→20°C→30°C→20°C.

	No NaCl, 5wt% Brij30		0.01M NaCl, 5wt% Brij30	
	Mean Diameter (nm)	PdI	Mean Diameter (nm)	PdI
Fresh-prepared (20°C)	91.07	0.056	86.9	0.070
Ageing (3 days)				
20°C	171.1	0.718	102.2	0.527
10°C	193.3	0.717	114.5	0.547
20°C	153.6	0.859	101.6	0.547
30°C	262.4	0.570	218.2	0.856
20°C	180.8	0.143	82.3	0.048

However, in this study, it is interesting to note that the nanoemulsions with the optimum surfactant concentration (7wt% Brij30 in a pure milliQ water system and 6wt% Brij30 in brine systems) behaved differently in the reversibility testing. In these systems and with the thermal equilibrium time was 5 minutes, the droplet sizes of ageing nanoemulsions were close to the freshly-prepared samples after the cooling and heating processes but their PdI were larger than the freshly-prepared samples after the heating process (Table 4.10). For example, in nanoemulsions with 0.1M NaCl and 6wt% Brij30, the droplet size of ageing nanoemulsions was 70.4nm (PdI=0.079) after the cooling process and 86nm (PdI=0.169) after the heating process (5 minutes thermal equilibrium time) while it was 72.1nm (PdI=0.086) for the freshly-prepared sample (Table 4.10). The PdI for a fresh-prepared sample was generally smaller than 0.1 but it became larger than 0.1 after the heating process in a 3-day ageing nanoemulsions with the optimum surfactant concentration. As the systems were able to reverse only the droplet size after the heating process, they were partially reversible.

Although those systems do not need any practical reversibility ability due to their high stability at 20°C, their behaviors in the reversibility testing are critical in some industries when the nanoemulsions are not able to be stored and delivered at 20°C. The reason for causing the partial reversibility ability remained an unsolved issue. It might attribute to their longer ΔT_{trans} (Figure 4.19), thus they needed longer time to experience the phase inversion process, which was a crucial process in the reversibility process and the generalised thermal equilibrium time (5 minutes) might not be enough to equilibrate those systems. Therefore, a longer thermal equilibrium time (10 minutes, doubled the general one) was carried out for system containing 0.1M NaCl and 6wt% Brij30 to justify the possible reason, where the result is shown in Table 4.10. The PdI (droplet size) was reduced to 0.126 (74.4nm) from 0.169

(86nm) when the thermal equilibrium time was longer in the heating process. There was still a partial reversibility system even thermal equilibrium time was doubled. However, the droplet size and Pdl of reversed ageing nanoemulsions with thermal equilibrium time was 10 minutes were closer to the freshly-prepared state than the system with 5 minutes. Therefore, complete reversibility ability was expected if the thermal equilibrium time was longer (more than two times to the general one) for the most stable nanoemulsions. This has shown that O/brine nanoemulsions with R=0.2 and wider transitional zone (larger ΔT_{trans}) required longer time to experience the phase inversion process in the reversibility testing.

Table 4.10: Reversibility testing for the most stable nanoemulsions with oil/aqueous=20/80 w/w after three days storage time. The test was done in a temperature sequence: 20°C→10°C→20°C→30°C→20°C. The thermal equilibrium time is 5minutes and 10 minutes in the heating process.

	No NaCl, 7wt% Brij30		0.1M NaCl, 6wt% Brij30	
	Mean Diameter (nm)	Pdl	Mean Diameter (nm)	Pdl
Fresh-prepared (20°C)	66.8	0.091	72.1	0.086
Ageing (3 days)				
20°C	66.4	0.080	74.8	0.011
10°C	57.9	0.038	61.5	0.041
20°C	66.0	0.065	70.4	0.079
30°C	551.2	1.000	716.9	1.000
20°C (5 minutes)	79.8	0.191	86.0	0.169
20°C (10 minutes)			74.4	0.126

4.7 Effect of NaCl concentration at different oil concentrations but fixed surfactant-to-oil ratio

This study was carried out to investigate the effect of sodium chloride on oil concentration while other conditions remained unchanged. The most stable nanoemulsions with R=0.2 have been found being systems with 7wt% Brij30 in a

pure milliQ water system⁵ and with 6wt% Brij30 in brine systems⁶ (Section 4.6), while the surfactant concentration was from 4wt% to 8wt% and salt concentration was from 0.001M to 0.1M. Those systems were chosen for further investigation in this section by increasing oil concentration (R). Figure 4.25 shows the dynamic conductivity curve of n-dodecane emulsion system with R=0.5 while the weight ratio of surfactant-to-oil being 0.3763. The figure shows that the curve did not have the 1st maximum at low temperatures. The 1st maximum indicated the formation of O/W emulsions, therefore there was no aqueous-continuous nanoemulsions but reverse emulsions produced in R=0.5. At constant R_{so} , O/W emulsions were produced at low temperature only when R was lower than 0.5, thus systems with R=0.3 and 0.4 were investigated in this section.

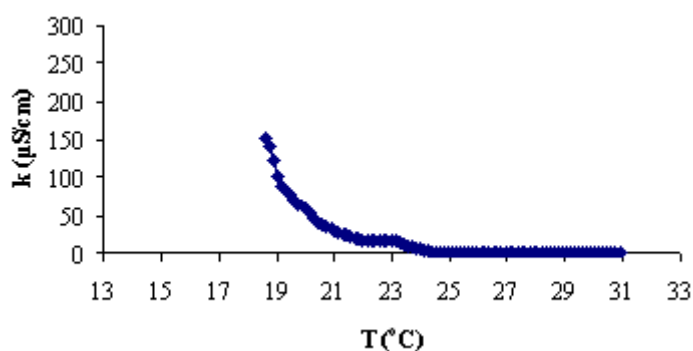


Figure 4.25 Dynamic conductivity values as a function of temperature for system with pure milliQ water, R=0.5 and fixed surfactant-to-oil ratio (0.3763).

4.7.1 Phase Inversion Process

Figure 4.26 shows the dynamic conductivity curve of emulsion systems (only pure water and 0.001M NaCl systems are shown) with oil concentration from R=0.2 to R=0.4, as a function of temperatures. The initial conductivity increased with oil concentrations in pure milliQ water system (Figure 4.26(a)) but remained unchanged in 0.001M NaCl system (Figure 4.26(b)) due to the higher conductive potential in sodium chloride system. The increase of conductivity in pure milliQ water system was due to the increase concentration of technical grade non-ionic surfactant while the oil concentration was increased at fixed R_{so} . The increase was different with decane system, where the initial conductivity decreased with oil concentrations (Kunieda *et al.*, 1996; Forgiarini *et al.*, 2001). In a brine system, the temperature at zero conductivity increased with increasing R (Figure 4.26(b)), which agreed with the results from Anton *et al* (2008) and Forgiarini *et al* (2001). In pure milliQ water system, the temperature of 1st maximum decreased from 20.8°C at R=0.2 to 19.7°C at R=0.3 (Figure 4.26(a)). Furthermore, the 2nd maximum was lower at R=0.3 while there was no 2nd maximum at R=0.4, in a pure milliQ water system. However, in brine

⁵ Surfactant-to-oil (R_{so}) ratio is 0.3763

⁶ Surfactant-to-oil ratio (R_{so}) is 0.3191

system, there was no 2nd maximum starting from R=0.3 (Figure 4.26(b)). Therefore, the phase inversion process for system with R=0.3 (at fixed R_{so}) was changed while the process for system with R=0.4 was unchanged by the addition of sodium chloride.

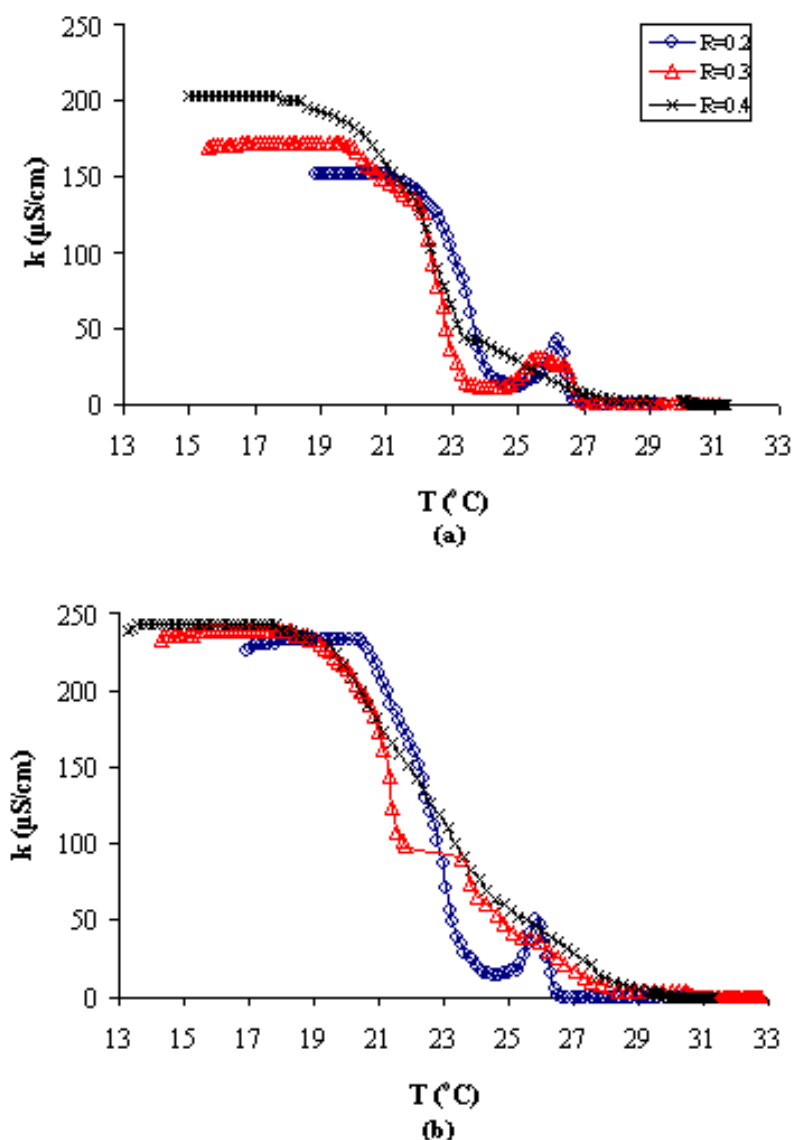


Figure 4.26: Dynamic conductivity values as a function of temperatures for systems with (a) pure milliQ water and (b) 0.001M NaCl and with different oil concentrations but fixed surfactant-to-oil ratio ($R_{so}=0.3191$).

Due to the shift of the temperatures at 1st maximum and 1st minimum, the PIT temperatures were expected to be changed with oil concentrations and by the addition of sodium chloride. A detailed study in the changes of PIT temperatures as a function of sodium chloride concentrations in R=0.2, 0.3 and 0.4 is shown in Figure 4.27. At $R_{so}=0.3763$ and pure milliQ water system, the PIT temperature decreased 2 $^{\circ}C$ when the oil concentration was increased from R=0.2 to 0.3; however, the temperature increased when the oil concentration was continuously increased to R=0.4. The results were different in 0.01M NaCl/Brij30/decane system, where the PIT temperature was continuously increased with increasing R (Forgiarini *et al.*, 2001). The difference was because of the reduction in the surfactant-to-oil ratio (fixed surfactant concentration)

in the decane system. The reduction of PIT temperature from $R=0.2$ to $R=0.3$ in present study may be because of the increase of surfactant concentration and therefore there was more amount of short ethylene oxide chain at oil-water interface (Izquierdo *et al.*, 2002).

At $R_{so}=0.3191$, the reduction of PIT temperature as a function of oil concentration was insignificant in pure milliQ water system and brine systems with 0.001M and 0.01M NaCl. However, the reduction was significant in 0.1M NaCl system since the dehydrating effect was more pronounced. The reduction of PIT temperature from pure milliQ water system to 0.1M NaCl system ($R_{so}=0.3191$) was 2.6°C in $R=0.3$ and 2.5°C in $R=0.4$ while only 0.8°C in $R=0.2$ (Figure 4.27). The surfactant concentration was considered large enough to interact with the water and oil phase to cause a small change in the PIT temperature (Forgiarini *et al.*, 2001). Therefore, the PIT temperature was more dependent on oil concentration (Anton *et al.*, 2007). There was a sharp decrease in the PIT temperature from 0.01M NaCl to 0.1M NaCl system, when R was 0.3 and 0.4 (whilst their PIT temperatures were nearly the same at $R=0.2$). It was because at higher oil concentration, more dehydrated non-ionic surfactant appeared in the system and caused the HLB of the system being decrease significantly, although there was fixed surfactant-to-oil ratio.

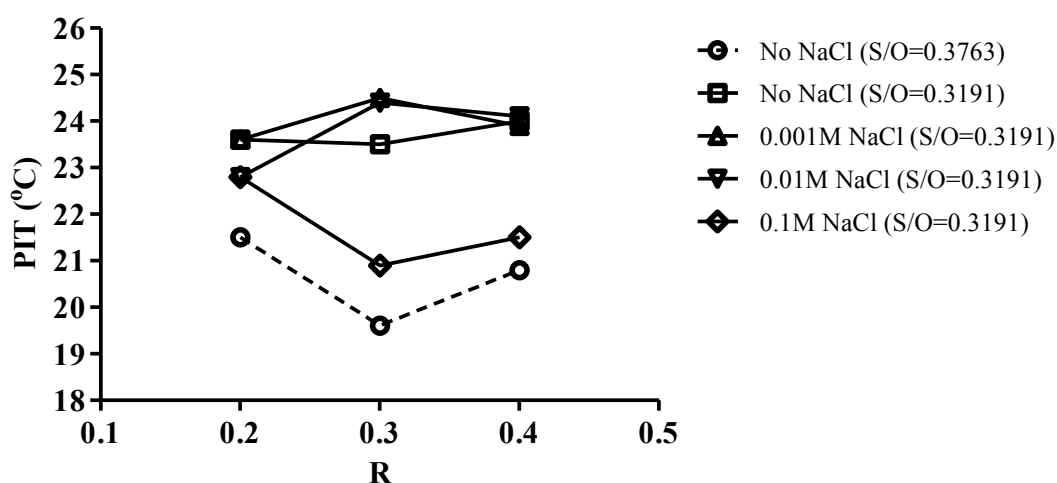


Figure 4.27: PIT temperature as a function of oil and sodium chloride concentrations with fixed surfactant-to-oil ratio.

The ΔT_{trans} as a function of oil and sodium chloride concentrations is shown in Figure 4.28. At $R_{so}=0.3763$ and in pure milliQ water system, the ΔT_{trans} was almost being half from 10.2°C to 5.8°C , when the oil concentration was increased to $R=0.3$ from 0.2. At $R_{so}=0.3191$ and fixed NaCl concentration, the ΔT_{trans} increased with oil concentration in either pure milliQ water or brine systems. For example, in 0.01M NaCl system, the ΔT_{trans} increased from 9.9°C to 11.6°C when the oil concentration was from $R=0.2$ to 0.3. The increase was due to the slower rate of decomposition of oil phase in the phase inversion process (Taisne and Cabane, 1998) at a constant sodium chloride concentration. At fixed $R_{so}=0.3191$ and when the sodium chloride concentration was increased from zero to 0.1M, the ΔT_{trans} was decreased 0.2°C at $R=0.3$ while there was 1.4°C at $R=0.4$. The decrease in ΔT_{trans} was more significant at higher oil concentration as the amount of the non-ionic surfactant was increased at a

fixed surfactant-to-oil ratio, thus the effect of the dehydrated non-ionic surfactant on HLB became more pronounced. In $R=0.2$ and 0.3 , the change of ΔT_{trans} were fluctuating due to the dehydrating non-ionic surfactant to hasten the phase inversion process (shorted ΔT_{trans}) and the excess surfactant to obstruct the heat transfer (longer ΔT_{trans}), which have been discussed in Section 4.5.1.

The effect of sodium chloride in phase inversion process became more significant in a system with higher oil concentration. In a fixed surfactant-to-oil ratio, although the coverage of the surfactant on the interface was expected unchanged with increasing oil concentration. However, the effect of the dehydrated non-ionic surfactant on the phase inversion process became more pronounced when the amount of surfactant was increased with the increase of oil concentration.

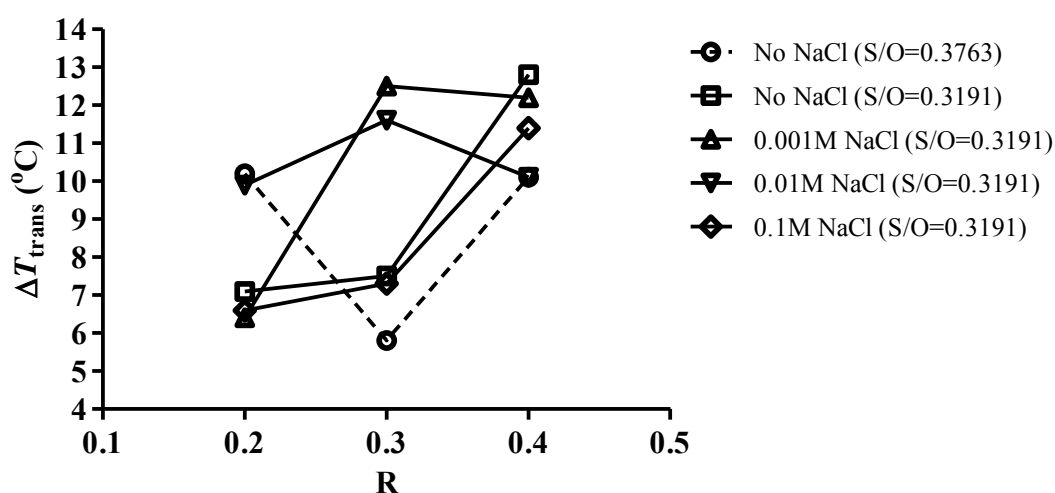


Figure 4.28: Width of transitional zone for system containing different oil and sodium chloride concentrations with fixed surfactant-to-oil ratio.

4.7.2 Formation and Stability of Nanoemulsions

As the PIT temperatures for systems with $R=0.3$ and 0.4 were higher than 15°C (Figure 4.27), the same method to produce n-dodecane nanoemulsions in previous sections was used in this section. The initial PSD curves of nanoemulsions with $R=0.2$ and 0.3 as a function of sodium chloride concentrations are shown in Figure 4.29. In the figure, there were two different R_{so} , which were 0.3763 and 0.3191 . At $R=0.2$, monodispersed nanoemulsions (single peak curve in PSD) with different sodium chloride concentrations were produced at both R_{so} (Figure 4.29(a)). However, when R was larger than 0.2 , nanoemulsions with two-peak PSD curve were found in pure milliQ water system at $R_{\text{so}}=0.3763$ (only $R=0.3$ is shown in Figure 4.29(b)). In present study, at $R_{\text{so}}=0.3191$, the initial PSD curve behaved the same as single peak in the systems with $R=0.2-0.4$ and sodium chloride concentration was $0-0.1\text{M}$. Furthermore, the 0.1M NaCl appeared to have the lowest volume percentage and widest size distribution in those three oil concentrations ($R=0.2, 0.3$ and 0.4). The polydispersity in $R_{\text{so}}=0.3763$ at R larger than 0.2 (Figure 4.29(b)) was because the PIT

temperatures in the systems were closer to the storage temperature (20°C) and so the systems were stored closer to the inversion zone and caused the instability.

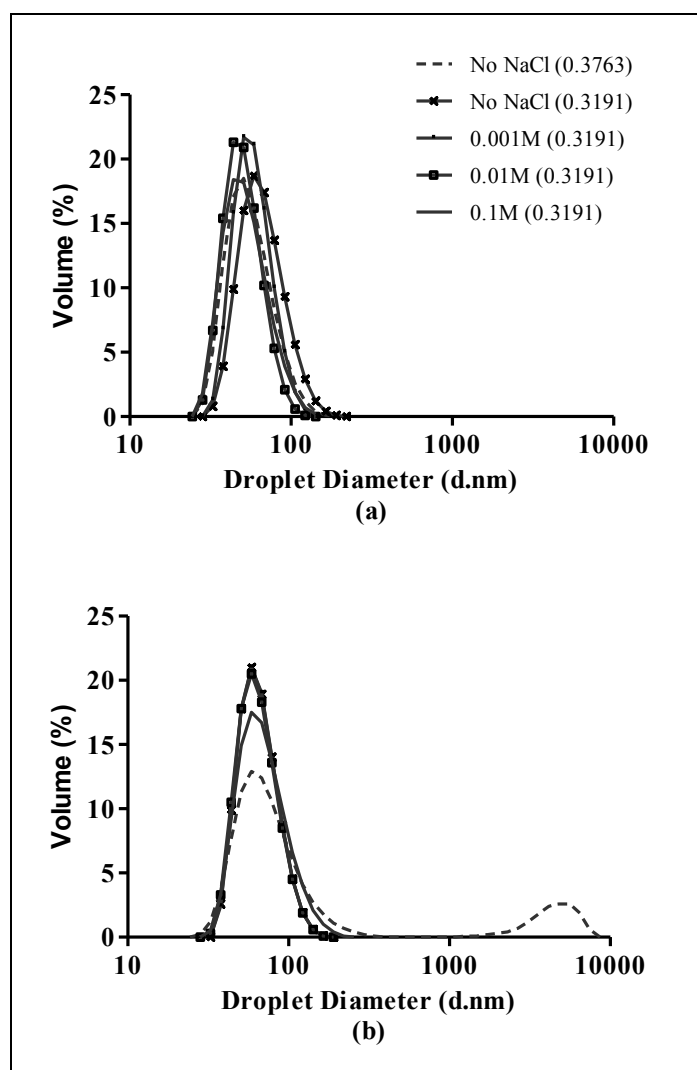


Figure 4.29: Initial size distribution at 20°C for n-dodecane nanoemulsions with (a) $R=0.2$ and (b) $R=0.3$ and two different fixed R_{s0} .

At 20°C, the initial droplet size and PDI of n-dodecane nanoemulsions as a function of oil concentration (at fixed R_{s0}), are shown in Figure 4.30. The figure also shows the effect of sodium chloride in the formation of the nanoemulsions. According to the figure, systems with $R=0.3$ and 0.4 produced the nanoemulsions with PDI smaller than 0.2 and droplet size smaller than 100nm at $R_{s0}=0.3191$ (Figure 4.30). At $R_{s0}=0.3763$, in pure milliQ water system with $R=0.3$ and 0.4 , the droplet size was larger than 100nm with PDI higher than 0.2 . The initial droplet size and PDI increased when oil concentration was increased from $R=0.2$ to 0.4 and the increment became more significant at high sodium chloride concentrations. For example, in 0.1M NaCl system, the PDI was lower than 0.1 at $R=0.2$ system but larger than 0.1 at $R=0.3$ and 0.4 systems. The increase in droplet size and PDI was because of the increase in diffusion rate when the interaction of droplets was increased with the oil concentration. Furthermore, the system with $R_{s0}=0.3763$ was monodispersed at a low R value (0.2) but polydispersed at a high R value (0.3) (Figure 4.29). The increment in droplet size with R_{s0} was because the oil constitutes the inner structure of the nano-droplets, which

is consistent with a direct O/W-type structure (Sadurni *et al.*, 2005). It has been suggested that system with an optimum R_{so} can produce the finest, smallest and most stable nanoemulsions and they are independent on oil concentration whilst any R_{so} apart from it will cause the droplet size and PDI to be increased (Morales *et al.*, 2003). In this study, 0.3191 may be considered as the optimum ratio to produce the n-dodecane nanoemulsions, as the droplet size (less than 100nm) and PDI (smaller than 0.2) were almost unchanged within the studied oil concentration.

At $R_{so}=0.3191$, the initial droplet size and PDI in nanoemulsions with $R=0.3$ were the same between systems without sodium chloride and with NaCl concentration at 0.001M and 0.01M. However, in $R=0.4$, the initial droplet size and PDI were larger in system with sodium chloride and were increased with the NaCl concentration. When 0.1M NaCl was used as a continuous phase, the initial droplet size and PDI were always appeared to be the largest among the brine system regardless the oil concentration in (where the oil concentration was $R=0.2-0.4$) in this study.

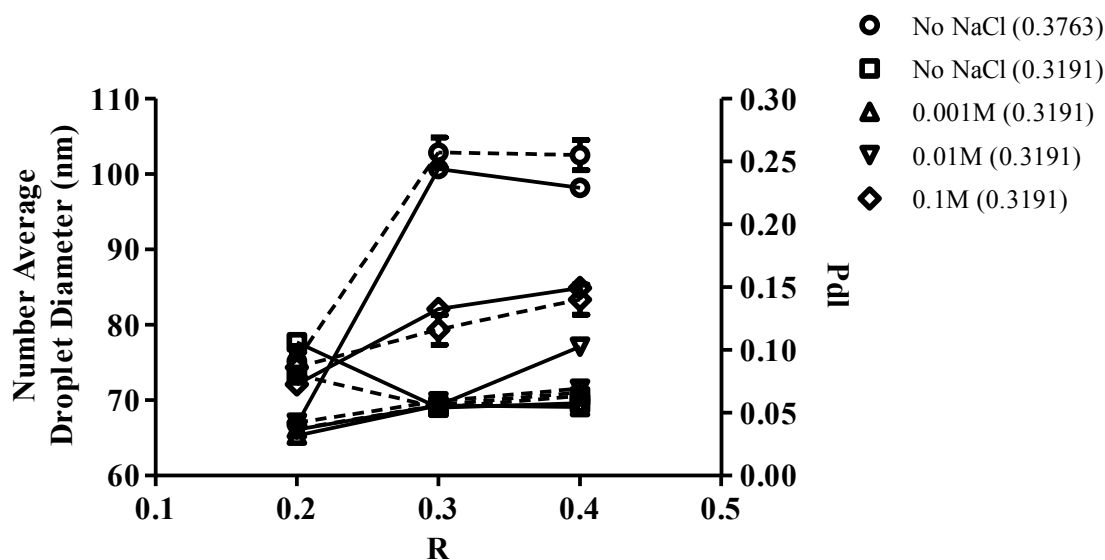


Figure 4.30: Initial droplet size and polydispersity index (PDI) as a function of oil concentrations (R) at 20°C for n-dodecane nanoemulsions with two R_{so} . Solid line: droplet diameter; dashed line: PDI.

The period for nanoemulsions with $R=0.2-0.4$ to have phase separation is shown in Table 4.11. When R_{so} was 0.3763, only nanoemulsions with $R=0.2$ and milliQ water remained one-phase transparent for 30 days while for $R=0.3$ and 0.4, the systems were phase separated very quickly. At $R_{so}=0.3191$, O/brine nanoemulsions with $R=0.2$ were stable without phase separation for more than 30 days; however, only 0.001M NaCl system at $R=0.3$ and 0.01M NaCl system at $R=0.4$ had the same stability. When the oil concentration increased, number of droplets increased; therefore, more sodium chloride was needed to cause the compression effect (increase the surfactant concentration on oil-water interface). Additionally, the osmotic pressure needed to be increased by the addition of sodium chloride, when the aqueous concentration was decreased (oil concentration increased). In addition to that, 0.001M NaCl appeared to have largest ΔT_{trans} among the brine systems at $R=0.3$ (Figure 4.28),

showing that the completeness of the solubilisation of n-dodecane during the inversion process and to produce a stable nanoemulsion system.

When the sodium chloride concentration was higher than 0.001M NaCl in R=0.3 or 0.01M NaCl in R=0.4, the stability was decreased as the dehydrating effect became more pronounced. For 0.1M NaCl system, the instability might also due to the lower PIT temperature (21°C in R=0.3 and 21.5°C in R=0.4), which was close to the storage temperature (20°C). In present study, $R_{so}=0.3191$ had been considered as an optimum ratio to produce nanoemulsions with smallest initial droplet size and lowest PdI in different oil concentrations (R=0.2-0.4). This optimum ratio was also verified by the systems with different salt concentrations such as to produce stable n-dodecane nanoemulsions in R=0.3 with 0.001M NaCl and in R=0.4 with 0.01M NaCl.

Table 4.11: Days taken for phase separation for n-dodecane nanoemulsions with different R and two fixed R_{so} . Storage temperature=20°C.

[NaCl] (M)	S/O	R = 0.2	R = 0.3	R = 0.4
0	0.3763	>30	-	-
0	0.3191	3	>30	3
0.001	0.3191	>30	>30	5
0.01	0.3191	>30	19	>30
0.1	0.3191	>30	6	20

4.7.3 Reversibility of Nanoemulsions

The reversibility testing for systems with different oil concentrations (R=0.2-0.4) and fixed R_{so} was carried out by the same method which was previously used: heating and cooling processes. Only systems at $R_{so}=0.3191$ and with sodium chloride were investigated, due to their high stability. Some selected results from the reversibility testing are shown in Table 4.12. According to the table, when thermal equilibrium time was 5 minutes, the system with R=0.3 and 0.001M NaCl was being partial reversible, as only the droplet size could be rejuvenated from ageing to freshly-prepared nanoemulsions. However, when the salt concentration was increased to 0.1M, the system with R=0.3 was irreversible in both droplet size and PdI.

When the thermal equilibrium time was increased from five minutes to ten minutes, the droplet size in system with R=0.3 and 0.001M NaCl was decreased to 72.9nm from 80.9nm and the PdI was decreased to 0.077 from 0.129 (Table 4.12), which were closer to the freshly prepared sample (69.3nm and 0.056 in droplet size and PdI). Therefore, it was another evident to show that more time was needed to reverse the properties of ageing nanoemulsions to freshly-prepared state if the system has larger ΔT_{trans} (as shown in Section 4.6.3 for the most stable nanoemulsions). Furthermore,

the number of droplet needed to be reversed was increased when the oil concentration was increased; resulting a longer thermal equilibrium time was needed to fully reverse the ageing nanoemulsions to their freshly-prepared state.

Table 4.12: Reversibility testing for selected systems with R=0.3 and different sodium chloride concentrations but (R_{os} (0.3191)) after three days storage time. The test was done in a temperature sequence: 20°C→10°C→20°C→30°C→20°C.

	0.001M (R = 0.3)		0.1M (R=0.3)	
	Mean Diameter (nm)	Pdl	Mean Diameter (nm)	Pdl
Freshly-prepared (20°C)	69.3	0.056	82.1	0.116
Ageing (3 days)				
20°C	68.8	0.055	80.7	0.075
10°C	62.8	0.040	69.6	0.040
20°C	91.2	0.184	77.3	0.063
30°C	3321.5	1.000	2253.5	1.000
20°C (5 minutes)	80.9	0.129	103.0	0.167
20°C (10 minutes)	72.9	0.077		

With the concentration of sodium chloride was 0.1M, there was partial reversible system in R=0.2. However, when the concentration of continuous phase decreased (R increased), the dehydrating and salting-out effect on the non-ionic surfactant were more pronounced. The properties of the non-ionic surfactant were more likely to be “damaged” permanently in systems with R=0.3 and 0.4 (relatively high sodium chloride concentration in high oil concentration). Moreover, the low stability in 0.1M NaCl systems at R=0.3 and 0.4 (phase separated in less than 30 days) has contributed to the ability to reverse the size and Pdl, and also the PIT temperature was close to the final temperature in heating and cooling process.

4.8 Effect of sodium chloride in a mixed surfactant system

It is well known that the surfactant concentration, oil concentration and the type of non-ionic surfactant are the important factors in the production of nanoemulsions by the PIT method (Shinoda and Saito, 1969; Izquierdo *et al.*, 2002; Izquierdo *et al.*, 2004). The effect of first two factors with the addition of sodium chloride had been investigated and discussed in previous sections. In this section, the effect of the type

of non-ionic surfactant with different sodium chloride concentrations was investigated. Mixed surfactant system has been proved to produce more stable nanoemulsions than single surfactant system (Uson *et al.*, 2004; Izquierdo *et al.*, 2005). Brij30 was chosen to be blended with another non-ionic surfactant, with a lower combined-HLB number, to create a new surfactant system. Brij30 is a non-ionic surfactant with a relatively high HLB number (9.7), which is used to produce aqueous-continuous nanoemulsions (Kabalnov, 1998). Therefore, Span 80 (S80) with HLB number is 4.3, which is widely used to produce oil-continuous nanoemulsions by CIP method (Wu *et al.*, 2001; Porras *et al.*, 2005), was chosen to blend with Brij30. The combined HLB number however would keep at a range of 8-18, which to produce aqueous-continuous nanoemulsions (Kabalnov, 1998).

Systems with R=0.2 were chosen for further investigation in this section. According to the results from previous sections, the most stable nanoemulsions were 7wt% Brij30 in pure milliQ water system and 6wt% Brij30 in brine systems. Those conditions would be remained unchanged; however, the surfactant system being used was the blended non-ionic surfactant instead of pure Brij30. The new HLB number (with the combination of two non-ionic surfactants) was calculated by Equation (4).

$$HLB_{mix} = HLB_1x \frac{m_1}{m_T} + HLB_2x \frac{m_2}{m_T} \quad (4)$$

where HLB_{mix} , HLB_1 and HLB_2 are the HLB values of the mixed surfactant and the first and second surfactant, m is the mass with the subscript 1 and 2 are the first and second surfactant, and m_T is total mass of two surfactants.

Two surfactants were mixed with equal mass (50/50 w/w with HLB=7) in the first attempt, the initial conductivity was very low at the starting temperature (13°C) and did not change with temperatures, thus there was no aqueous-continuous emulsions produced, due to the low HLB number. In the second attempt, the weight percentage of S80 was decreased to 20wt% (80wt% of Brij30), which the HLB number was changed to 8.6, the initial conductivity was higher than the equal mass system. However, there was no sudden change in the conductivity curve when the system was heated up. Furthermore, the system (20/80) was cooled down to 8.5°C and the conductivity was increased. Therefore, the PIT temperature was expected to be lower than 0°C and oil-continuous emulsions or transitional phases were produced at the starting temperature. Due to the limitation of the storage temperature, the mass fraction of S80 needed to be decreased to create a surfactant system with higher HLB and higher PIT temperature. After several trials, it has been found that the highest mass percentage of S80 to produce aqueous-continuous emulsions at the starting temperature (13°C) was 10wt%. With 10wt% of S80, the final HLB number of the non-ionic surfactant system itself was 9.16 (90wt% of Brij30), which is a value to favour the production of aqueous-continuous emulsions.

4.8.1 Phase Inversion Process

Figure 4.31 shows the dynamic conductivity curve of emulsion system with different surfactant system. The figure also shows the effect of the addition of sodium chloride (only 0.01M NaCl system is shown in Figure 4.31(b)). According to the figure, in a

mixed surfactant (Brij30/S80) with pure milliQ water system, the height of the 2nd maximum was small when the surfactant concentration was 6wt% but there was no 2nd maximum when the concentration was 7wt%; however the 2nd maximum existed in both of the surfactant concentrations in a pure-surfactant system (Brij30). In pure milliQ system and with surfactant concentration was 6wt%, the temperature of 1st maximum was decreased from 20.1°C to 11.6°C when the HLB number of the surfactant system was decreased from 9.70 to 9.16 (Figure 4.31(a)). Therefore, the phase inversion process occurred at lower temperature when the surfactant system was a mixture of Brij30 and Span80.

In a brine system, in a blended-surfactant system, the shape of the conductivity curve was identical with the one in pure milliQ water system, where there was small 2nd maximum in 6wt% of surfactant and no 2nd maximum in 7wt% surfactant. The addition of sodium chloride in the mixed-surfactant emulsion system did not have any effect in the formation of transitional phase (WIII). Comparing the system with surfactant concentration was 7wt% in mixed-surfactant system, the temperature of 1st maximum was 13.4°C in pure milliQ water system while it was 11.6°C in 0.01M NaCl system, and therefore, the phase inversion occurred at lower temperature when sodium chloride was added into the emulsion system. The change of the conductivity behaviour as a function of HLB number in this study, agreed with the 0.01M NaCl/isohehexadecane system (Izquierdo *et al.*, 2005), where the phase inversion process occurred at lower temperature in system with smaller HLB number.

By blending a W/O emulsifier (S80) in an emulsion system, although the change of the HLB number was small (from 9.7 to 9.16), the conditions for the production of nanoemulsions with the PIT method was dramatically changed, where the changes in PIT and transitional temperatures are shown in Figure 4.32 and Figure 4.33. The PIT temperature was lower in mixed-surfactant system and by increasing the surfactant or sodium chloride concentrations, the PIT temperature was decreased. In present study, the reduction of PIT temperature in the 0.01M NaCl system was 15.4°C/HLB when the surfactant concentration was 6wt% and 8.9°C/HLB when the surfactant concentration was 7wt%. It has been reported that the PIT temperature was reduced by 19°C/HLB in a 0.01M NaCl/C₁₂E₄:C₁₂E₆/isohehexadecane system and with the surfactant concentration was 4wt% (Izquierdo *et al.*, 2005). The reduction from present study was close to the published result although different systems have been used; therefore, the change of PIT temperature was seem like more dependent on the HLB number of surfactant system than the emulsion system.

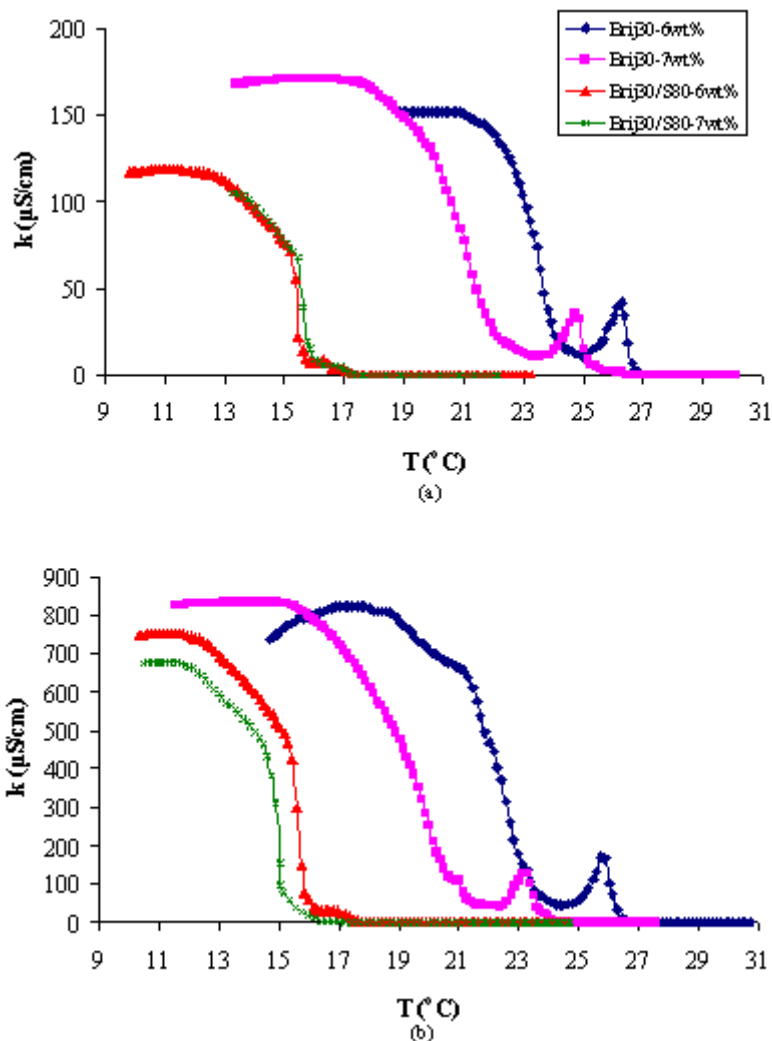


Figure 4.31: Conductivity values as a function of temperatures for systems with (a) pure milliQ water and (b) 0.01M NaCl and with different types of surfactant. Oil/aqueous=20/80 w/w.

The largest reduction of PIT temperature from a pure milliQ water to a brine system was 2°C where the system was with mixed surfactant and surfactant concentration was 6wt%. Furthermore, the PIT temperature for a brine system in Brij30/S80 system was always lower than a pure milliQ water system (Figure 4.32), which was similar with pure Brij30 system. It was because the blended surfactant system contained more Brij30 (90wt%), and so the result was similar to the pure-Brij30 system.

According to Figure 4.33, ΔT_{trans} almost decreased in half from pure-surfactant to mixed-surfactant emulsion system, at a constant surfactant concentration. The decrease of ΔT_{trans} was more significant in higher surfactant and sodium chloride concentrations. For a pure milliQ water system, when the surfactant system changed from Brij30 to the mixture of Brij30 and S80, the decrease in ΔT_{trans} was $2.4^{\circ}\text{C}/\text{HLB}$ when the surfactant concentration was 6wt% while it was $10.6^{\circ}\text{C}/\text{HLB}$ when the surfactant concentration was 7wt%. In the system containing sodium chloride, the decrease of ΔT_{trans} increased from $3.3^{\circ}\text{C}/\text{HLB}$ to $9.8^{\circ}\text{C}/\text{HLB}$ when the concentration was increased from 0.001M to 0.1M NaCl, at a surfactant concentration was 6wt%.

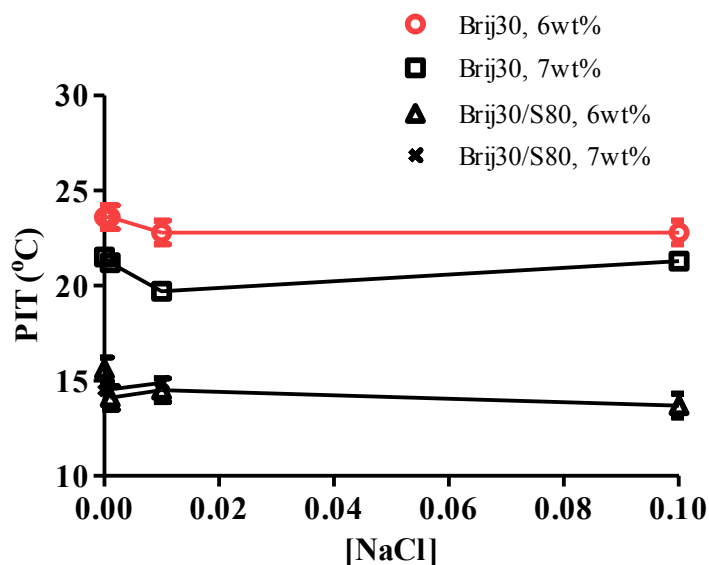


Figure 4.32: PIT temperature as a function of sodium chloride concentrations. Surfactant concentrations are 6wt% and 7wt% for mixed-surfactant (90/10w/w Brij30/S80) emulsion system and pure-surfactant (Brij30) emulsion system. Oil/aqueous=20/80 w/w.

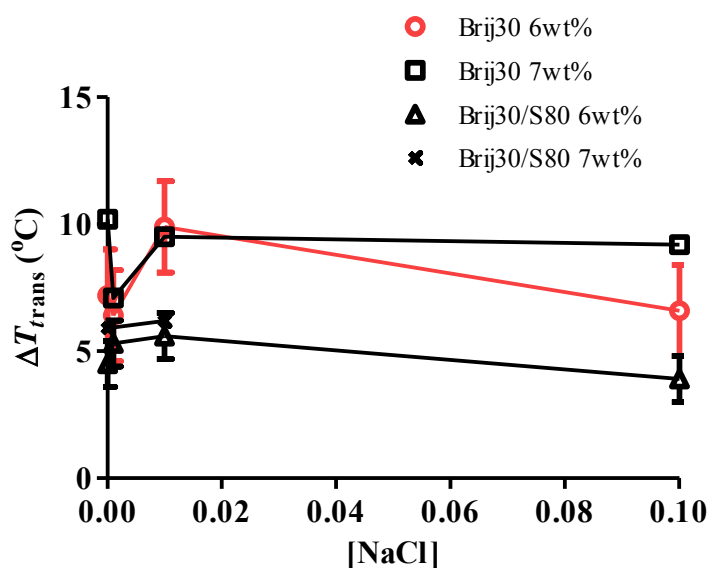


Figure 4.33: Width of transitional zone for systems containing different sodium chloride concentrations. Surfactant concentrations are 6wt% and 7wt% for mixed-surfactant system (90/10w/w Brij30/S80) and pure-surfactant (Brij30) emulsion system. Oil/aqueous=20/80 w/w.

The high hydrophobic property from Span80 in the mixed surfactant system decreased the solubility of the surfactant in aqueous phase and the hydrophilic chain in the surfactant system was decreased. Therefore, in the production of n-dodecane nanoemulsions, the PIT temperature and ΔT_{trans} was depressed with the addition of S80. In addition to that, when sodium chloride was added and the hydrophilic chain of Brij30 was dehydrated, there was a combination of hydrophobic effect from S80 and

dehydrated Brij30 to cause the reduction of PIT temperature and hasten the phase inversion process (smaller ΔT_{trans}).

4.8.2 Formation and Stability of Nanoemulsions

The PIT temperature for mixed surfactant system was lower than 15°C, which has been shown in Figure 4.32, so that the method to produce n-dodecane nanoemulsion was different with the previous sections. An aqueous-continuous emulsion system with mixed surfactant was gradually heated to oil-continuous emulsions then followed by a quenching process to cool the system to 5°C to produce aqueous-continuous nanoemulsions and stored at 10°C, while the cooling temperature was 15°C and storage temperature was 20°C had been used in previous sections.

The initial size distribution for the mixed-surfactant emulsion system is shown in Figure 4.34. All systems were polydispersed (two peaks in the distribution graph), whereby the polydispersity was more significant in 0.001M and 0.1M NaCl system (at 6wt% mixed-surfactant). The polydispersity was due to the addition of a hydrophobic surfactant (S80) and also the salting-out effect from sodium chloride, making the solubility of the surfactant in aqueous phase was too low to produce a fine aqueous-continuous nanoemulsions. The polydispersity of 0.01M NaCl system was relatively low among the brine systems (insert figure in Figure 4.34), suggesting that 0.01M NaCl may be a sodium chloride concentration to produce finer O/brine nanoemulsions among the brine systems. a similar sodium chloride concentration to produce finer n-dodecane nanoemulsions had been reported in previous sections (Section 4.5 and 4.6)

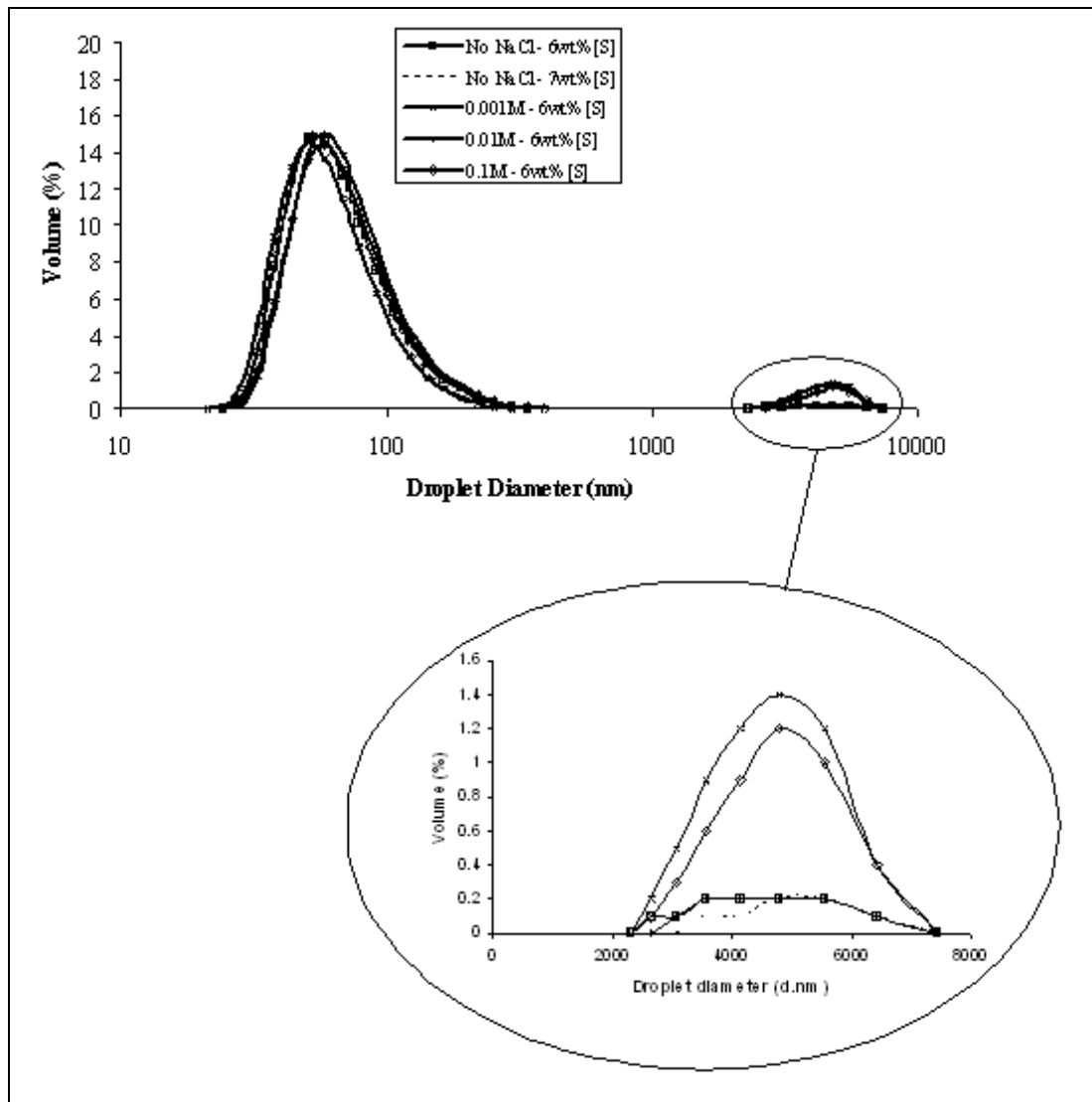


Figure 4.34: Initial size distribution at 10°C for n-dodecane nanoemulsions with different sodium chloride concentrations and in a mixed surfactant system (90/10w/w Brij30/S80). Surfactant concentrations are 6wt% and 7wt% for pure milliQ water system while only 6wt% for brine systems. Oil/aqueous=20/80 w/w.

Figure 4.35 indicates the changes of droplet sizes and PDI when the surfactant system was changed from Brij30 to Brij30/S80, as a function of NaCl concentrations. Only systems with surfactant concentration was 6wt% are shown to investigate the effect of sodium chloride on the droplet size and PDI. Droplets with less than 100nm and PDI lower than 0.1 were produced in a pure milliQ water system but they were larger than 100nm and with PDI higher than 0.1 in a mixed-surfactant system. It has been reported that a system with mixed surfactant and with an optimum HLB number (by mixing ratio of surfactants) is able to produce aqueous-continuous nanoemulsions with the smallest initial droplet sizes (Porrás *et al.*, 2004; Izquierdo *et al.*, 2005; Liu *et al.*, 2006; Morais *et al.*, 2006; Sajjadi, 2006). However, in present study, the mixed-surfactant emulsion system produced coarser nanoemulsions than pure-surfactant system (Figure 4.35). It may be because the system was not in the optimum HLB number. However, the current results (droplet size and PDI increased in mixed surfactant system) showed some similarity with n-isohexadecane system at low surfactant concentration (4wt%) (Izquierdo *et al.*, 2005). A detailed comparison could not be

made as the mixed surfactant system used in Izquierdo *et al* (2005) was with both surfactants were O/W emulsifier (HLB number larger than 6) while in present study, there was a mixture between O/W and W/O emulsifier.

In a pure-Brij30 system, the droplet size was smaller and PDI was lower when sodium chloride was added and when they increased with the increase of NaCl concentration. In a mixed surfactant system, the droplet size increased from 103.7nm at no NaCl system to 106.5nm at 0.1M NaCl system. The increment was considered significant with a standard deviation was 0.63nm. From a pure milliQ water system to 0.1M NaCl system, the system became more polydispersed as the PDI was increased from 0.177 to 0.203 (with a standard deviation of 0.012). According to Figure 4.35, the effect of sodium chloride in the formation of n-dodecane was less significant then the effect from surfactant type (or different HLB number in a surfactant system). It may due to the effect of the hydrophobic tail in S80 was more significant than the salting-out effect by the addition of sodium chloride.

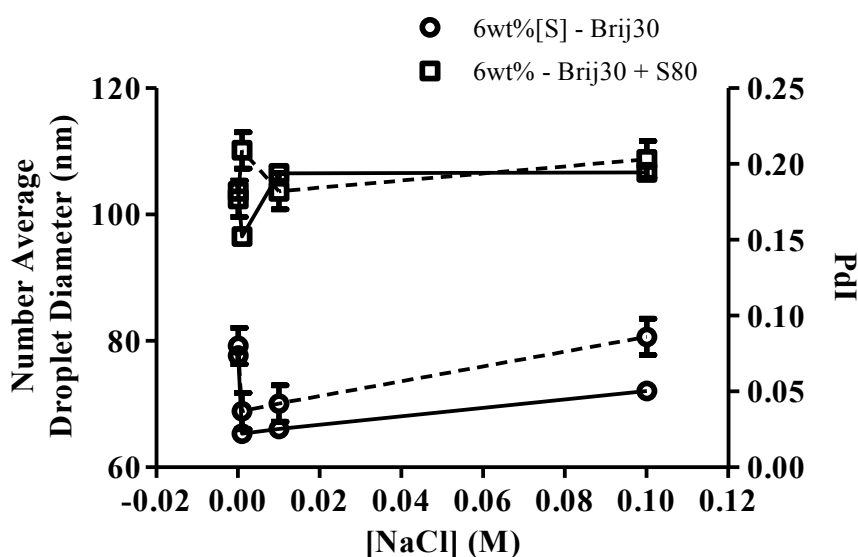


Figure 4.35: Initial droplet size and polydispersity index (PDI) as a function of sodium chloride concentrations at 10°C for n-dodecane nanoemulsions in different surfactant systems. The total surfactant concentration is 6wt%. Oil/aqueous=20/80 w/w. (Solid line: droplet diameter; dashed line: PDI)

As the combined HLB number in present study may not be the optimum HLB number to produce nanoemulsions, the stability of the nanoemulsions with mixed surfactant (Brij30/S80) was decreased, where the days taken for phase separation are shown in Table 4.13. The only stable nanoemulsions in mixed-surfactant system was pure milliQ water system with the surfactant concentration was 7wt%, which was the same with the pure-Brij30 system (Table 4.7). However, the droplet size and PDI were much larger in mixed-surfactant system. The mixed surfactant systems with the surfactant concentration was 6wt% were phase separated after one day they had been prepared (Table 4.13). Therefore, the growth rate in droplet size and PDI was unable to be determined. In this study, it has shown that nanoemulsions with smaller initial droplet size, narrower size distribution and higher stability could only be produced if only one surfactant, which was HLB number greater than 6, was used.

Table 4.13: Days taken for phase separation for n-dodecane nanoemulsions (oil/aqueous=20/80 w/w) stored at 10°C with different sodium chloride concentrations. Surfactant mixing ratio is 90/10 Brij30/S80 (in weight).

[S] (wt%)	No NaCl	0.001M NaCl	0.01M NaCl	0.1M NaCl
6	1	1	1	1
7	>30	-	-	-

4.8.3 Reversibility of Nanoemulsions

As the mixed surfactant/NaCl nanoemulsions systems were phase separated after one day they have been prepared, the reversibility testing was unable to be carried out (in a period of three days). Although the nanoemulsions with pure milliQ water and surfactant concentration was 7wt% were remained one phase for more than 30 days, the system without sodium chloride has been shown previously to have no ability to reverse the droplet size and PDI (Chapter 4.5.3).

4.9 Effect of different electrolyte

In this section, the effect of the addition of calcium chloride on the formation, stability and reversibility of n-dodecane nanoemulsions was investigated with the surfactant concentration being 6wt% and the R being 0.2. Only Brij30 was used in this study as the mixed-surfactant system was unable to produce stable nanoemulsions (Section 4.8). Furthermore, the result from this study was compared with those obtained from sodium chloride system.

4.9.1 Phase Inversion Process

Figure 4.36 shows the shape of the dynamic conductivity curve of calcium chloride system was same with pure milliQ water system, where there were 1st maximum, 2nd maximum, 1st minimum and 2nd minimum. The temperature of the 1st maximum of the conductivity curve decreased from 20°C in pure milliQ water system to 18.9°C in 0.01M CaCl₂ system, and also to 18.3°C in 0.1M NaCl system. The decrease of the temperature with CaCl₂ concentration behaved similarly with sodium chloride system, indicating the phase inversion process occurred at lower temperature, when calcium chloride was added into an emulsion system and when the concentration was being increased. Furthermore, the phases involved in the phase inversion process in CaCl₂ system was the same as those in NaCl system.

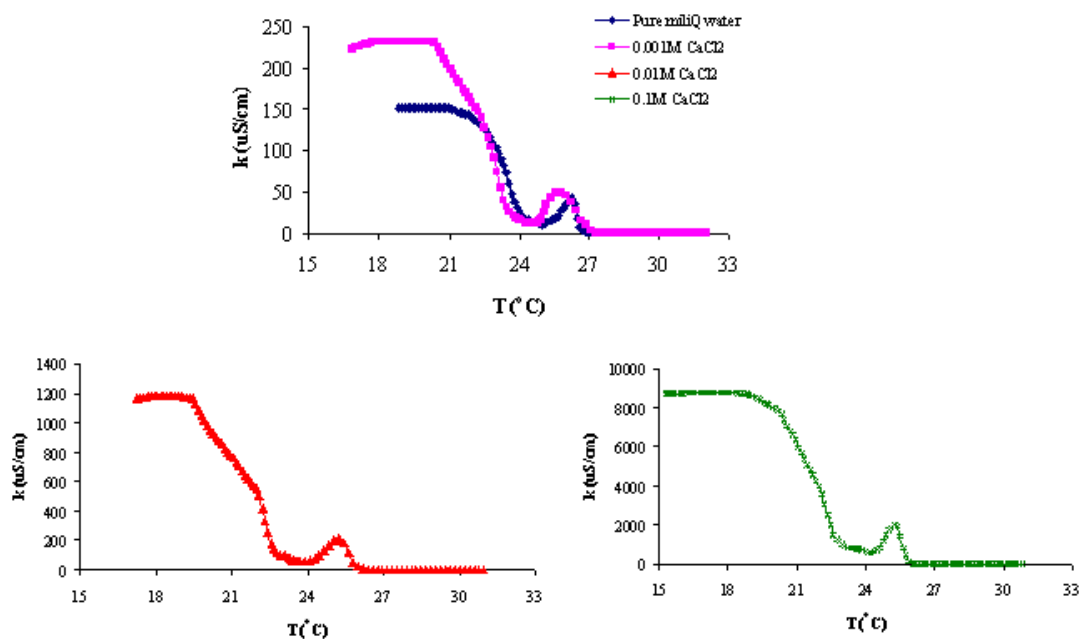


Figure 4.36: Dynamic conductivity value as a function of temperatures for systems with different calcium chloride concentrations. Surfactant concentration is 6wt% of Brij30. Oil/aqueous=20/80 w/w.

From Figure 4.36, the PIT temperature of CaCl₂ and NaCl systems are shown as a function of electrolyte concentrations, and it is found that the temperature was lower in calcium chloride system compared to the pure milliQ water system. The PIT temperature was reduced 1.3°C in calcium chloride system while it was only 0.8°C in sodium chloride system, when the electrolyte concentration was increased from zero to 0.1M and with a surfactant concentration was 6wt% Brij30 (Figure 4.37). It has been reported that the reduction of the PIT temperature was larger in sodium chloride system than in calcium chloride system in a heptane-water emulsion system containing 3wt% of polyoxyethylene (9.7) nonylphenylether (Shinoda and Takeda, 1970). The dehydrating effect of the hydrophilic tail in a non-ionic surfactant is mainly caused by the cation from an electrolyte, and so the larger reduction in calcium chloride system in present study may be because of the higher degree of dehydration by calcium cation (+2) than sodium cation (+1). With a standard deviation of 0.63°C, the difference in the reduction between both electrolyte systems was actually insignificant. Although the dehydrating effect from calcium chloride was doubled than sodium chloride at same concentration (higher charged of cation in CaCl₂), the insignificant difference was due to the low electrolyte concentration (0.63wt% in CaCl₂ and 0.33wt% in NaCl). In addition, the CaCl₂ concentration was up to 10wt% in the heptane-water emulsion system (Shinoda and Takeda, 1970). Therefore, the reduction of the PIT temperature between calcium chloride and sodium chloride system in present study was almost the same.

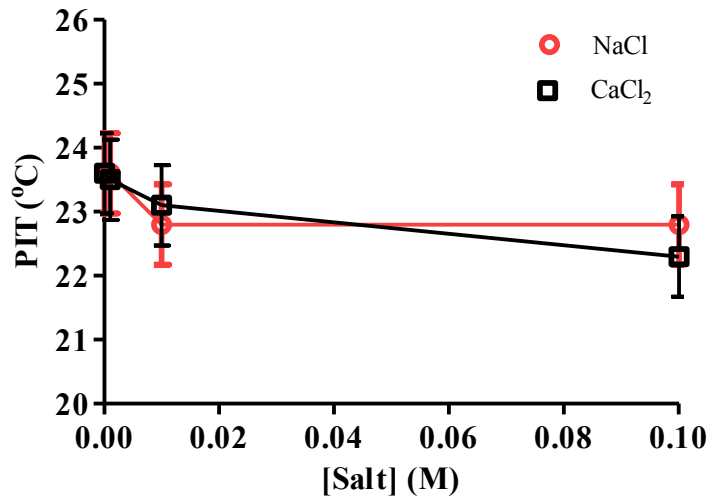


Figure 4.37: PIT temperature as a function of different electrolyte and electrolyte concentrations. Surfactant concentration is 6wt% of Brij30. Oil/aqueous=20/80 w/w.

The change of ΔT_{trans} as a function of NaCl and CaCl₂ concentration in emulsion systems containing 6wt% Brij30 is shown in Figure 4.38. There was a maximum ΔT_{trans} existed at the 0.01M CaCl₂ system, which was the same with the 0.01M NaCl system. However, the maximum ΔT_{trans} in calcium chloride system was not appeared as significant as it was in sodium chloride system. Between the pure milliQ water and 0.01M system, the ΔT_{trans} was increased 2.7°C in sodium chloride system while it was increased 1.1°C in calcium chloride system. The smaller difference in calcium chloride system was due to the larger cation (+2) in calcium chloride, and so shorter time was needed to experience the phase inversion process with the same electrolyte concentration. The system with maximum ΔT_{trans} was expected to have high stability due to the completeness solubilisation of n-dodecane during the phase inversion process, which has been shown in sodium chloride system in Section 4.6.

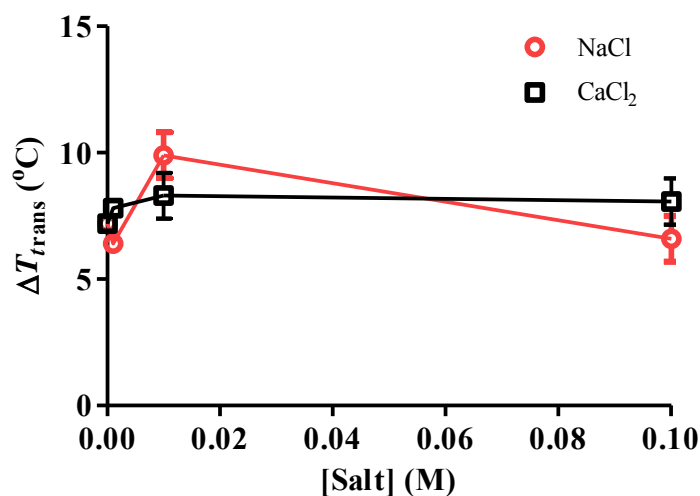


Figure 4.38: Width of transitional zone as a function of electrolyte concentration for systems containing different electrolyte. Surfactant concentration is 6wt% of Brij30. Oil/aqueous=20/80 w/w.

4.9.2 Formation and Stability of Nanoemulsions

According to Figure 4.39, which shows the initial droplet size distribution of n-dodecane/CaCl₂ nanoemulsions, there were monodispersed nanoemulsions in 0.001M-0.1M calcium chloride system. Furthermore, from the figure, the 0.1M CaCl₂ system consisted of the widest but single-peak PSD curve. These results were found as the same with sodium chloride system, therefore, monodispersed n-dodecane nanoemulsions with R=0.2 and 6wt% Brij30 could be produced by either adding sodium chloride or calcium chloride. Figure 4.40 which represents the initial droplet size and PDI as a function of NaCl and CaCl₂ concentration, showing that nanoemulsions with larger droplet sizes were produced in calcium chloride system comparing to sodium chloride system. From 0.001M to 0.1M, the droplet size was 65-72nm in sodium chloride system but it was 69-77nm in calcium chloride system, whereby the PDI was almost the same in both systems, which was 0.04-0.09. The increase of the droplet size in calcium chloride system was considered significant by a standard deviation being 0.73nm. The larger droplet size might due to the higher cation charge (+2) in calcium chloride system, to cause more free ion existed in the bulk solution and promote the diffusion of n-dodecane between droplets; and to create a non-ionic surfactant with higher degree of dehydration. In addition, the initial droplet size and PDI increased with electrolyte concentration in both electrolyte systems due to the increase of the dehydrating/salting-out effect on the non-ionic surfactant.

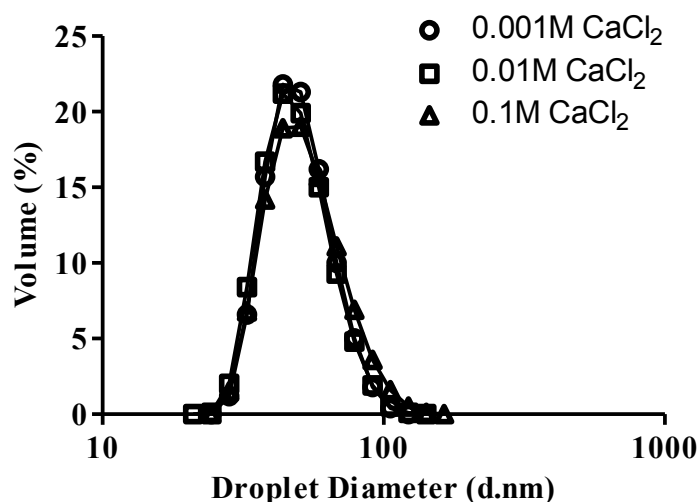


Figure 4.39: Initial size distribution of n-dodecane nanoemulsions (R=0.2, 6wt% [Brij30]) at 20°C with different calcium chloride concentrations. Oil/aqueous=20/80 w/w.

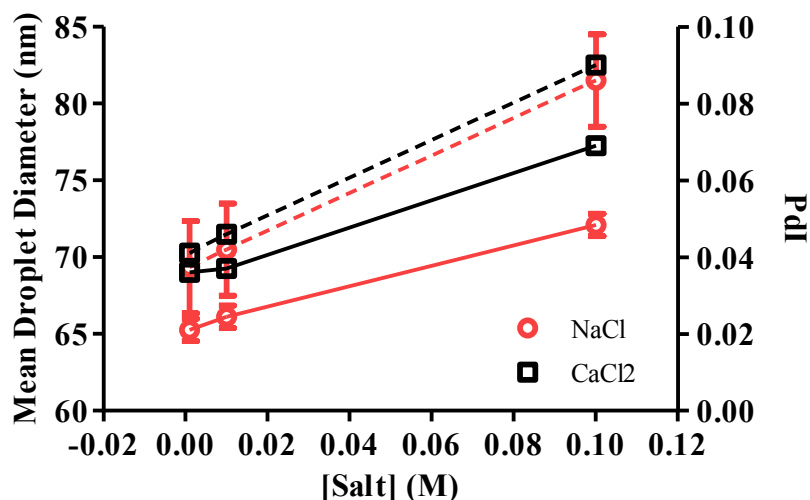


Figure 4.40: Initial droplet size and polydispersity index (PdI) as a function of electrolyte concentrations at 20°C for n-dodecane nanoemulsions with 6wt% of Brij30. Oil/aqueous=20/80 w/w. (Solid line: droplet size; dashed line: PdI)

At 20°C, n-dodecane nanoemulsions with R=0.2 and 6wt% Brij30 could be stored more than 30 days when NaCl concentration was 0.001M, 0.01M and 0.1M (Table 4.7), however, only 0.001M and 0.01M CaCl₂ systems could be stored more than 30 days without phase separation. The 0.1M CaCl₂ system was phase separated after it has been prepared for three days. The growth of the droplet size and PdI at 20°C in calcium chloride system is presented in Figure 4.41. The droplet size and PdI remained unchanged during the studied period when the CaCl₂ concentration was 0.001M or 0.01M. Among the calcium chloride systems, 0.1M CaCl₂ system contained the largest droplet with highest PdI when it was freshly prepared (Figure 4.40) and when the system was aged for three days (Figure 4.41). The phenomenon in droplet size and PdI in 0.1M CaCl₂ system was same with the 0.1M NaCl system. The growth of 0.1M CaCl₂ has shown a very interesting result in Figure 4.41, where the droplet size and PdI were the smallest at the day before the system was phase separated showing that the instability of 0.1M NaCl system was spontaneous. It may be no nanoemulsions but bicontinuous phase or multiphase in 0.1M CaCl₂ system as the storage temperature (20°C) was located in the zone of 1st falling in the conductivity curve (Figure 4.36). The result in droplet size and PdI obtained from DLS may be due to the dilution of bicontinuous phase by the continuous medium (Taylor and Ottewill, 1994; Forgiarini *et al.*, 2001; Morales *et al.*, 2003; Tadros *et al.*, 2004).

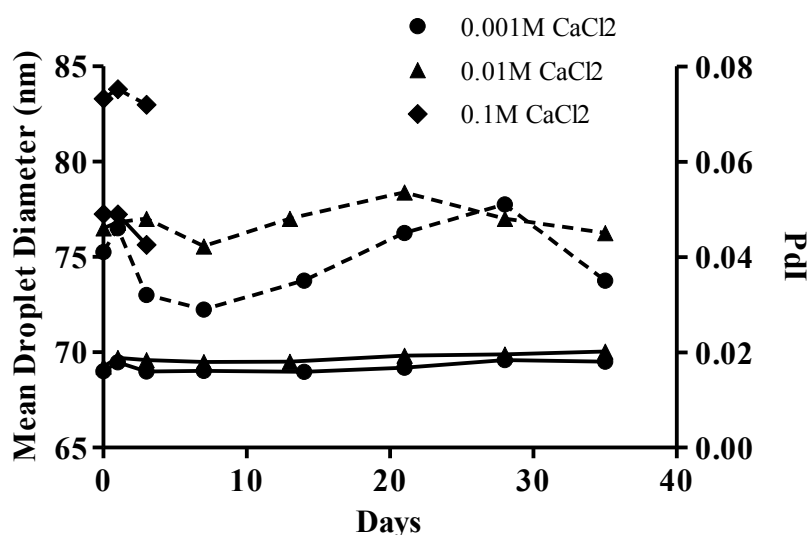


Figure 4.41: The stability of n-dodecane nanoemulsions containing 6wt% Brij30 and different calcium chloride concentrations. Oil/aqueous=20/80 w/w. All measurement was done at 20°C. (Solid line: droplet size; dashed line: PDI)

There may be an optimum electrolyte concentration existed to produce the most stable n-dodecane nanoemulsions with $R=0.2$ and 6wt% Brij30. In sodium chloride system, the most stable nanoemulsions have been shown to be produced in 0.001M, 0.01M and 0.1M (Table 4.7 and Figure 4.22(b)). From the results obtained in present section, the optimum CaCl_2 concentrations to produce the n-dodecane nanoemulsions with same stability were 0.001M and 0.01M. Therefore, as a preliminary conclusion, the optimum electrolyte concentration/concentrations was/were different in different electrolyte system, although the surfactant and oil concentrations were the same.

As shown in Table 4.14, the zeta potential was decreased (in absolute value) when the electrolyte concentration was increased. Therefore, the tendency of flocculation was increased when more electrolytes were added and so nanoemulsions with larger droplet size, higher PDI (in either freshly prepared or ageing state) and lower stability were produced. The potential was higher (in absolute value) in calcium chloride system compared to the sodium chloride system. As the cation charge of calcium chloride was higher than sodium chloride, the free ion in the bulk solution was stronger. Thus, the repulsive force was stronger in calcium chloride system. Since the repulsive force in calcium chloride was stronger, the instability in 0.1M CaCl_2 may due to the higher dehydrating effect or the formation of WIII region (bicontinuous phase or multiphase).

The effect of different electrolyte in the formation and stability of nanoemulsions was more significant than in the phase inversion process (PIT temperature). From present study, it has shown that nanoemulsions with better quality (size and PDI) and higher stability could be produced in a sodium chloride system rather than in a calcium chloride system. This has suggested that the higher cation charge in calcium ion has bigger impact on the surface on the nanoemulsions, and to cause the instability.

Table 4.14: Zeta potential for some selected nanoemulsions with sodium chloride and calcium chloride and with different electrolyte concentrations. Oil/aqueous=20/80 w/w and surfactant concentration is 6wt% Brij30. Storage temperature=20°C.

Salt	[Salt] (M)	Zeta Potential (mV)
NaCl	0.01	-0.950
NaCl	0.1	-0.2336
CaCl ₂	0.01	-1.544
CaCl ₂	0.1	-0.527

4.9.3 Reversibility of Nanoemulsions

Table 4.15 shows the reversibility of n-dodecane/CaCl₂ nanoemulsions, after they were aged for three days. The figure indicates the nanoemulsions (R=0.2, 6wt% Brij30) with calcium chloride were irreversible. From 0.001M to 0.1M CaCl₂ system, the droplet size (PDI) at 20°C was less than 80nm (less than 0.1) in a freshly prepared sample but the size larger than 80nm (larger than 0.1) in a 3-days ageing nanoemulsions and after the heating process. Unlike sodium chloride system, there were partially reversible system in 0.001M and 0.01M NaCl but irreversible system in 0.1M NaCl, after a 5-minute thermal equilibrium process (Table 4.9). The higher degree of the dehydration effect (by Ca⁺²) on the hydrophilic tail in a non-ionic surfactant was possible to cause a permanent damage (dehydrating) to the non-ionic surfactant. Therefore, the properties of the non-ionic surfactant were unable to be reversed after the phase inversion process, which was done by the heating process.

Table 4.15: Reversibility testing for the nanoemulsions with oil/aqueous=20/80 w/w and 6wt% Brij30 after three days storage time. The test was done in a temperature sequence: 20°C→10°C→20°C→30°C→20°C. Storage temperature = 20°C.

	0.001M CaCl ₂		0.1M CaCl ₂	
	Mean Diameter (nm)	PdI	Mean Diameter (nm)	PdI
Freshly-prepared (20°C)	69.0	0.041	77.3	0.090
Ageing (3 days)				
20°C	69.0	0.032	75.6	0.050
10°C	64.3	0.036	67.2	0.043
20°C	70.3	0.034	77.5	0.073
30°C	2696.5	1.000	1855.0	0.995
20°C	85.7	0.102	107.7	0.181

4.10 Different dilution agent (sequence of the addition of electrolyte)

In this section, the effect of the dilution agent was investigated. The nanoemulsions were diluted by their parent continuous phase when they were undergoing the measurement of particle size and PdI (Section 4.5 - 4.9). From this study, the effect of the sequence of the addition of electrolyte, before or after the emulsification process, was also investigated.

The droplet size and PdI as a function of dilution agent (different CaCl₂ concentrations) in 0.001M, 0.01M and 0.1M CaCl₂ systems are shown in Figure 4.42. The droplet sizes and PdI were smaller when calcium chloride-continuous nanoemulsions were diluted in a continuous phase with a lower CaCl₂ electrolyte concentration or in a pure milliQ water, but larger droplets with higher PdI were obtained when they were diluted with a continuous phase with higher CaCl₂ concentration (Figure 4.42). For example, in 0.01M CaCl₂ system, the droplet size and PdI of n-dodecane nanoemulsions at 20°C were 69.7nm and 0.048 when they were diluted with the parent continuous phase, while the droplet size and PdI were decreased to 64nm and 0.032 when they were diluted in pure milliQ water, however, the droplet size and PdI were increased to 77.1 and 0.072 when they were diluted in 0.1M CaCl₂. The hydration and dehydration effect by electrolyte are reversible

(Dukhin and Sjoblom, 1996), and the results obtained from present study have shown the same behaviour. When the electrolyte concentration of the continuous phase decreases, the dehydrated non-ionic surfactant would be hydrated again (or the degree of dehydration was decreased) and promoted the formation of smaller and finer nanoemulsions. This phenomenon was more clearly shown in 0.1M CaCl_2 system, as the droplet size and Pdl were smaller in all dilution agents than the one which was diluted in the parent continuous phase (Figure 4.42).

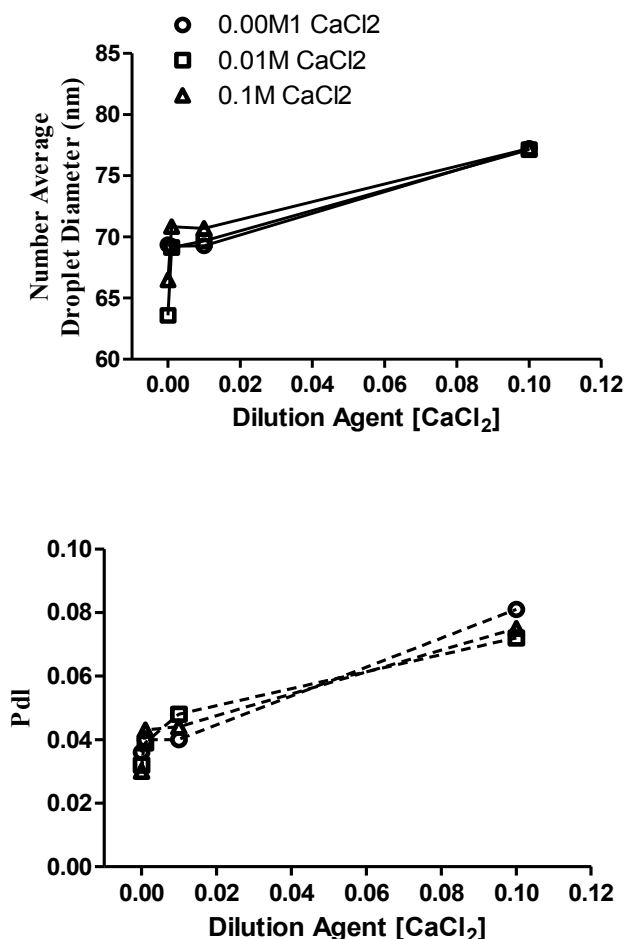


Figure 4.42: Droplet size and Pdl for calcium chloride-continuous nanoemulsions with different dilution agents. Oil/aqueous=20/80 w/w and 6wt% Brij30.

It has been known that nanoemulsions with continuous phase with different electrolyte concentration have different droplet sizes and Pdl due to the different interfacial tension and dehydrating effect. However, in present study, with same dilution agent but different nanoemulsions, the droplet size and Pdl were similar in dilution agent with low CaCl_2 concentration; while the size and Pdl became the same in the dilution agent with high concentration (0.1M CaCl_2), as shown in Figure 4.42. For example, when 0.001M and 0.01M CaCl_2 systems were diluted in 0.1M CaCl_2 solution, their droplet sizes and Pdl were 77nm and 0.08, which were the same with the 0.1M CaCl_2 nanoemulsions. Therefore, it has shown that the same quality of calcium chloride-continuous nanoemulsions could be produced by adding the calcium

chloride either before or after the emulsification process, which also has been showed by previous researchers in aqueous NaCl-heptane system (Binks *et al.*, 2000).

As in the production of nanoemulsions, the hydrophilic tail is exposed to the aqueous phase and so the degree of dehydration (or hydration) can be tailored by changing the electrolyte concentration of the continuous phase. Since the dehydrating effect by the dilution agent was not significant in low and intermediate electrolyte concentration, the droplet size and PDI were the same only at high CaCl₂ concentration. From the results above, it is possible to initially produce pure milliQ water nanoemulsions, then to produce the desired nanoemulsions (with particular droplet size and PDI) by adding different dilution agent (different electrolyte concentration), which is a good practice in nanoemulsion/emulsion industries.

4.11 Effect of storage temperature

Following the previous study about the investigation in optimum storage temperature (Ee *et al.*, 2007), some nanoemulsion system were selected to store at 10°C in this section to investigate the effect of different storage temperature on the stability of n-dodecane nanoemulsions. 0.001M-0.1M CaCl₂ and 0.01M NaCl nanoemulsions with 6wt% Brij30 and R=0.2 were chosen. The nanoemulsions were prepared by the same method which was used in previous sections but stored at 10°C instead of 20°C. The droplet sizes and PDI were measured at 10°C as a function of time to determine the stability.

From previous sections, it has been found that the freshly-prepared samples for all chosen systems were transparent and the nanoemulsions remained one-phase transparent for more than 30 days at 20°C (except 0.1M CaCl₂). When those systems were stored at 10°C, their appearance changed from transparent to translucent, followed by milky; while for 0.1M CaCl₂ system, it changed to milky from translucent. Although their appearances were changed, they remained in one-phase more than 30 days.

The droplet sizes of the selected systems as a function of storage time is shown in Figure 4.43(a). From the figure, the droplet size was increased with time in system containing 0.001M CaCl₂, 0.01M CaCl₂ and 0.01M NaCl, therefore the change in the appearance of the sample (from transparent to milky) may be due to the larger droplets. The PDI as a function of storage time, which is shown in Figure 4.43(b), shows the PDI remained constant at short storage period (20 days in 0.01M NaCl or CaCl₂ and 30 days in 0.001M CaCl₂); but it increased sharply at long storage period (eg. from 0.091 to 0.193 in 0.01M CaCl₂ system). Furthermore, as shown in Figure 4.44, where the size distribution as a function of storage time of 0.01M CaCl₂ system is shown, the PSD curve was wider and with lower volume percentage when the sample was stored for 10 days. Then, the curve became two peaks from one peak after the system was stored more than 20 days at 10°C. According to previous sections (Chapter 4.6 and 4.9), during a storage period of 30 days, there were no droplet size and PDI changes in system with 0.01M NaCl or CaCl₂ and 0.001M CaCl₂. Thus the systems were less stable when the storage temperature was decreased to 10°C. Shinoda and Saito (1968) have shown that a cyclohexane emulsion with higher stability was obtained if the storage temperature was much lower than the PIT

temperature (the lower the storage temperature, the more stable the system is). However, in present study, the result agreed more with the decane emulsion system (Ee *et al.*, 2007), where there was an optimum storage temperature for emulsions and emulsions were unstable if the storage temperature was lower than the optimum one.

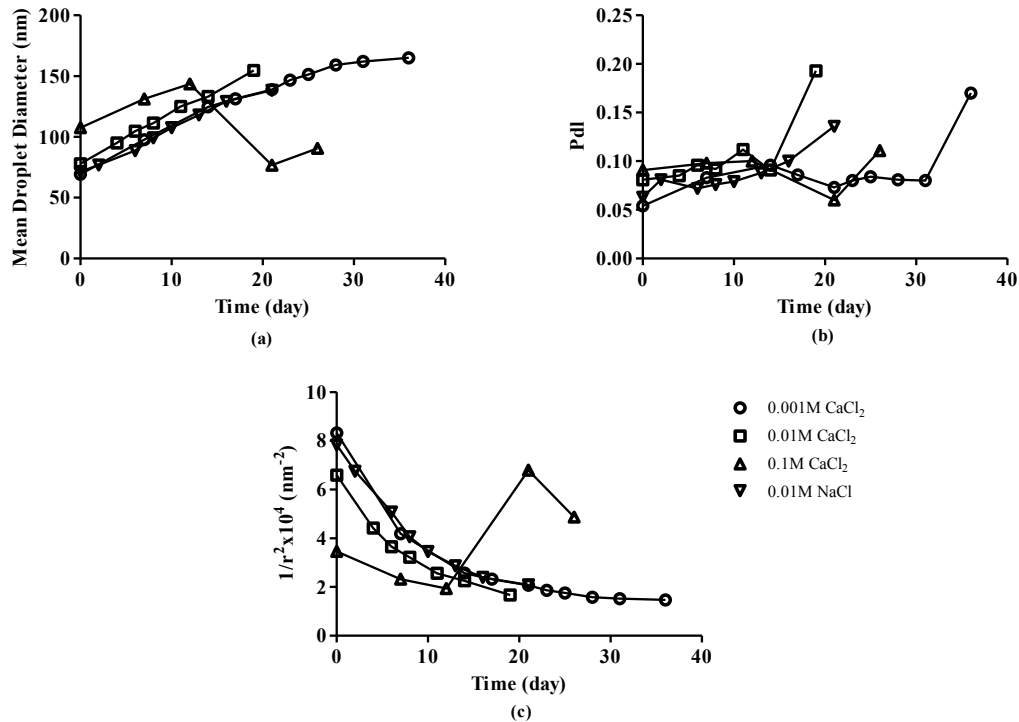


Figure 4.43: The growth of (a) mean droplet diameter; (b) PDI; and (c) $1/r^2$ as a function of time for nanoemulsions (6wt% Brij30 and $R=0.2$) with different electrolyte and electrolyte concentrations. Storage and measuring temperature = 10°C .

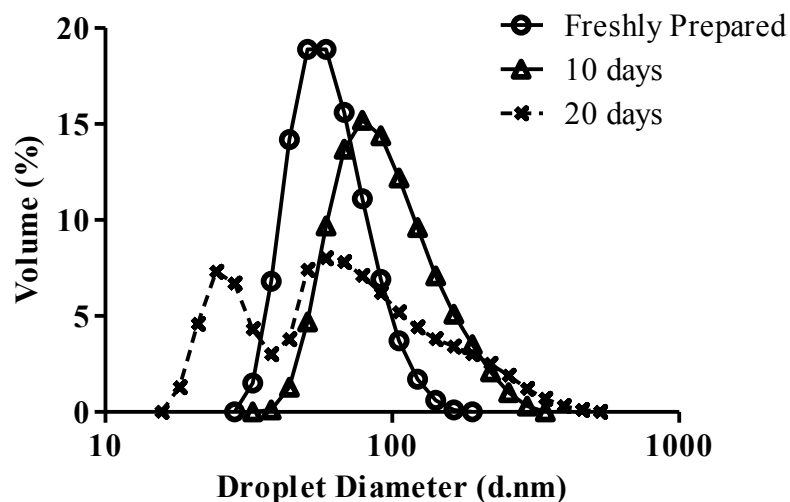


Figure 4.44: Size distribution as a function of storage period for n-dodecane nanoemulsions ($R=0.2$, 6wt% Brij30) at 10°C with aqueous 0.01 CaCl₂ as continuous phase. Storage and measuring temperature = 10°C .

The plot of $1/r^2$ -t (Figure 4.43(c)) and r^3 -t (Figure 4.45) were used to determine the destabilising factor for the chosen systems. The change of $1/r^2$ as a function of time was same in systems containing 0.001M and 0.01M CaCl_2 as well as the system with 0.01M NaCl. In Figure 4.45, only data from 0.1M CaCl_2 was not able to be correlated into a straight line, where the regression values of the other systems are shown in Table 4.17. Therefore, within the chosen systems (except 0.1M CaCl_2), they were destabilised by a same mechanism as the trend of the changes in Figure 4.43(c) and Figure 4.45 were the same. According to the graphs, the destabilising factor in present systems was Ostwald ripening, as a straight line was able to be fitted into the data with high regression (up to 98%) in an r^3 -t plot (Table 4.17). Furthermore, it has been suggested that the size distribution of a nanoemulsion system is time independent if Ostwald ripening is the only destabilising factor (Taylor and Ottewill, 1994; Taisne and Cabane, 1998; Porras *et al.*, 2004; Uson *et al.*, 2004), which the result from Figure 4.43(b) shows the independency of PDI with time during the short storage period. In addition, as shown in Table 4.16, the zeta potential for 0.01M NaCl or CaCl_2 systems at 10°C were negative (repulsive force was larger than attractive force) after they were aged for five days, showing that coalescence was unlikely to cause the instability during the short storage period.

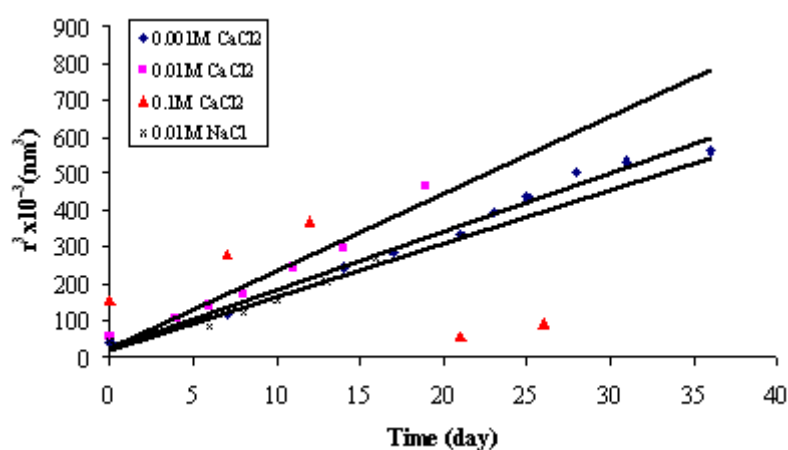


Figure 4.45: The change of r^3 as a function of time at 10°C for nanoemulsions with 6wt% Brij30, $R=0.2$ and different electrolyte and electrolyte concentrations.

Table 4.16: Zeta potential at 10°C for nanoemulsions (5-day ageing) with sodium chloride and calcium chloride while the electrolyte concentration was 0.01M. Oil/aqueous=20/80 w/w and with 6wt% Brij30.

Salt	[Salt] (M)	Zeta Potential (mV)
NaCl	0.01	-1.187
CaCl_2	0.01	-1.826

When the storage period was longer than 20 days in 0.01M NaCl or CaCl_2 systems and 30 days in 0.001M CaCl_2 system, the PDI was no longer independent on the storage period (Figure 4.43(c)). Therefore, Ostwald ripening was no longer dominant in destabilising the nanoemulsions. During the longer storage period, coalescence may

be the main destabilising factor as the droplet size had been increased (distance between droplets was decreased) from Ostwald ripening during the short storage period, and so the possibility of droplets to have collision was increased. Since the measurement in droplet size was stop after the sample had become polydispersity (two peak in PSD curve), the main mechanism to cause the instability during the long storage period was yet unclear.

Figure 4.46 shows the structures of the nanoemulsions with 0.01M CaCl₂ (6wt% Brij30) and with pure milliQ water (7wt% Brij30), after they had been aged for five days. From the figure, it shows that Ostwald ripening destabilised the n-dodecane/0.01M CaCl₂ nanoemulsions, where the droplet size was monodispersed with a perfect spherical shape in the system. Figure 4.46(a) also clarified the results obtained from Figure 4.43(b) and Figure 4.44, where nanoemulsions with low PDI were produced. Additionally, the system without calcium chloride and with 7wt% Brij30 was observed to have no perfect spherical structures but only “star-fish” liked structures (Figure 4.46(b)). The width of the “tentacle” of each “star-fish” liked structure was almost in the same size with the individual droplet size (in freshly-prepared sample). From the result above, after a 5-day storage period, the main destabilising factor in calcium chloride system (R=0.2, 6wt% Brij30) may be Ostwald ripening while it was flocculation in a pure milliQ water system (R=0.2, 7wt% Brij30) when the nanoemulsions were stored at 10°C.

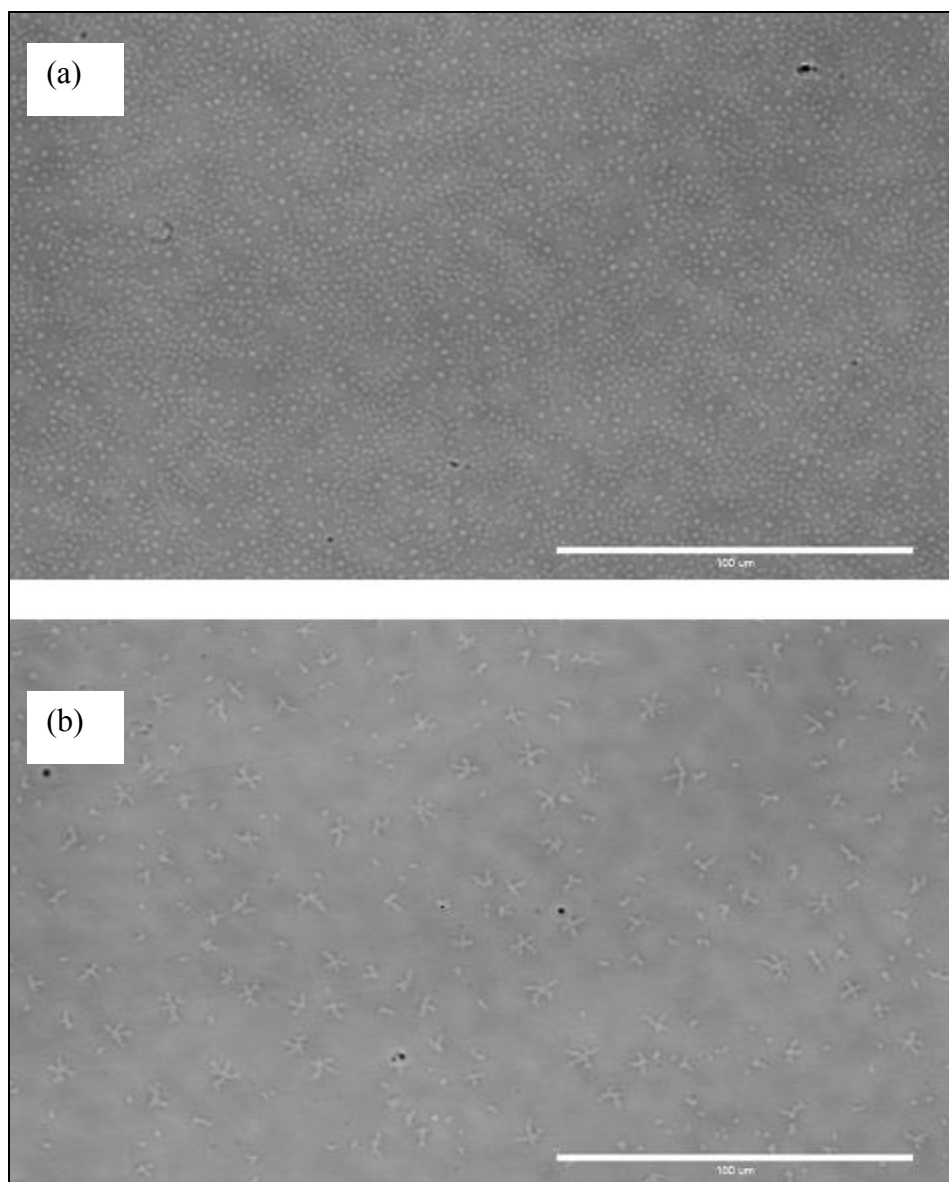


Figure 4.46: Microscope image for ageing nanoemulsions at 10°C with (a) 0.01M CaCl₂ and 6wt% Brij30 ; and with (b) pure milliQ water and 7wt% Brij30. Oil/aqueous=20/80 w/w. Storage period = 5 days.

Ostwald ripening rate (ω) can be determined by the slope of the straight line which is fitted into the data in the plot of r^3-t (Equation 1), and the rates in chosen systems are shown in Table 4.17. The Ostwald ripening rate increased from $5.69 \times 10^{-20} \text{ m}^3/\text{s}$ to $7.56 \times 10^{-20} \text{ m}^3/\text{s}$ when the calcium chloride concentration was increased from 0.001M to 0.01M. Additionally, the rate increased from $5.19 \times 10^{-20} \text{ m}^3/\text{s}$ to $7.56 \times 10^{-20} \text{ m}^3/\text{s}$ from NaCl to CaCl₂ system (Table 4.17). The increasing rate with increasing CaCl₂ concentration was because the higher diffusion rate with the larger amount of free radical cation presented in the continuous phase. The lower rate in NaCl was because of the lower interfacial tension (Equation 1). Furthermore, the larger dehydrating effect from Ca²⁺ has caused more instability in CaCl₂ than in NaCl system, while both of the electrolytes have the same concentration at 0.01M. At storage temperature was 10°C, 0.01M NaCl nanoemulsions were the most stable system among the chosen systems, with the lowest Ostwald ripening rate and the longest period taken from turning one-phase transparent to one-phase milky (Table 4.17).

Table 4.17: Time to turn milky, regression and Ostwald ripening rate (ω), of nanoemulsions with 6wt% Brij30 (R=0.2) and with different electrolyte and electrolyte concentrations. Storage and measurement temperature = 10°C.

System	Time to turn milky (day)	Regression	ω ($\times 10^{20}$ m ³ /s)
0.001M CaCl ₂	9	0.9847	5.69
0.01M CaCl ₂	6	0.9651	7.56
0.1M CaCl ₂	7	-	-
0.01M NaCl	10	0.9766	5.19

The changes of droplet size and PDI were inconsistent in 0.1M CaCl₂ system (Figure 4.43(a) and (b)) indicating the system was unstable at 10°C. Additionally, 0.1M CaCl₂ system consisted of the largest initial droplet size and PDI among the chosen systems at 10°C. From Figure 4.43(c) and Figure 4.45, the instability factor in 0.1M CaCl₂ system was neither coalescence nor Ostwald ripening. The instability of the system may be because of the highly dehydrated non-ionic surfactant causing the break down of the aqueous continuous-nanoemulsions or because of the system tended to produce oil-continuous emulsions. However, it is interesting to note that 10°C was a better storage temperature than 20°C for 0.1M CaCl₂ system, as the days taken for phase separation was more than 30 days which was longer than the one stored in 20°C.

In present study, a phase separated 0.1M CaCl₂ system was moved from 20°C to 10°C. The sample was changed to one-phase translucent from 2-phase after it had been stored for seven days in 10°C. Then, it changed from one-phase translucent to one-phase milky when it was stored for another seven days, which was the same period for the system stored at 10°C at the beginning (Table 4.17). Therefore, 0.1M CaCl₂ nanoemulsions at 10°C were able to revert to their freshly-prepared state by a heating process.

5.0 Conclusion

This research provided a preliminary study on the effect of electrolyte in n-dodecane nanoemulsions. This study was carried out with some limited conditions such as only two surfactants (Brij30 and S80), two electrolytes (NaCl and CaCl₂), two storage temperatures (10°C and 20°C) and narrow oil concentration (R=0.2-0.4), surfactant concentration (4-8wt% Brij30), as well as electrolyte concentration (0.001, 0.01 and 0.1M). Although the results obtained from this study were limited to some specified conditions, there were some interesting results which would form a basis for more detailed study. An overview of the results is outlined below:

1. In an aqueous-0.01M NaCl/6wt% Brij30/n-dodecane emulsion system, there were O/W emulsions produced at low temperature but W/O emulsions at high temperature. From the O/W to W/O emulsions, there were microemulsions, lamellar liquid crystal (LLC), and bicontinuous phase (L₃) formed during the phase inversion process.
2. In an O/W emulsion system, PIT temperature decreased when surfactant or oil concentration was increased but when the HLB number of a surfactant system was decreased. In a mixed surfactant system (Brij30/S80) containing 10wt% S80 and R=0.2, the PIT temperature, ΔT_{trans} , and stability changed dramatically in the system with 6wt% surfactant, due to the highly hydrophobic property of S80. Furthermore, in O/W nanoemulsions with R=0.2, flocculation may be the destabilised factor in 10°C with 7wt% Brij30 but it was destabilised by Ostwald ripening in 20°C with 4wt% Brij30.
3. Among the systems with R=0.2 and 4wt% Brij30, 0.03M NaCl produced the most stable nanoemulsions with smallest droplets and lowest PdI, when the NaCl concentration was changed from zero to 0.1M. With the addition of sodium chloride, the surfactant concentration needed to produce the most stable n-dodecane nanoemulsions (R=0.2) was reduced, from 7wt% to 6wt% Brij30. The most stable nanoemulsions were found to be linked with the maximum ΔT_{trans} , where the complete solubilisation of n-dodecane occurred during the phase inversion process. Furthermore, nanoemulsions with PdI smaller than 0.1 were produced in 4-6wt% Brij30 in sodium chloride system but in 4-7wt% Brij30 in pure milliQ water system. The smaller surfactant range may be because of the compression effect from sodium chloride to cause a higher concentration of Brij30 on the interface. At higher oil concentration, more sodium chloride was needed to stabilise the system. However, in the mixed surfactant system, the addition of sodium chloride did not affect the stability.
4. The effect of calcium chloride in nanoemulsions with R=0.2 and 6wt% Brij30 was more significant than sodium chloride in the present study, due to its larger cation effect (Ca⁺² compared to Na⁺¹) in every mole of electrolyte added. Although the PIT temperature and ΔT_{trans} was similar in both electrolyte systems, the droplet size was larger in calcium chloride system and only 0.001M and 0.01M CaCl₂ produced the most stable nanoemulsions; whilst the most stable nanoemulsions were produced in 0.001M, 0.01M and 0.1M NaCl with the same amount of surfactant.

5. At $R=0.2$, only nanoemulsions with sodium chloride had the ability to reverse the droplet size and PDI, from ageing to freshly prepared, by a heating process (where the system was experienced phase inversion process). However, nanoemulsions with 6wt% Brij30, only droplet size was reversible (partially reversible) by a 5-minute thermal equilibrium time, which may be insufficient to process the large ΔT_{trans} in the phase inversion process. When the oil concentration increased (at fixed R_{so}), there were partially reversible systems in low NaCl system for $R=0.3$ and 0.4 . However, there were irreversible systems in 0.1M NaCl system when R was larger than 0.2 . Nanoemulsions produced by mixed surfactant system were too unstable to undergo the reversibility testing.
6. When the n-dodecane nanoemulsions were with $R=0.2$ and 6wt% Brij30, the droplet size and PDI of 0.01M CaCl_2 system were smaller if it was diluted by pure milliQ water or 0.001M CaCl_2 but they were larger when the dilution agent was 0.1M CaCl_2 . Furthermore, the droplet size and PDI of 0.01M NaCl system with dilution agent was 0.1M NaCl were the same with the 0.1M NaCl system. Although the result only applied to calcium chloride system and in certain range of CaCl_2 concentration, the droplet size and PDI of nanoemulsions with calcium chloride were the same when the electrolyte was added either before or after the emulsification process.
7. 20°C appeared to be a better storage temperature than 10°C for 0.01M NaCl and 0.001M and 0.01M CaCl_2 systems. At 10°C , the droplet size and PDI were increased with time and the destabilising factor may be Ostwald ripening during the short storage period. However, 10°C may be a better storage temperature to store 0.1M CaCl_2 system as it remained one phase for longer period and appeared to have the reversibility ability at that temperature.

In conclusion, in this study, the addition of sodium chloride and calcium chloride does affect the formation and stability of aqueous/non-ionic surfactant/n-dodecane nanoemulsions produced by the PIT method. However, the advantages over the effects only appeared at certain electrolyte concentration (in NaCl or CaCl_2) and at certain surfactant concentration. The addition of electrolyte needed to be carried out carefully to prevent highly dehydrating the non-ionic surfactant. More works need to be done in order to fully understand the effects of electrolyte on the aqueous-continuous nanoemulsions.

6.0 Recommendations for Future Research

In this research, the effect of the electrolyte on the formation, stability and reversibility of nanoemulsions produced by the PIT method were only investigated by the addition of sodium chloride and calcium chloride. The dispersed phase was n-dodecane while the non-ionic surfactants used were Brij30 and Span80, and with narrow oil and surfactant concentrations. In order to fully develop the knowledge about the effect of the electrolyte on the production of aqueous-continuous nanoemulsions by the PIT method, several issues are raised out as follow:

- Electrolyte with the same (eg. K^+) or higher (eg. Al^{3+}) cation charge can be used to further investigate the effect of the strength of cation.
- Wider electrolyte concentration (up to 1M or 10M) and with smaller increments (gradual increase of 0.001M) are recommended to investigate the effect of electrolyte in more depth.
- Oil with different carbon numbers (eg. decane, kerosene, hexane) and with different structures (eg. hexane and isohexane) can be used to investigate the effect of oil as well as the effect of electrolyte.
- Investigation on different non-ionic surfactant (either hydrophilic or hydrophobic) with different concentration or different combination of surfactant (different HLB number) should be carried out.
- The ability to reverse the other physical properties (eg. viscosity) from ageing to freshly prepared O/W nanoemulsions need to studied, to fully understand the ability in nanoemulsions produced by the PIT method.
- An optimum storage temperature can be determined by conducting experiments with wider temperature range.
- Longer investigation period should be studied for the system with $CaCl_2$, $R=0.2$, 6wt% Brij30 and with storage temperature of $10^\circ C$, to understand the destabilising factor during the long storage period.
- The effect of a fourth component (co-surfactant such as alcohol or particles) can be studied along with the current systems.
- An optimum condition (with the combination of the optimum surfactant system (optimum HLB number), surfactant concentration, storage temperature, and electrolyte concentration) should be developed to produce stable nanoemulsions with certain oil and fixed oil concentration.
- A study of nanoemulsions with larger volume will provide a better understanding to emulsion industries.

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