Challenges Associated in Stability of Food Grade Nanoemulsions

P. Karthik[ab], P.N. Ezhilarasi[ab] & C. Anandharamakrishnan[ab]

[a] Department of Food Engineering, CSIR-Central Food Technological Research Institute (CFTRI)

[b] AcSIR- Academic of Scientific and Innovative Research, CSIR-CFTRI Campus, Mysore–570 020, India

Accepted author version posted online: 26 Jun 2015.

CrossMark

Click for updates

PLEASE SCROLL DOWN FOR ARTICLE

Challenges Associated in Stability of Food Grade Nanoemulsions

P. Karthik[1, 2], P.N. Ezhilarasi[1, 2] and C. Anandharamakrishnan[1, 2]*

[1]Department of Food Engineering,

CSIR-Central Food Technological Research Institute (CFTRI),

[2]AcSIR- Academic of Scientific and Innovative Research, CSIR-CFTRI Campus,

Mysore-570 020, India.

Correspondence: C. Anandharamakrishnan,

Ph: +91-821-2513910

Fax: +91-821-2517233

*E.Mail: anandhram@cftri.res.in,

c.anandharamakrishnan@gmail.com

Food grade nanoemulsions are being increasingly used in the food sector for their physico-chemical properties towards efficient encapsulation, entrapment of bioactive compounds, solubilization, targeted delivery and bioavailability. Nanoemulsions are considered as one of the important vehicles for the sustain release of food bioactive compounds due to their smaller size (nm), increased surface area and unique morphological characteristics. Nanoemulsification is an ideal technique for fabricating the bioactive compounds in a nano form. Formation and stabilization of nanoemulsion depends on the physicochemical characteristics of its constituents including oil phase, aqueous phase and emulsifiers. This review is mainly focused on the instability mechanisms of nanoemulsion such as, flocculation, Ostwald ripening, creaming, phase separation, coalescence, and sedimentation. Further, the major factors associated with

these instability mechanisms like ionic strength, temperature, solubilization, particle size distribution, particle charge, pH strength, acid stability and heat treatment are also discussed. Finally, safety issues of food grade nanoemulsions are highlighted.

Keywords Nanoemulsion, Stability, Instability mechanisms, Emulsification, Food bioactive components

INTRODUCTION

Nanotechnology holds potential to transfigure the scientific research in the world. The term 'nano' refers to the magnitude of 10^{-9} m (Quintanilla-Carvajal et al., 2010). Decreasing the particle size to the nanoscale range increases surface-to-volume ratio and results in increased reactivity with changes in mechanical, electrical, and optical properties (Neethirajan and Jayas, 2010). The changes in properties of materials from larger scale to nano scale broaden its applications by many folds. Extensive research has been carried out in nanoparticle generation processes and its application in the various fields. The current applications of nanotechnology in food systems include nanocomposites for food packaging, nano-biosensors for detection of contamination and quality deterioration, nanoencapsulation and nanoemulsion for controlled delivery of nutraceuticals (Chen, et al. 2006; Sanguansri and Augustin, 2006; Sozer and Kokini, 2009; Weiss et al., 2006).

Nanoemulsions are one of the most interesting fields of application, since they can act as carriers or delivery systems for bioactive compounds. Nanoemulsions are heterogeneous systems consisting of two immiscible liquids, with one phase being dispersed as nanometric droplets into another continuous liquid phase with the droplet sizes in the range of 50 to 1000 nm (Sanguansri and Augustin, 2006). A schematic representation of a nanoemulsion is shown in Fig. 1. The appearance of nanoemulsion can be transparent, translucent or milky white form and gives kinetic stability in storage conditions. Based on the relative spatial organization of the oil and aqueous phases, there are two basic types of nanoemulsions. The oil-in-water (O/W) nanoemulsion comprises of oil droplets dispersed in the water phase whereas water droplets

dispersed in an oil phase is referred as water-in-oil (W/O) nanoemulsion (McClements and Rao, 2011). Nanoemulsions, microemulsion and conventional emulsions are discriminated based on their size, composition and preparation techniques. Composition of nanoemulsions includes the major constituents such as emulsifiers, continuous (water or solvent) and dispersed (oil) phases of the system. The nanoemulsification techniques are based on the low energy and high energy approaches. Nanoemulsions can either be used directly in the liquid state or converted to powder form using drying techniques such as spray drying and freeze drying after emulsification (Ezhilarasi et al., 2013). Thus, nanoemulsion systems could be considered as an initial step for nanoparticle generation by crystallization, solvent evaporation and conventional drying methods (spray drying or freeze drying).

Generally, emulsion based delivery systems are applied to protect the bioactive compounds against adverse environmental conditions, increase the storage and product stability, and maintain their functional activity (Madene et al., 2006). The nanoencapsulation of bioactive compounds through nanoemulsions is an effective approach for improving the bioavailability through controlled delivery and maintaining the physio-chemical stability. Nanoemulsions have shown a greater ability to increase the bioavailability of lipophilic substances encapsulated within them such as DHA (Zimet et al., 2011), fish oil (Belhaj et al., 2010), beta-carotene (Yuan et al., 2008), curcumin (Wang et al., 2008), etc., Controlled delivery function can in turn be attributed to small particle size and high surface-to-volume ratio of the nanoemulsions (Acosta, 2009). Moreover, nanoemulsion also has the ability to deliver the bioactive compounds to the intradermal layers through diffusion. Nanoemulsion can also be used as an antimicrobial agent due to their improved bioactivity and shown wide application in antibiotics, disinfectants and

antiseptics (Lawrence and Rees, 2012; Teixeira et al., 2007). The property of anti-adherence and anti-biofilm behavior shows that the nanoemulsion could be helpful for the development of anti-carcinogenic agents as well (Ramalingam et al., 2012). These recent advances in the field of nanoemulsion are opening up new applications in many areas such as pharmaceuticals, cosmetics, food and agriculture.

Despite the many advantages of nanoemulsion, the main limitation that reduces its wide application is stability (Gutierrez et al., 2008). Nanoemulsions are thermodynamically unfavorable systems due to the positive free energy associated with creating the oil-water interface, and have a tendency to break down over time, due to gravitational separation, flocculation, coalescence, and/or Ostwald ripening. The rates at which these processes occur are often considerably different in nanoemulsions than in conventional emulsions because of particle size and curvature effects (McClements, 2005). Among these destabilization mechanism, Ostwald ripening is one of the major ageing processes involved in nanoemulsion (Taylor, 1998; Katsumoto et al., 2000). Irreversible increase in droplet size may occur due to Ostwald ripening and coalescence (Abismail et al., 1999; Delmas et al., 2011). These destabilization mechanism of emulsions are related to the different forces in the system such as gravitational forces, inter-particle repulsive, attractive forces, flow forces, and molecular forces (Chiralt, 2009). Furthermore, major factors associated with these destabilization mechanism are ionic strength, temperature, solubilization, particle size distribution, pH strength and heat treatment, etc. The kinetics of destabilization of nanoemulsions is a slow process than the microemulsion due to their tiny droplet size. Maintaining the stability of nanoemulsion is crucial, as it can determine

the bioactivity and bioavailability of the encapsulated compound and other applications of nanoemulsion (Anandharamakrishnan, 2013).

Despite the existence of many fundamental studies related to the stability of nanoemulsion, the extreme variability and complexity of the system necessitates the exploration of technologically applicable solution for these destabilization mechanism. Improving the stability of nanoemulsion to achieve technological success for large scale production of nanoemulsion and wide application remains to be a significant challenge. Many reviews and research papers have been published on the food grade nanoemulsion (Sanguansri and Augustin, 2006; McClements and Rao, 2011; Silva et al., 2012; Ezhilarasi et al., 2013). However, better understanding of stabilization and destabilization mechanism of nanoemulsion and its associated factors are more essential to optimize the preparation techniques and storage conditions of nanoemulsion. Therefore, the main aim of this review is to focus on the various instability mechanisms of the nanoemulsion, its associated factors, nanoemulsification techniques and safety issues.

NANOEMULSIONS

Nanoemulsion being non-equilibrium systems cannot be formed spontaneously, and it requires energy input (either external or internal source) (Walstra, 1996). The techniques for nanoemulsion production are broadly categorized as either high energy or low energy approaches depending on the underlying principle (Fig. 2, step: 1). These techniques used in the preparation of nanoemulsion greatly influence the droplet size, and other instability mechanisms through

operating conditions and composition of the emulsion system. In general, nanoemulsions require reasonably lower surfactant concentrations (5-10%) for preparation than microemulsion (20% and higher) (Tadros et al., 2004).

High Energy Approaches (HEA)

High energy approaches utilize mechanical devices capable of generating intense disruptive forces that mix and disrupt oil and water phases leading to the formation of the tiny oil droplets e.g., high pressure valve homogenizers, microfluidizers and sonication methods (Wooster et al., 2008; Leong et al., 2009). Intense energies are required to generate disruptive forces higher than the restoring forces to hold the droplets in spherical shapes (Schubert and Engel, 2004). In high energy methods, the nanoemulsion droplet size depends on the intensity and duration of energy input, type and concentration of emulsifiers, interfacial tension, and relative viscosities of disperse and continuous phases (McClements, 2005). Smaller droplets are usually obtained by increasing the intensity or duration of homogenization, by increasing the concentration of emulsifier used, or by controlling the viscosity ratio (Schubert and Engel, 2004; Wooster et al., 2008; Mohd-Setapar et al. 2013).

High pressure valve homogenizers require extremely high pressure and multiple passes to produce nanoemulsion with required droplet size (McClements and Rao, 2011). It breaks the large droplets into smaller ones by a combination of intensive disruptive forces such as shear stress, cavitation and turbulent flow conditions acting on them (Stang et al., 2001). Using high pressure homogenization technique, β-carotene, thyme oil and curcumin nanoemulsions were

produced at various operating conditions (Yuan et al., 2008; Wang et al., 2008; Ziani et al., 2011). A microfluidizer is almost similar in design to high pressure homogenizer with a slight difference is in the flow of emulsion channels. Using microfluidization technique, nanoemulsion of different bioactive compounds were produced (Buranasuksombat et al., 2011; Salvia-Trujillo et al., 2013; Qian et al., 2012). Similarly, in the sonication methods, high intensity ultrasonic waves (frequency > 20 kHz) form emulsions of nano droplets size (70 nm). The droplet size decreased with increasing sonication time, power level, and emulsifier concentration (Kentish et al. 2008). High energy approaches are mostly used to prepare nanoemulsion due to its capability of large scale production.

Low Energy Approaches (LEA)

The low energy methods rely on the internal chemical energy of the system for emulsion production (Tadros et al., 2004; Sole et al., 2006). In low energy approaches, nanoemulsions are formed as a result of phase transitions that occur during the emulsification process when the environmental conditions are altered (either temperature or composition) (Yin et al., 2009). The low energy approaches include spontaneous emulsification, phase inversion temperature (PIT), phase inversion composition (PIC), and emulsion inversion point (EIP) techniques. In low energy methods, the emulsion droplet size depends on system composition (surfactant–oil–water ratio, surfactant type, ionic strength) and environmental conditions (temperature, time history, stirring speeds) (Anton et al., 2008; Anton and Vandamme, 2009).

The spontaneous emulsification methods involve spontaneous formation of nanoemulsion on the mixture of two liquids (organic phase and an aqueous phase) at a particular temperature due to the movement of a water miscible component from the organic phase into the aqueous phase (Anton et al., 2008). Phase inversion methods utilize the chemical energy released as a result of phase transitions taking place during an emulsification process. Nanoemulsion have been formed by inducing phase inversion in emulsion from a W/O to O/W form or *vice versa* by either changing the temperature (PIT method) or the composition (PIC method) (Solans and Sole, 2012). In EIP method, catastrophic-phase inversion is induced by altering the ratio of the oil-to-water phases (Fernandez et al., 2004; Thakur et al., 2008). Using low energy emulsification process, Anton et al., (2008); Anton and Vandamme, (2009); Porras et al., (2008) reported nanoemulsion of lower droplet size. However, in each nanoemulsion techniques, different strategies are need to be optimized to achieve the desirable droplet size. Low energy approaches are more effective in a production of smaller droplet sizes, more cost effective than high energy methods, but they are limited in the types of oils and emulsifiers that can be used.

STABILITY OF NANOEMULSION AND ITS ASSOCIATED FACTORS

The instability of nanoemulsion is undesirable and creates various problems on storage for a longer period (Sharma et al., 2010) and during application. The major instability mechanisms of nanoemulsion are flocculation, creaming, sedimentation, coalescence, Ostwald ripening, and phase separation (Fig. 2, step: 2). All major instability mechanism of nanoemulsion studies has been described in Table.1.

Ostwald Ripening

Ostwald ripening is a process where the larger droplets are formed in the emulsion at the expense of tiny droplets due to molecular diffusion of oil between droplets through the continuous phase. This was due to the Kelvin effect, where small emulsion droplets have property of increasing oil solubility than the larger droplets due to the difference in Laplace pressures. The Ostwald ripening is mainly influenced by solubility of oil in the continuous phase which can be depicted by LSW kinetics theory (Lifshitz-Slyozov-Wagner) (Lifshitz and Slyozov, 1961; Wagner, 1961; Wooster et al., 2008). The molar volume of oil (V_m) influences the Ostwald ripening to a large extent. The low molar volume of oil (200-350 $cm^3\ mol^{-1}$) was soluble in water and resulted in destabilization by Ostwald ripening, while large molar volume of oil (long chain triglyceride, $\sim$900 $cm^3\ mol^{-1}$) which is insoluble in water prevents Ostwald ripening (Wooster et al., 2008). Kabalnov (2001) states that rate of Ostwald ripening is proportional to the interfacial tension. The change in mean droplet diameter (d) with time (t), owing to Ostwald ripening of a single component emulsified lipid in the steady state system is specified by the following equation (1) (Taylor, 1998):

$$d^3 - d_0^3 = \overline{\omega} = \frac{32}{9}\alpha cDt \qquad (1)$$

where, $\overline{\omega}$ represents the Ostwald ripening rate, α (=$2\gamma V_m/RT$), γ is the interfacial tension, V_m is the molar volume of the lipid, R is the gas constant and T is the absolute temperature, c is the

water solubility of the lipid and D is the translational diffusion coefficient of the lipid in the water.

In general, the Ostwald ripening commonly occurs in beverage emulsions which contain water soluble flavor oils, short chain triglycerides, and essential oils (Orange oil, D-limonene, etc.,) but does not occur in dairy based emulsions due to the presence of water insoluble triglyceride oils (Dickinson, 2009). Therefore, this instability of nanoemulsions can be effectively prevented by the addition of ripening inhibitor (non-polar molecule) where it is soluble in oil phase and insoluble in water phase like long chain triacylglycerol (corn , soy, sunflower, fish oil, etc.) (Li et al., 2009; McClements and Rao, 2011). Wooster et al., (2008) studied the impact of long chain triglycerides (Peanut oil), and n-alkane oil on Ostwald ripening of nanoemulsion. It was reported that peanut oil nanoemulsion (120 nm) was physically stable for more than 3 months of storage with no Ostwald ripening. However, n-alkane (Tributyrin) nanoemulsion had higher Ostwald ripening rate. Further, the addition of peanut oil into the soluble n-alkane oil (tricaprylin) emulsion made them more stable against Ostwald ripening and thermodynamic property. The large molar volume of LCT oils acts as kinetic barrier to Ostwald ripening and there was no change in particle size.

Similarly, Ziani et al., (2011) reported the increase in mean particle diameter (>7000 nm), creaming and oiling off in thyme oil nanoemulsions after 3 days of storage. The nanoemulsions were highly susceptible to Ostwald ripening due to the relatively high water solubility, rapid creaming and low density of thyme oil. Further, the addition of more than 75% of corn oil (a hydrophobic material with low water solubility) into nanoemulsion prevented the Ostwald ripening. Similarly, Delmas et al., (2011) studied Ostwald ripening process in the nanoemulsion

with a mixture of oil compounds (soybean oil or Labrafac). Nanoemulsion was stored at different temperatures (25, 40, 60, and 70 °C) to monitor the size evolution and Ostwald ripening rate (extracted over 90 h experiment). The Ostwald ripening showed a linear relationship between droplet radius and time, that indicates them as major destabilization mechanism (Fig. 3a). Also, it was reported that Ostwald ripening rates are temperature-dependent, and it gave an exponential relationship with the inverse of storage temperature (Fig. 3b). Ostwald ripening plots was linear at short time (t<20 h) storage of nanoemulsion, whereas nonlinearity was observed at long time storage, and the rate of droplet growth reduced by addition of insoluble surfactant. It was suggested that, incorporation of insoluble surfactant in sufficient amount in nanoemulsion stabilize them against Ostwald ripening. Further, the stability of nanoemulsions was due to kinetic suppression of the Ostwald and coalescence processes.

Rao and McClements (2012) investigated the effect of lemon oil fold (1X, 3X, 5X and 10X based on the degree of distillation of oil) on the formation and stability of nanoemulsion. Nanoemulsion with lower fold oils (1X, 3X and 5X) was found to be highly unstable and susceptible to Ostwald ripening during storage. However the higher oil fold (10X) nanoemulsion was stable against Ostwald ripening due to very low water solubility that inhibited the droplet growth. Recently, Eid et al., (2013) studied the effect of surfactant (sucrose laureate), glycerol and oil concentration on stability of olive oil nanoemulsion. Nanoemulsion with 50% oil concentration exhibited smaller droplets size with good uniformity compared to 36% oil concentration. However, in respect to the surfactant concentration, the nanoemulsion (50% of oil concentration) with 15% surfactant concentration showed larger droplet size and uniformity compared to 20% and 25% surfactant concentration. The specific formulation of nanoemulsion

(50% of oil concentration and 15% surfactant concentration) found to be unstable for Ostwald ripening. This is may be because of concentration of surfactant and oil composition. In the contrary, Ahmed et al., (2012) reported that there was no Ostwald ripening in curcumin nanoemulsion (less than 200 nm) composed of 50:50 of long chain triacylglycerols (LCT): short chain triacylglycerols (SCT). The nanoemulsion was also stable to particle growth and gravitational separation. It was due to mixing of high water solubility (SCT) oil into relatively low water solubility (LCT) oil during emulsion preparation. This effect is due to a compositional ripening process which responds to the Ostwald ripening process.

The nanoemulsion containing large molar volume of insoluble oils is more stable against Ostwald ripening as they can act as kinetic barrier to Ostwald ripening. Moreover, the nanoemulsions containing highly distilled oil were also stable towards Ostwald ripening due to low water solubility. However, the nanoemulsion with higher oil and lower surfactant concentration are prone to Ostwald ripening.

Coalescence

Coalescence occurs when two droplets collides each other and finally merge together and it is the destabilization mechanism over the time evolution of droplet size that express different behaviors from perfectly homogeneous growth (monomodal distribution with average size increases in time) to strongly heterogeneous growth (plurimodal distribution with the possibility of early phase separation). In general, nanoemulsions are more stable towards coalescence than the microsized emulsions (Capek, 2004) as particle size decreases, the rate of coalescence

increases due to more Brownian motion (Povey and Ding, 2010). The rate of droplet coalescence (related to droplet stability) is determined by the ability of the surfactant to rapidly adsorb to the surface of newly formed droplets; which in turn governed by surfactant surface activity and concentration (Tadros et al., 2004). Increase in surfactant concentration can increase the attractive forces between droplets in nanoemulsion, owing to a depletion mechanism that leads to droplet growth through increased coalescence (Mondain-Monval et al., 1996). Moreover, coalescence leads to irreversible rupture of the droplet interface that induces the droplets to merge together and eventually result in phase separation (Heurtault et al., 2003). Britten and Giroux, (1991) stated that coalescence was slower than creaming and flocculation in case of protein stabilized emulsions; however, it was strongly accelerated in the presence of shear stress. There are various factors (e.g. salt concentration, pH, composition, formulation and storage condition) that influence the occurrence of coalescence in nanoemulsion.

Jafari et al., (2006) evaluated the efficiency of sonication and microfluidization technique on the stability of nanoemulsion. It was reported that the high rate of collision during microfluidization leads to increase in droplet size with higher coalescence. This phenomenon of increase in droplet size and droplets coalescence beyond certain energy input level during emulsification is named as "over-processing". Furthermore, the coalescence was observed in ultrasonicated emulsion exhibiting bimodal droplet size distribution. In a subsequent publication, the authors analyzed the stability of d-limonene nanoemulsion prepared with various dispersed-phase volume fraction of d-limonene (5 to 15%) by microfluidization at 21, 42 and 63 MPa (2 cycles) (Jafari et al., 2007). At the highest pressure (63 Mpa), increased re-coalescence of new droplets occurred as a result of increased energy input, shorter residence time of

emulsion, and higher coalescence frequency without any influence of dispersed phase concentration. Furthermore, the droplet coalescence was observed with an increased emulsion droplet size in the optical microscopic image (Fig. 4). Kentish et al., (2008) also reported the similar phenomenon of droplet coalescence and cavitational bubble cloud formation in flaxseed oil nanoemulsion (135 nm) beyond the optimum power input level of ultrasonication. Moreover, low surfactant (Tween-40) contents also resulted in droplets coalescence.

Besides the operating factors of emulsion preparation techniques, the nature of surfactants, its concentration can also affect the occurrence of coalescence. Takegami et al., (2008) investigated the influence of co-surfactants such as sodium palmitate and sucrose palmitate on a lipid nanoemulsion (50 nm) prepared from soybean oil and phosphatidylcholine. The phosphatidylcholine stabilized lipid nanoemulsion showed an increase in mean droplet size due to coalescence during the storage period of 12 months. Moreover in a saline solution, the coalescence proceeded very rapidly, with an increase in mean droplet size to more than 150 nm within 0.5 h. However, the coalescence of nanoemulsion, were reduced with the application of sodium palmitate and sucrose palmitate, and that could maintain a mean droplet size around 50 nm for 12 months. Even in a saline solution, the mean droplet size could be maintained within 100 nm after 24 h. Sodium palmitate reduced the diameter of nanoemulsion and sucrose palmitate prevented the coalescence and enhanced the storage stability. Likewise, Wulff-Perez et al., (2009) analyzed the stability of natural oils (soybean, olive or sesame oil) nanoemulsions (below 500 nm) at different surfactant (Pluronic F68) concentration. Below certain concentration of Pluronic F68, emulsions destabilized quickly with occurrence of coalescence due to

insufficiency of surfactant to stabilize the emulsion, which made the droplets come closer and finally merge.

Similarly, Mao et al., (2009) examined the effect of different surfactants on the stability of β-carotene nanoemulsion (115 to 303 nm). Octenyl succinate starch (OSS) stabilized nanoemulsion had higher stability followed by those stabilized by whey protein isolate (WPI) and Tween-20, whilst decaglycerol monolaurate stabilized nanoemulsion had the least stability. OSS and WPI are large molecule emulsifiers that can form mechanically strong interfacial layers and cause steric hindrance to prevent droplet coalescence. Moreover, droplets in WPI emulsions often carry a large number of ions (with a zeta potential of -17.3 mV) and OSS solution had a relatively high viscosity that might improve their stabilizing properties. Likewise, Henry et al., (2010) examined the effect of different surfactants and its concentration on the processes of droplet breakup and coalescence in decane nanoemulsion. The surfactants used were phosphatidylcholine, phosphatidylglycerol, whey protein concentrate, and β-lactoglobulin. At higher emulsifier concentrations (1%), there was no coalescence except for phosphatidylcholine stabilized emulsion. However, in the phosphatidylcholine stabilized emulsions, coalescence on re-processing occurred up to the highest concentration (2%). Likewise, Anjali et al., (2012) studied the effect of different oil and surfactant (Tween-20) ratio (1: 0.3, 1: 1.5 and 1: 3) on the stability of neem oil nanoemulsion (31 to 25 nm). The nanoemulsion prepared with higher surfactant content (1: 3 ratio of oil and surfactant) had least droplet size (31 nm) and found to be stable against coalescence.

Similarly, Rao and McClements (2011) analyzed the stability of sucrose monopalmitate (SMP) stabilized lemon oil nanoemulsions at various storage temperatures. It was found that,

nanoemulsions were stable at refrigerator (5 °C) and ambient (23 °C) temperatures, but exhibited coalescence at elevated temperatures (40 °C) with a steep increase in mean particle diameter that occurred after 3 days of storage. This effect has been attributed to the dehydration of SMP when the system is heated. Qian et al., (2012) compared the stability of nanoemulsion droplets stabilized by non-ionic surfactant (Tween-20) and globular protein (β-lactoglobulin). Tween-20 stabilized nanoemulsion had a slight increase in the particle size (55-60 nm) during 15 days of storage. This effect has been attributed to coalescence when the non-ionic surfactant coated droplets are maintained at temperatures approaching the phase inversion temperature.

From the above studies, it is clear that, energy input beyond certain level during the emulsification process, results in over-processing of emulsion that leads to droplet coalescence. The nature of surfactants, its concentration, and the ratio of oil to surfactant are shown to influence coalescence of nanoemulsion. Lower surfactant concentration leads to coalescence due to insufficiency of surfactant to stabilize the emulsion. Emulsifiers like OSS, WPI and sucrose palmitate had exhibited higher stability of nanoemulsion against coalescence. Moreover, the emulsion with higher emulsifier concentration reduced the interfacial free energy and provided a mechanical barrier to coalescence. Furthermore, the stability of the emulsions to droplet coalescence increased, as the storage temperature was decreased.

Flocculation

Flocculation is one of the destabilization mechanisms of emulsions during long term storage process. Flocculation is the process, where two or more droplets associate with each

other, but maintain their individual integrity. It tends to occur when the attractive interactions between droplets dominate the long range repulsive interactions, but not the short range repulsive interactions. Fig. 5 illustrates the graphical view of flocculation phenomenon occurring in the nanoemulsion. The main attractive interactions in food emulsions are van der Waals, depletion and hydrophobic forces, whereas the main repulsive interactions are electrostatic and steric forces (McClements, 2005). The flocculation of droplets is far more vulnerable to aggregation and leads to increase in the droplet size (Liu et al., 2006). The emulsion flocculation rate will increase as the height of the repulsive energy barrier (wEB) decrease, within a reasonable time (<24 h) for wEB < 15 kT (McClements, 2005).

Jafari et al., (2007) reported that the addition of a Tween-20 (surfactant) to Hi cap (biopolymer) stabilized D-limonene nanoemulsion helped to reduce the emulsion size significantly during microfluidization. However, it leads to extensive flocculation due to surfactant biopolymer interactions and emulsifier displacement. Moreover, the extensive flocculation leads to creaming (phase separation). This can be due to mixture of surfactants and biopolymers present in an emulsion that compete each other to adsorb at oil-water interface and lowered the interfacial tension. Likewise, the type of surfactant and concentration also influences the flocculation. Similarly, Wulff-Perez et al., (2009) reported the flocculation of the natural oil nanoemulsions above the certain concentration of Pluronic F68 (3.1×10^{-3} mol/l). A quick phase separation was observed without any coalescence. It has been stated as depletion-flocculation mechanism, in which the excess of surfactant results in micelles formation, that make the droplets come closer via depletion. They do not merge due to their surfactant layers, but are close enough to flocculate and cream, giving rise to two different phases. Comparatively, Mao et al.,

(2009) reported no significant flocculation in Tween-20 stabilized β-carotene nanoemulsions due to the lower concentration of Tween-20. Recently, Qian et al., (2012) reported a slight increase in mean particle size (78-80 nm) of the β-lactoglobulin stabilized β-carotene nanoemulsions after 15 days of storage. This has been stated as the droplet flocculation resulting from the increased surface hydrophobicity of protein coated droplets when the globular proteins unfold. Moreover, there was also an increase in the hydrophobic attraction between lipid droplets, which can promote a droplet flocculation.

Besides the nature of the surfactant and its concentration, the ionic strength of emulsion created by salts can also influence the occurrence of flocculation. The major reason for the droplet flocculation in the presence of salt is the reduction in electrostatic repulsion and gradual increase in the hydrophobic attraction between the droplets during storage (Damodaran and Anand, 1997; Kim et al., 2002). Takegami et al., (2008) investigated the influence of co-surfactants on a lipid nanoemulsion (50 nm) prepared from soybean oil and phosphatidylcholine. Flocculation occurred in sodium palmitate stabilized lipid nanoemulsion during the storage period of 12 months as well as storage in saline solution. However, there was no flocculation in sucrose palmitate stabilized nanoemulsion during the 12 months of storage. Sucrose palmitate enhanced the storage stability by preventing the flocculation as well as coalescence in the nanoemulsion.

Similarly, Rao and Mcclements (2011) examined the influence of different salt (NaCl) concentration (0-200 mM) on the stability of SMP stabilized lemon oil nanoemulsion during the storage period. After 1 day storage, nanoemulsion had relatively low mean particle diameter and stable to phase separation. However, particle aggregation was observed after periods of a week

and a month storage at higher salt concentrations such as 100 mM and 50 mM NaCl, respectively. This effect has been attributed to the reduction in electrostatic repulsion between the negatively charged nanoemulsion droplets at increasing salt concentrations leading to flocculation and coalescence. Recently, Rao et al., (2013) investigated the influence of carrier oil composition (ratio of digestible to indigestible oil) on the physical stability of β-carotene nanoemulsion (less than 150 nm) in the simulated gastrointestinal conditions. The mean particle diameter of β-carotene nanoemulsion was increased appreciably (d_{43} > 1 μm) after incubation in artificial saliva (pH 7), due to extensive flocculation along with appreciable reduction in the negative charge of the nanoemulsions. The changes in droplet aggregation and charge within the mouth phase have been attributed to the presence of salts and mucin within the saliva. Salts may reduce the ζ –potential of droplets due to electrostatic screening whereas mucin may change the droplet charge by adsorbing to the droplet surfaces. As a result, electrostatic repulsion between the droplets decrease leading to flocculation.

The surfactant and biopolymer interaction lead to the flocculation of droplets in the emulsion. Moreover, the higher surfactant concentration resulted in flocculation of nanoemulsion. Hence, the concentration of surfactant needs to be optimized in the emulsification process to prevent flocculation. Increase in ionic strength also resulted in flocculation due to higher salt concentration that reduced the electrostatic repulsion between nanoemulsion droplets. Nanoemulsions were shown to flocculate in mouth phase due to the presence of salts and mucin in the saliva.

Phase Separation

The formation of two clear phases with separated boundary in the nanoemulsion is termed as phase separation. It is one of the physical indications of nanoemulsion instability that can be visually observed. The emulsion droplets have a different density to the liquid that environs them and net gravitational forces acts on them (Hunter, 1989; Robins, 2000), which in turn may lead to the phase separation. Phase separation (immiscibility) exhibits undesirable spontaneous separation of two layers, and often, induces specific morphology to the solution that results in immiscible blend with interesting rheological properties. The phase separation kinetics is to be determined through rheological behavior of the interconnected oil droplet regions and it involves an increase in strength of depletion attraction and gel-like droplet network formation. The addition of polysaccharide (chitosan and gum arabic) in different ratios leads to the formation of droplet network (high viscous) that prevents or even inhibits phase separation (Moschakis et al., 2010). In general, the types of emulsifiers, storage period and temperature influence the phase separation mechanism in emulsion (Karthik and Anandharamakrishnan, 2013).

Lee et al., (2011) prepared triacylglycerol oil (corn oil or fish oil) nanoemulsion using WPI as an emulsifier and compared the nano and conventional emulsions. It was observed that mean particle diameter increased around the isoelectric point (*PI*) (4.5<pH<5.5) of WPI. Different patterns of phase separation were found near the *PI* in both types of emulsions (Fig. 6). At pH 4.5 to 5.5 the distinct cream layer was observed on top of the conventional emulsion but cream layer was not formed at pH 4.5 and 5 in the nanoemulsion. Instead, there was an opaque suspension of aggregated particles was formed. Further, the droplet aggregation which occurred

at pH 5.5 reduced the nanoemulsion transparency, but not sufficient enough to induce phase separation. Similarly, Ahmed et al., (2012) studied the effect of long, medium, and short chain triacylglycerols on the stability of curcumin nanoemulsions. There was no phase separation in the nanoemulsion containing LCT or MCT (medium-chain triacylglycerols) stored at refrigerator (4 °C) over 10 days. But, nanoemulsion containing SCT showed phase separation within a few hours, and it could be improved by adding 50% of LCT into the lipid phase. This instability mechanism is due to Ostwald ripening which is related to higher water solubility of the SCT (low molecular weight triacylglycerol). Likewise, Ziani et al., (2011) reported the phase separation in thyme oil nanoemulsion with increase in the mean particle diameter (>7000 nm) after 3 days of storage. Recently, Ghosh et al. (2013) studied the stability of basil oil nanoemulsion against phase separation by varying the emulsification time with different surfactant concentration. It was experimental that different ratios of oil and surfactant such as 1:1, 1:2, 1:3 and 1:4 yielded droplet diameter of 57, 48, 45, and 29 nm for 5 min of sonication respectively. However, for the same ratios of oil and surfactant (1:1, 1:2, 1:3, and 1:4) the increase in sonication time to 15 min reduced the droplet diameter (41, 31, 29 and 29 nm). All these nanoemulsions were stable for one month, and phase separation occurred after one month of storage. Recently, Kim et al., (2013) investigated the stability of lemongrass oil nanoemulsion prepared by different concentration of Lemon oil (0.5% to 4.0%) with Tween-80 as surfactant. The mean droplet size of nanoemulsion increased from 56 to 88 nm as the oil concentration increased from 0.5- 4%. There was no significant change in stability of 0.5% lemon oil emulsion, whereas phase separation was observed in 4 % lemon oil emulsion. This can be due to higher concentration of oil and increasing level of mean droplet diameter.

Contradictorily, Sugumar et al., (2013) reported no phase separation during storage and centrifugation studies in the eucalyptus oil nanoemulsion (17 nm). This has been attributed to the smaller droplet size and higher physical stability of the nanoemulsion. Similarly, Anjali et al., (2012) also reported no phase separation in different formulations of neem oil nanoemulsion. All the formulated nanoemulsions were found to be physically stable at room temperature due to the significant role of steric effect. Moreover, Dey et al., (2012) reported no phase separation in EPA and DHA rich fish oil nanoemulsion. This was due to very low interfacial tension between oil and water and small droplet size that gives kinetic stability as well as long shelf life. Likewise, Liang et al., (2013) also observed no phase separation in modified starches stabilized β‑carotene nanoemulsions over 30 days of storage. The mean diameters of nanoemulsions were increased at 25 ℃ storage, whereas no significant change was observed at 4 ℃. This enhanced physical stability of nanoemulsions was attributed to the lower storage temperature and higher dispersed molecular density of modified starch that yielded thicker and denser layer around the oil droplets.

Nanoemulsions, with higher water solubility oil (SCT) were prone to phase separation due to increase in their mean droplet size. This could be prevented by the addition of lower water solubility oil (LCT) into the emulsion mixture. Altering the preparation technique, pH, oil, surfactant ratio and sonication time can influence the droplet size and stability towards phase separation. Storage of nanoemulsion at lower temperature can also effectively improve the stability against phase separation. Moreover, few reported studies, states that there was no phase separation in the nanoemulsion.

Sedimentation

The emulsion droplets have higher density than the surrounding liquid, inclined to downward movement, and referred as sedimentation. Normally, droplets of oil in water emulsion tend to move upwards (cream), whereas those of water in oil tend to move downwards (sediment) (McClements, 2005). Both the problems are highly related with a gravitational force over the emulsion droplets. Emulsions of tiny droplet size tend to have less gravitational force with higher Brownian motion that may act against the gravity and helps to prevent the sedimentation during storage (Tadros, 2009). Despite this fact, the external factors like composition of emulsion system, emulsification techniques, storage temperature and period can lead to sedimentation in the nanoemulsion.

Teo et al., (2010) investigated the storage stability of vitamin-E loaded palm oil esters based nanoemulsions (80-200 nm) with different formulation of pluronic F-68. The formulation with higher emulsifier (2.4 %) yielded more stable nanoemulsion during the 4 weeks of the storage period at 45 °C. However, other formulations exhibited creaming and sedimentation on storage at 45 °C during 5 weeks. Most of the formulations showed instability on storage at room temperature (25 °C), but none of the formulations showed phase separation on storage at 5 °C for 4 weeks. Similarly, based on various types of oil, emulsifier and neutral co-solvents, Li et al., (2012) studied the formation and stability of polymethoxyflavones loaded nanoemulsions (200 nm). A thin yellow layer was observed at the bottom of the stored nanoemulsions that confirmed the presence of sediment (Fig. 7). Moreover, after one month of storage, more yellow layer was observed at the bottom of the test tubes. This was due to low solubility of polymethoxyflavones

in both water and oil phases at ambient temperatures. It turns to form crystals in a nanoemulsion that leads to sediment. Recently, Ng et al., (2013) produced tocotrienol loaded palm oil esters based nanoemulsion that exhibited sedimentation and the rate of sedimentation was measured by centrifugal force which is related to the percent of transmission with time. The sedimentation process is influenced by oil phase concentration, centrifugal conditions, dispersed particles and storage periods.

In the contrary, sedimentation was not observed in curcumin nanoemulsion containing long-chain triacylglycerols (LCT) or medium-chain triacylglycerols (MCT) as the lipid phase (Ahmed et al., 2012). This is due to low water solubility effect of LCT and MCT as discussed earlier. Tang and Sivakumar (2013) also reported ferrous fumarate nanoemulsion (multiple water-in-oil-in-water emulsion of 600 nm) without sedimentation during storage at -4 °C for 10 days. This indicates its higher stability towards gravitational separation.

The nanoemulsions containing low water soluble oil were stable towards sedimentation during storage. Moreover, the rate of sedimentation of nanoemulsion increases with increase in concentration of oil. Besides, the stability of nanoemulsion against sedimentation depends on the storage period, temperature, droplet size and compositions.

Creaming

Creaming occurs when dispersed particles either settle or float in the lower or upper portion of continuous phase respectively, and becomes more opaque/creamier (Lieberman et al., 1989). The creaming process is measured in terms of increase in cream layer thickness of the

emulsion. Decrease in an average droplets size reduces the creaming velocity (Stokes law) and improves the stability of the emulsion (Desrumaux and Marcand, 2002). McClements (2005) reported that increase in particle size can increase the creaming velocity of droplets within emulsions. The creaming stability of nanoemulsion is measured by the value of creaming volume percentage C and it is calculated using the following equation (2) by Roland et al., (2003).

$$C = 100 \frac{(V_t - V_s)}{V_t} \qquad (2)$$

Where, V_t (mL) represents the total volume of the sample and V_s (mL) is the volume of the lower phase layer (serum). Therefore, the C value of 100 indicates the stable emulsion.

Sessa et al., (2011) investigated the stability of encapsulated resveratrol by peanut oil based nanoemulsions with different formulations. The creaming volume percentage (C) over time was articulate with size of droplets. It was reported, that creaming volume percentage was increasing (which decrease the stability) by increasing the storage temperature of nanoemulsions towards 4, 30 and 55 ℃. On the other hand, it also depends on their formulation and composition. Similarly, Ziani et al., (2011) reported the occurrence of creaming in thyme oil nanoemulsion. The rapid creaming was due to relatively large droplet size (d > 5000 nm), low density of thyme oil (ρ = 923 kg m^{-3}) and higher density of water (ρ = kg m^{-3}). Recently, physical stability of grape marc polyphenols encapsulated nanoemulsion was investigated with respect to creaming volume percentage over time under different storage (4, 30 and 55 ℃) conditions (Sessa et al., 2012). It was found that creaming volume percentage increased with increase in storage temperature of the nanoemulsions as discussed earlier. Moreover, unstable behavior in the particle size measurements was observed.

Likewise, Rao and McClements (2012) studied the stability of lemon oil nanoemulsion stored at different temperature condition for up to 12 h. The lower oil fold (1X, 3X and 5X) nanoemulsions were highly unstable to droplet growth and increased in particle size. However, higher oil fold (10X) are stable to droplet growth with a slight change in mean particle size. Moreover, the creamy layer appeared at the top of the lower oil fold emulsions, but no change in 10X emulsion. Similarly, curcumin nanoemulsion creaming stability was investigated by Ahmed et al., (2012). There was no creamy layer observed in LCT and MCT nanoemulsion over storage period of 24 h and remained to be homogeneous optical yellowish in appearance. SCT nanoemulsions were unstable to droplet growth and creaming with rapid separation of creamy or oily layer. This instability is due to the Ostwald ripening as discussed earlier. Recently, Tang and Sivakumar (2013) investigated creaming instability of ferrous fumarate nanoemulsion. It was reported that no creaming instability in the emulsion stored at -4 ℃ up to 10 days. However, at 45 ℃ storage, slower colour development with a gradual creaming was observed. As the time progresses, it turned yellowish white colour during the 10 days of storage. Oil rich layer formed (1.6 vol %) at the top of emulsions due to the downward movement of the water droplets after 10 days of storage at 45 ℃.

Contradictorily, Donsi et al., (2011) studied the effect of different emulsifiers on the storage stability of essential oil based nanoemulsion (74 to 366 nm). There was no creaming and change in droplet diameter during storage for 4 weeks in all the nanoemulsions. Similarly, Li et al., (2012) also reported the non-occurrence of creaming in polymethoxyflavones nanoemulsion over storage period of 24 h to 1 month. Uniform opaque appearance was observed throughout their volume of prepared emulsion. This is because of uniform smaller droplet size distribution.

Also, Dey et al., (2012) reported no creaming in EPA and DHA rich fish oil nanoemulsion through stability studies. This was because of kinetic stability as discussed earlier. Likewise, there was no creaming appearance in β‑carotene nanoemulsions due to the emulsifier property and storage conditions as discussed earlier (Liang et al., 2013).

The creaming stability of nanoemulsion reduced with an increase in storage temperature, but it mainly depends on the composition of nanoemulsion. Moreover, the creaming stability of nanoemulsion varied with the degree of distillation of oil. The nanoemulsions are more stable towards creaming while stored at lower temperature. However, few studies (Ahmed et al., 2012; Donsi et al., 2011; Li et al., 2012; Dey et al., 2012) reported no creaming instability in the nanoemulsion during storage period.

SAFETY OF NANOEMULSION

The current state of knowledge about the unique properties of engineered nano materials does not give exact inclusion or exclusion criteria for nano specific risk evaluation. The biological fate of a nanoemulsion particle may depend on the nature of the core and shell material. On exposure of nanoemulsion to the various physiological conditions, the droplets in nanoemulsions may change appreciably as they pass through the gastrointestinal tract (Johnson, 2001). There may be changes in the composition, size distribution, aggregation state, electrical charge, interfacial properties, and physical state of the nanoemulsion droplets. Currently, there is a knowledge gap in understanding the characteristics of specific nanoparticle, their biological fate in the gastrointestinal tract, and more research is needed to establish their potential toxicity

and safety (Hagens et al., 2007; Bouwmeester et al., 2009). It is also important to understand the properties of nanoemulsions that influence the release and absorption of the encapsulated components.

CONCLUSIONS

Nanoemulsions have great potential in the development of innovative food product due to their unique characteristics and higher stability over conventional emulsions. The various instability mechanism of nanoemulsion such as flocculation, creaming, sedimentation, coalescence, Ostwald ripening, and phase separation are reported to be influenced by several factors like ionic strength, temperature, solubilization, particle size distribution, particle charge, pH strength and heat treatment. Most of these physiochemical factors and instability mechanism are found to be interrelated. The over processing of emulsions and reduction in surfactant concentration leads to coalescence in the nanoemulsion. Higher ionic strength resulted in flocculation of emulsion droplets. The rate of sedimentation of nanoemulsion increased with the increase in concentration of oil. The nanoemulsions are unstable towards all destabilization mechanism at higher storage temperature and decreases in storage temperature are shown to improve their stability. The stability of nanoemulsion against Ostwald ripening, phase separation, sedimentation is improved by the addition of low solubility oil (LCT) in the emulsion. Moreover, the coalescence and flocculation of the nanoemulsion can be improved by optimizing the type of surfactants, its concentration, ratio with the oil and operating conditions of emulsification techniques. Optimizing the conditions for the enhanced stability of nanoemulsion helps in the

ACCEPTED MANUSCRIPT

development of high-throughput production and the widespread application in food, beverage and pharmaceutical industries based on their specific needs.

ACKNOWLEDGMENTS

ACCEPTED MANUSCRIPT

REFERENCES

Abismail, B., Canselier, J. P., Wilhelm, A. M., Delmas, H. and Gourdon, C., (1999). Emulsification by ultrasound: drop size distribution and stability. *Ultrason Sonochem*. **6:**75-83.

Acosta, E. (2009). Bioavailability of nanoparticles in nutrient and nutraceutical delivery. *Curr Opin Colloid In*. **14:**3-15.

Ahmed, K., Li, Y., McClements, D. J. and Xiao, H. (2012). Nanoemulsion-and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chem*. **132:**799-807.

Anandharamakrishnan, C. (2013). Techniques for nanoencapsulation of food ingredients. Springer, New York.

Anjali, C. H., Sharma, Y., Mukherjee, A. and Chandrasekaran, N. (2012). Neem oil (Azadirachta indica) nanoemulsion-a potent larvicidal agent against Culex quinquefasciatus. *Pest Manag Sci*. **68:**158-163.

Anton, N., Benoit, J. P., Saulnier, P. (2008). Design and production of nanoparticles formulated from nano-emulsion templates-a review. *J Control Release*. **128:**185-199.

Anton, N. and Vandamme, T. F. (2009). The universality of low-energy nanoemulsification. *Int J Pharmaceut*. **377:**142-147.

Belhaj, N., Arab-Tehrany, E. and Linder, M. (2010). Oxidative kinetics of salmon oil in bulk and in nanoemulsion stabilized by marine lecithin. *Process Biochem*. **45:**187-195.

Bouwmeester, H., Dekkers, S., Noordam, M. Y., Hagens, W. I., Bulder, A. S. and Heer, C., et al. (2009). Review of health safety aspects of nanotechnologies in food production. *Regul Toxicol Pharm.* **53:**52-62.

Britten, M. and Giroux, H. J. (1991). Coalescence index of protein-stabilized emulsions. *J Food Sci.* **56:**792-795.

Buranasuksombat, U., Kwon, Y. J., Turner, M. and Bhandari, B. (2011). Influence of Emulsion Droplet Size on Antimicrobial Properties. *Food Sci Biotechnol.* **20:**793-800.

Capek I. (2004). Degradation of kinetically-stable o/w emulsions. *Adv Colloid Interface Sci.* **107:**125-155.

Chen, L., Remondetto, G. E., Subirade, M. (2006). Food protein-based materials as nutraceutical delivery systems. *Trends Food Sci Tech.* **17:**272-283.

Chiralt. (2009). Food emulsion. **In** : Food Engineering - Volume II, Gustavo V. Barbosa-Canovas. Eds., EOLSS, USA.

Damodaran, S. and Anand, K. (1997). Sulfhydryl-disulfide interchange-induced interparticle protein polymerization in whey protein-stabilized emulsions and its relation to emulsion stability. *J Agr Food Chem.* **45:**3813-3820.

Delmas, T., Piraux, H., Couffin, A. C., Texier, I., Vinet, F., Poulin, P. and Bibette, J. (2011). How to prepare and stabilize very small nanoemulsions. *Langmuir.* **27:**1683-1692.

Desrumaux, A. and Marcand, J. (2002). Formation of sunflower oil emulsions stabilized by whey proteins with high-pressure homogenization (up to 350 Mpa): effect of pressure on emulsion characteristics. *Int J Food Sci Tech.* **37:**263-269.

Dey, T. K., Ghosh, S., Ghosh, M., Koley, H., Dhar, P. (2012). Comparative study of gastrointestinal absorption of EPA & DHA rich fish oil from nano and conventional emulsion formulation in rats. *Food Res Int.* **49:**72-79.

Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloid.* **23:**1473-1482.

Donsi, F., Annunziata, M., Sessa, M. and Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *LWT-Food Sci Technol.* **44:**1908-1914.

Eid, M., Mustafa, A., Ali Elmarzugi, N., A., G., I., B. and Ali El-Enshasy H., E., S., H., A., M. (2013). Preparation and Evaluation Of Olive Oil Nanoemulsion Using Sucrose Monoester. *Int J Pharm Pharm Sci.* 5.

Ezhilarasi, P. N., Karthik, P., Chhanwal, N. and Anandharamakrishnan, C. (2013). Nanoencapsulation techniques for food bioactive components: a review. *Food Bioprocess Tech.* **6:**628-647.

Fernandez, P., Andre, V., Rieger, J. and Kuhnle, A. (2004). Nano-emulsion formation by emulsion phase inversion. *Colloids Surf A Physicochem Eng Asp.* **251:**53-58.

Ghosh, V., Mukherjee, A. and Chandrasekaran, N. (2013). Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. *Ultrason Sonochem.* **20:**338-344.

Gutierrez, J. M., Gonzalez, C., Maestro, A., Sole, I., Pey, C. M. and Nolla, J. (2008). Nano-emulsions: New applications and optimization of their preparation. *Curr Opin Colloid In.* **13:**245-251.

Hagens, W. I., Oomen, A. G., de Jong, W. H., Cassee, F. R. and Sips, A. J. (2007). What do we (need to) know about the kinetic properties of nanoparticles in the body?. *Regul Toxicol Pharm*. **49:**217-229.

Henry, J. V., Fryer, P. J., Frith, W. J. and Norton, I. T. (2010). The influence of phospholipids and food proteins on the size and stability of model sub-micron emulsions. *Food Hydrocolloid*. **24:**66-71.

Heurtault, B., Saulnier, P., Pech, B., Proust, J. E. and Benoit, J. P. (2003). Physico-chemical stability of colloidal lipid particles. *Biomaterials*. **24:**4283-4300.

Hunter, R. J. (1989).Foundations of colloid science, vol.2, Oxford University Press, Oxford, UK.

Jafari, S. M., He, Y. H. and Bhandari, B. (2006) Nano-emulsion production by sonication and microfluidization-A comparison. *Int J Food Prop*. **9:**475-485.

Jafari, S. M, He, Y. H. and Bhandari, B. (2007). Optimization of nano-emulsions production by microfluidization. *Eur Food Res Technol*. **225:**733-741.

Johnson, L. R. (2001).Gastrointestinal Physiology, 6th Edition. Mosby, St. Louis, Missouri.

Kabalnov, A. (2001). Ostwald Ripening and Related Phenomena. *J Dispersion Sci Technol*. **22:**1-12.

Karthik, P. and Anandharamakrishnan, C. (2013). Microencapsulation of Docosahexaenoic Acid by Spray-Freeze-Drying Method and Comparison of its Stability with Spray-Drying and Freeze-Drying Methods. *Food Bioprocess Tech*. **6:**2780-2790.

Katsumoto, Y., Ushiki, H., Mendiboure, B., Graciaa, A. and Lachaise, J. (2000). Evolutionary behavior of miniemulsion phases: II. Growth mechanism of miniemulsion droplets. *J Phys: Condens Matter*. **12:**3569.

Kentish, S., Wooster, T. J., Ashokkumar, M., Balachandran, S., Mawson, R. and Simons, L. (2008). The use of ultrasonics for nanoemulsion preparation. *Innov Food Sci Emerg*. **9:**170-175.

Kim, H. J., Decker, E. A. and McClements, D. J. (2002). Impact of protein surface denaturation on droplet flocculation in hexadecane oil-in-water emulsions stabilized by beta-lactoglobulin. *J Agr Food Chem*. **50:**7131-7137.

Kim, I. H., Lee, H., Kim, J. E, Song, K. B., Lee, Y. S., Chung, D. S. and Min, S. C. (2013). Plum Coatings of Lemongrass Oil-incorporating Carnauba Wax-based Nanoemulsion. *J Food Sci*. **78:**E1551-E1559.

Lawrence, M. J. and Rees, G. D. (2012). Microemulsion-based media as novel drug delivery systems. *Adv Drug Deliver Rev*. **45:**89-121.

Lee, S. J., Choi, S. J., Li, Y., Decker, E. A. and McClements, D. J. (2011). Protein-stabilized nanoemulsions and emulsions: Comparison of physicochemical stability, lipid oxidation, and lipase digestibility. *J Agr Food Chem*. **59:**415-427.

Leong, T. S. H., Wooster, T. J., Kentish, S. E. and Ashokkumar, M. (2009). Minimising oil droplet size using ultrasonic emulsification. *Ultrason Sonochem*. **16:**721-727.

Li, Y., Le Maux, S., Xiao, H. and McClements, D. J. (2009). Emulsion-based delivery systems for tributyrin, a potential colon cancer preventative agent. *J Agr Food Chem*. **57:**9243-9249.

Li, Y., Zheng, J., Xiao, H. and McClements, D. J. (2012). Nanoemulsion-based delivery systems for poorly water-soluble bioactive compounds: Influence of formulation parameters on polymethoxyflavone crystallization. *Food hydrocolloid*. **27:**517-528.

Liang, R., Shoemaker, C. F., Yang, X., Zhong, F. and Huang, Q. (2013). Stability and Bioaccessibility of β-Carotene in Nanoemulsions Stabilized by Modified Starches. *J Agr Food Chem*. **61:**1249-1257.

Lieberman, H. A., Rieger, M. M. and Banker, G. S. (1989). Pharmaceutical Dosage Forms: Disperse Systems, Vol. 2. Mercel Dekker, New York.

Lifshitz, I. M. and Slyozov, V. V. (1961). The kinetics of precipitation from supersaturated solid solutions. *J Phys Chem Solids*. **19:**35-50.

Liu, W., Sun, D., Li, C., Liu, Q. and Xu, J. (2006). Formation and stability of paraffin oil-in-water nano-emulsions prepared by the emulsion inversion point method. *J Colloid Interface Sci*. **303:**557-563.

Madene, A., Jacquot, M., Scher, J. and Desobry, S. (2006). Flavour encapsulation and controlled release - A review. *Int J Food Sci Tech*. **41:**1-21.

Mao, L., Xu, D., Yang, J., Yuan, F., Gao, Y. and Zhao, J. (2009). Effect of small and large molecules emulsifiers on the characteristics of β-carotene nanoemulsions prepared by high pressure homogenization. *Food Technol Biotech*. **47:**336-342.

McClements, D. J. and Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Crit Rev Food Sci*. **51:**285-330.

McClements, D. J. (2005). Food emulsions: Principles, practice, and techniques. (2nd ed.). CRC Press, Boca Raton, FL.

Mohd-Setapar, S. H., Nian-Yian, L., Kamarudin, W. N. W., Idham, Z. and Norfahana, A. T. (2013). Omega-3 emulsion of Rubber (Hevea brasiliensis) seed oil. *Agric Sci*. **4:**84.

Mondain-Monval, O., Leal-Calderon, F. and Bibette, J. (1996) Forces between emulsion droplets: role of surface charges and excess surfactant. *J de Physique II*. **6:**1313-1329.

Moschakis, T., Murray, B. S. and Biliaderis, C. G. (2010). Modifications in stability and structure of whey protein-coated o/w emulsions by interacting chitosan and gum arabic mixed dispersions. *Food Hydrocolloid*. **24:**8-17.

Neethirajan, S. and Jayas, D. S. (2010). Nanotechnology for the food and bioprocessing industries. *Food Bioprocess Tech*. **4:**39-47.

Ng, S. H., Woi, P. M., Basri, M. and Ismail, Z. (2013). Characterization of structural stability of palm oil esters-based nanocosmeceuticals loaded with tocotrienol. *J Nanobiotechnology*. **11:**27.

Porras, M., Solans, C., Gonzalez, C. and Gutierrez, J. M. (2008). Properties of water-in-oil (W/O) nano-emulsions prepared by a low-energy emulsification method. *Colloid Surf A*. **324:**181-188.

Povey, M. and Ding, Y. L. (2010). Nanodispersion and method of formation thereof. WO/2010/038087.

Qian, C., Decker, E. A., Xiao, H. and McClements, D. J. (2012). Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chem*. **132:**1221-1229.

Quintanilla-Carvajal, M. X., Camacho-Diaz, B. H., Meraz-Torres, L. S., Chanona-Perez, J. J., Alamilla-Beltran, L., Jimenez-Aparicio, A. and Gutierrez-Lopez, G. F. (2010). Nanoencapsulation: a new trend in food engineering processing. *Food Eng Rev*. **2:**39-50.

Ramalingam, K., Amaechi, B. T., Ralph, R. H. and Lee, V. A. (2012). Antimicrobial activity of nanoemulsion on cariogenic planktonic and biofilm organisms. *Arch Oral Biol.* **57:**15-22.

Rao, J., Decker, E. A., Xiao, H. and McClements, D. J. (2013). Nutraceutical nanoemulsions: influence of carrier oil composition (digestible versus indigestible oil) on β-carotene bioavailability. *J Sci Food Agr.* **93:**3175-3183.

Rao, J. and McClements, D. J. (2011). Food-grade microemulsions, nanoemulsions and emulsions: Fabrication from sucrose monopalmitate & lemon oil. *Food Hydrocolloid.* **25:**1413-1423.

Rao, J. and McClements, D. J. (2012). Impact of lemon oil composition on formation and stability of model food and beverage emulsions. *Food chem.* **134:**749-757.

Robins, M. M. (2000). Lipid emulsions. *Grasas y aceites*. **51:**26-34.

Roland, I., Piel, G., Delattre, L. and Evrard, B. (2003). Systematic characterization of oil-in-water emulsions for formulation design. *Int J Pharmaceut.* **263:**85-94.

Salvia-Trujillo, L., Rojas-Grau, A., Soliva-Fortuny, R. and Martin-Belloso, O. (2013). Effect of processing parameters on physicochemical characteristics of microfluidized lemongrass essential oil-alginate nanoemulsions. *Food Hydrocolloid*. **30:**401-407.

Sanguansri, P. and Augustin, M. A. (2006). Nanoscale materials development–a food industry perspective. *Trends Food Sci Tech.* **17:**547-556.

Schubert, H. and Engel, R. (2004). Product and formulation engineering of emulsions. *Chem Eng Res Des.* **82:**1137-1143.

Sessa, M., Casazza, A. A., Perego, P., Tsao, R., Ferrari, G. and Donsi, F. (2012). Exploitation of polyphenolic extracts from grape marc as natural antioxidants by encapsulation in lipid-based nanodelivery systems. *Food Bioprocess Tech*. **6:**2609-2620.

Sessa, M., Tsao, R, Liu, R., Ferrari, G and Donsi, F. (2011). Evaluation of the stability and antioxidant activity of nanoencapsulated resveratrol during in vitro digestion. *J Agr Food Chem*. **59:**12352-12360.

Sharma, N., Bansal, M., Visht, S., Sharma, P. K. and Kulkarni, G. T. (2010). Nanoemulsion: A new concept of delivery system. *Chron of Young Scientists*.**1:**2.

Silva, H. D., Cerqueira, M. A. and Vicente, A. A. (2012). Nanoemulsions for food applications: development and characterization. *Food Bioprocess Tech*. **5:** 854-867.

Solans, C. and Sole, I. (2012). Nano-emulsions: Formation by low-energy methods. *Curr Opin Colloid In*.**17:**246-254.

Sole, I., Maestro, A., Pey, C., Gonzalez, C., Solans, C. and Gutierrez, J. M. (2006). Nano-emulsions preparation by low energy methods in an ionic surfactant system. *Colloids Surf A Physicochem Eng Asp*. **288:**138-143.

Sozer, N. and Kokini, J. L. (2009). Nanotechnology and its applications in the food sector. *Trends Biotechnol*. **27:**82-89.

Stang, M., Schuchmann, H. and Schubert, H. (2001). Emulsification in high-pressure homogenizers. *Eng Life Sci*. **1:**151-157.

Sugumar, S., Nirmala, J., Ghosh, V., Anjali, H., Mukherjee, A. and Chandrasekaran, N. (2013). Bio-based nanoemulsion formulation, characterization and antibacterial activity against food-borne pathogens. *J Basic Microb*. **53:**677-685.

Tadros, T., Izquierdo, P., Esquena, J. and Solans, C. (2004). Formation and stability of nano-emulsions. *Adv Colloid Interface Sci*. **108:**303-318.

Tadros, T. F. (2009). Emulsion science and technology: a general introduction. Emulsion science and technology. Wiley-VCH, Weinheim.

Takegami, S., Kitamura, K., Kawada, H., Matsumoto, Y., Kitade, T., Ishida, H. and Nagata, C. (2008). Preparation and characterization of a new lipid nano-emulsion containing two cosurfactants, sodium palmitate for droplet size reduction and sucrose palmitate for stability enhancement. *Chem. Pharm. Bull*. **56:**1097-1102.

Tang, S. Y. and Sivakumar, M. (2013). A novel and facile liquid whistle hydrodynamic cavitation reactor to produce submicron multiple emulsions. *AIChE J*. **59:**155-167.

Taylor, P. (1998). Ostwald Ripening in Emulsions. *Adv Colloid Interface Sci*. **75:**107-163.

Teixeira, P. C., Leite, G. M., Domingues, R. J., Silva, J., Gibbs, P. A. and Ferreira, J. P. (2007). Antimicrobial effects of a microemulsion and a nanoemulsion on enteric and other pathogens and biofilms. *Int J Food Microbiol*. **118:**15-19.

Teo, B. S. X., Basri, M., Zakaria, M. R. S., Salleh, A. B., Rahman, R. N. and Rahman, M. B. (2010). A potential tocopherol acetate loaded palm oil esters-in-water nanoemulsions for nanocosmeceuticals. *J Nanobiotechnology*, **8(4):**1-11.

Thakur, R. K., Villette, C., Aubry, J. M. and Delaplace, G. (2008). Dynamic emulsification and catastrophic phase inversion of lecithin-based emulsions. *Colloids Surf A Physicochem Eng Asp*. **315:**285-293.

Wagner, C. (1961). Theorie der Alterung von Niederschlagen durch Umlosen (Ostwald-Reifung). *Z Elektrochem*. **65:**581-591.

Walstra, P. (1996).Emulsion stability. **In :** Encyclopedia of emulsion technology, Becher, P., Eds., Marcel Dekker, New York.

Wang, X., Jiang, Y., Wang, Y. W., Huang, M. T., Hoa, C. T. and Huang, Q. (2008). Enhancing anti-inflammation activity of curcumin through O/W nanoemulsions. *Food Chem*. **108:**419-424.

Weiss, J., Takhistov, P. and McClements, D. J. (2006). Functional materials in food nanotechnology. *J Food Sci*. **71:**R107-R116.

Wooster, T. J., Golding, M. and Sanguansri, P. (2008). Impact of oil type on nanoemulsion formation and Ostwald ripening stability. *Langmuir*. **24:**12758-12765.

Wulff-Perez, M., Torcello-Gomez, A., Galvez-Ruiz, M. J. and Martin-Rodriguez, A. (2009). Stability of emulsions for parenteral feeding: Preparation and characterization of o/w nanoemulsions with natural oils and Pluronic f68 as surfactant. *Food Hydrocolloid*. **23:**1096-1102.

Yin, L., Chu, B., Kobayashi, I. and Nakajima, M. (2009). Performance of selected emulsifiers and their combinations in the preparation of beta-carotene nanodispersions. *Food Hydrocolloid.* **23:**1617-1622.

Yuan, Y., Gao, Y., Zhao, J. and Mao, L. (2008). Characterization and stability evaluation of [beta]-carotene nanoemulsions prepared by high pressure homogenization under various emulsifying conditions. *Food Res Int.* **41:**61-68.

Ziani, K., Chang, Y., McLandsborough, L. and McClements, D. J. (2011). Influence of surfactant charge on antimicrobial efficacy of surfactant-stabilized thyme oil nanoemulsions. *J Agr Food Chem*. **59:**6247-6255.

ACCEPTED MANUSCRIPT

Zimet, P., Rosenberg, D. and Livney, Y. D. (2011). Re-assembled casein micelles and casein nanoparticles as nano-vehicles for ω-3 polyunsaturated fatty acids. *Food hydrocolloid.* **25:**1270-1276.

Table 1 Different stability studies of food grade nanoemulsion

Food bioactive components	Types of instability studies	Emulsifiers and oil phase	Emulsification Techniques	Droplet size	Factors influencing	References
Thyme Oil	1.Ostwald ripening 2.Phase separation 3.Creaming	1. Tween-80 2. Lauric arginate 3. Sodium dodecyl sulfate	1. High speed blending 2. High pressure homogenization	7-196 nm	Droplet size and solubility	Ziani et al. (2011)
Olive oil	Ostwald ripening	Sucrose monoesters	Mixing	Less than 200 nm	Composition and droplet size	Eid et al. (2013)
Lemon oil	1.Ostwald ripening 2.Creaming	Tween-80	1. High speed stirring 2. High pressure homogenization	112-296 nm	Droplet growth and composition	Rao and McClements, (2012)

Curcumin	1.Ostwald ripening 2.Phase separation 3.Creaming 4.Sedimentation	β-lactoglobulin Oil phase: Long chain triacylglycerols (LCT), medium chain triacylglycerols (MCT) and short chain triacylglycerols (SCT)	1. High speed blending 2. High pressure homogenization	Less than 200 nm	Solubility	Ahmed et al. (2012)
Peanut oil	Ostwald ripening	1. Sodium dodecylsulphate (SDS) 2. Tween-80 3.	1. Silverson rotor-stator mixing 2. Microfluidization	40 nm	Solubility and droplet size	Wooster et al. (2008)

		Polyethylene glycol Other Oils: Tributyrin, tricaproin, tricaprylin, tricaprin, trilaurin, dodecane, tetradecane, hexadecane and octadecane				
Soybean oil	Ostwald ripening	PEG stearates Myrj s40, Myrj s50 and Myrj s100. Other oil:	Ultrasonication	150 nm	Emulsification process, temperature, type of emulsifiers and concentrati	Delmas et al. (2011)

ACCEPTED MANUSCRIPT

		Labrafac			on	
d-limonene	Coalescence	1. Modified starch 2. Sodium caseinate 3. Whey protein hydrolysate 4. Whey protein concentrate	1. High speed blending 2. Microfluidization	150-600 nm	Droplet size, emulsification method, mixer of biopolymer surfactant interaction	Jafari et al. (2007)
Flaxseed oil	Coalescence	Tween-40	1. Homogenisation 2. Ultrasonication	135 nm	Droplet size and emulsification method	Kentish et al. (2008)
Soybean oil and phosphatidylcholine	Coalescence	1. Sodium palmitate 2. Sucrose palmitate	Sonication	50 nm	Droplet size, storage period and	Takegami et al. (2008)

		3. Sucrose laurate 4. Sucrose myristate 5. Sucrose stearate 6. Tween-80			emulsion composition	
Natural oils (Sesame, olive soybean oil)	Coalescence	Pluronic F68	Ultrasonication	Below 500 nm	Surfactant concentration and droplet size	Wulff-Perez et al. (2009)
β-carotene	1.Coalescence 2.Flocculation	1. Tween-20 2. Decaglycerol monolaurate 3. Whey protein isolate 4. Octenyl succinate	1. Blending 2. Two stage valve homogenization	115-303 nm	Emulsifier properties and concentration	Mao et al. (2009)

		starch Oil phase: MCT oil				
d-limonene	Coalescence	1. Whey protein concentrate 2. Modified starch	1. High-speed blending 2. Microfluidization 3. Ultrasonication	150-700 nm	Emulsifier properties, droplet size and emulsification method	Jafari et al. (2006)
Lemon oil	Coalescence	Sucrose monopalmitate	1. Magnetic stirring 2. High-shear mixing 3. High pressure homogenization	Less than 200 nm	Temperature, salt concentration and particle charge	Rao and McClements, (2011)

β-carotene	Coalescence	1. β-lactoglobulin 2. Tween-20 Oil phase: orange oil	1. High speed blending 2. High pressure microfluidization	79 nm	Particle size	Qian et al. (2012)
β-carotene	Flocculation	1. Sucrose monoester 2. Lysolecithin Oil phase: 1. Corn oil 2. Lemon oil	1. High-speed mixing 2. High pressure homogenization	Less than 150 nm	Particle size and pH	Rao et al. (2013)
Triacylglycerol oil (corn oil or fish oil)	Phase separation	Whey protein isolate	1. High-shear mixing 2. High pressure homogenization	66-325 nm	Particle size and pH	Lee et al. (2011)

Basil oil	Phase separation	Tween-80	Ultrasonication	29 nm	Emulsification time and Droplet size	Ghosh et al. (2013)
Eucalyptus oil	Phase separation	Tween-80	Ultrasonication	17 nm	-	Sugumar et al. (2013)
Lemongrass Oil	Phase separation	Tween-80	Dynamic high pressure processing	57-89 nm	Droplet diameter and oil concentration	Kim et al. (2013)
Neem oil	Phase separation	Tween-20	Ultrasonication	31-251 nm	-	Anjali et al. (2012)
EPA and DHA rich fish oil	1. Phase separation 2. Creaming	1. Tween-20 2. Span-80	1. High speed homogenization 2.Ultrasonication	79-94 nm	Interfacial tension and droplet size	Dey et al. (2012)

β-Carotene	1. Phase separation 2. Creaming	Modified Starch Oil phase: MCT	1.High speed homogenization 2.High pressure homogenization	142-157 nm	Storage temperature and droplet diameter	Liang et al. (2013)
Vitamin E	Sedimentation	1. Pluronic F-68 2. Tween 80 Oil phase: palm oil esters	High shear homogenization	80-200 nm	Storage temperature and period	Teo et al. (2010)
Tocotrienol	Sedimentation	1. Tween-80 2. Span-80 3. Xanthan gum Oil phase: Palm oil esters	1. Homogenization 2. Ultrasonication	-	Migration of emulsion droplet	Ng et al. (2013)

Polymethoxy-flavone	1.Sedimentation 2.Creaming	1. β-lactoglobulin 2. Lyso-lecithin 3. Dodecyl trimethyl ammonium bromide 4. Tween 20 and 85. Oil phase: Corn oil, MCT and orange oil.	1.High speed blending 2. High pressure homogenization	less than 200 nm	Solubility, composition and droplet size distribution	Li et al. (2012)
Ferrous fumarate	1.Sedimentation 2.Creaming	1. Span 80 2. Cremophor EL, 3. Capryol 90,	1. Sonication 2. Homogenization 3. Liquid whistle	600 nm	Storage temperature, emulsification process and	Tang and Sivakumar, (2013)

		4. Labrasol	hydrodynami c cavitation reactor		compositio n	
		Oil phase: Maisine 35-1				
Resveratrol	Creaming	1. Soy lecithin Solec IP 2. Soy lecithin Lecinova, 3. Glycerol monooleate 4. Sugar ester P1670 5. Defatted soy lecithin Solec FS-B 6. Polysorbate Tween 20	1. High speed homogenizati on 2. High pressure homogenizati on	less than 180 nm	Emulsificat ion compositio n, storage temperatur e and droplet size	Sessa et al. (2011)

		Oil phase: Peanut oil				
Grape Marc Polyphenols	Creaming	1. Defatted soy lecithin Solec FS-B 2. Soy lecithin Solec IP 3. Glycerol monooleate Oil phase: sunflower oil (or) palm oil	1. High shear homogenizati on 2.High pressure homogenizati on	176-1,330 nm	Storage temperatur e and compositio n as mentioned above	Sessa et al. (2012)

Essential oils (D-limonene and mixture of terpenes)	Creaming	1. Soy lecithin 2. Tween-20 3. Glycerol Monooleate 4. Modified starch Oil phase: 1. Sunflower oil 2. Palm oil	1. High shear homogenizati on 2. High pressure homogenizati on	75-365 nm	Droplet size	Donsi et al. (2011)
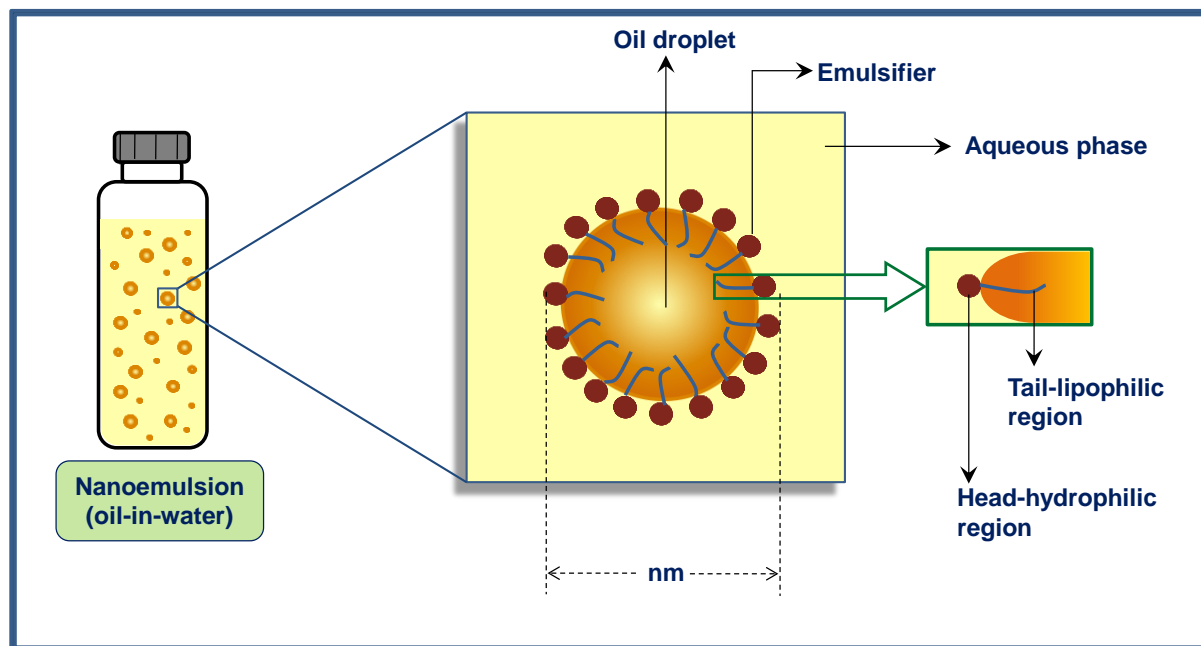
Figure 1 Schematic representation of oil in water nanoemulsion.
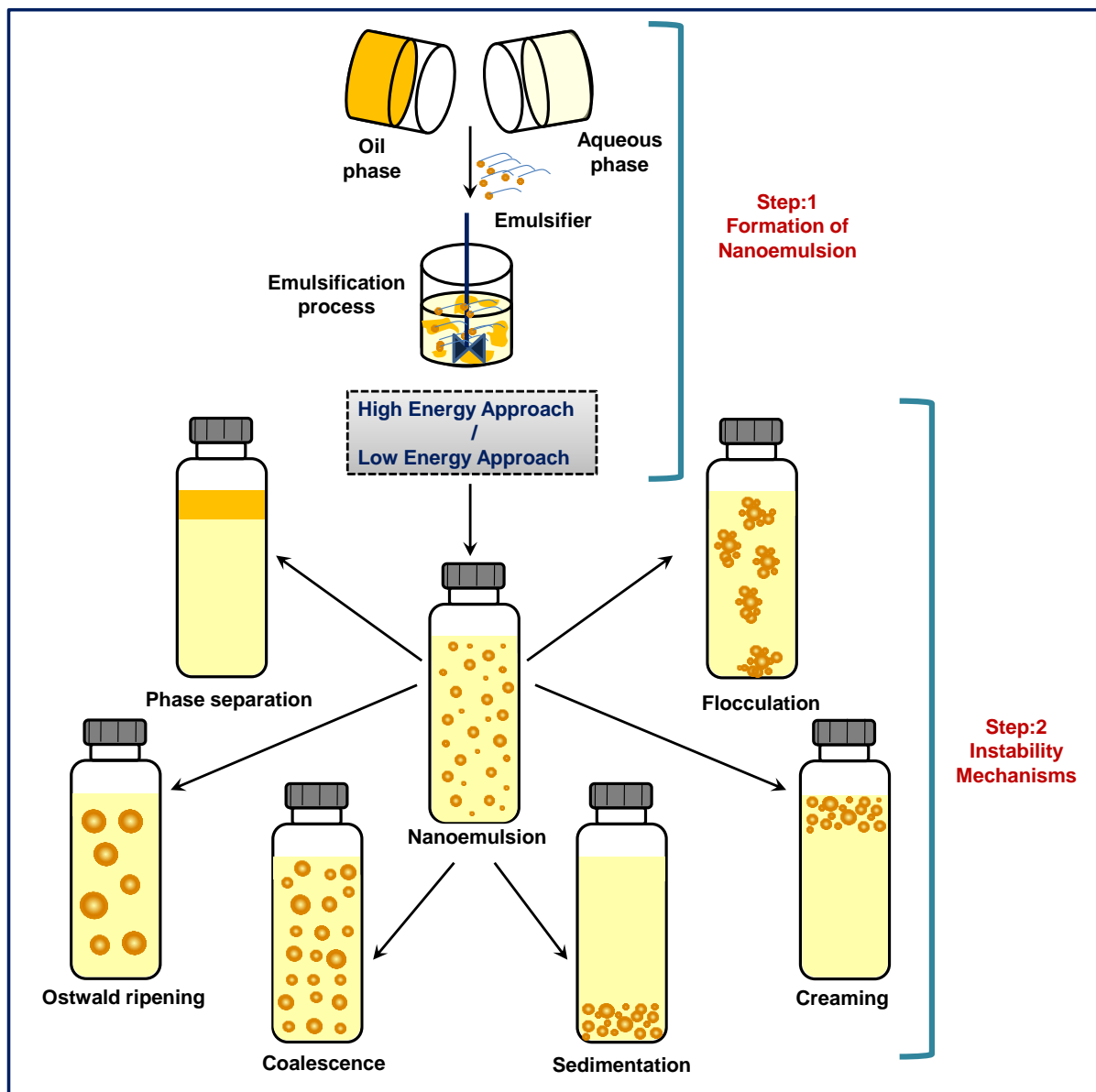
Figure 2 Step: 1 represents the formation of nanoemulsions through high energy approach or low energy approach. Step: 2 represents the breakdown processes of nanoemulsion through different types of mechanisms such as, creaming, phase separation, coalescence, sedimentation, flocculation and Ostwald ripening.

ACCEPTED MANUSCRIPT
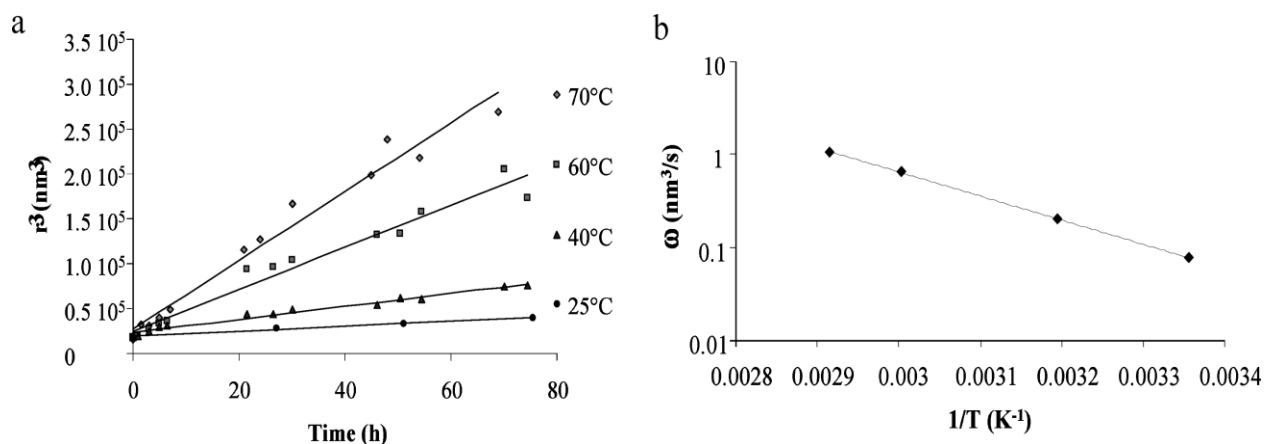
Figure 3 Illustration of Ostwald ripening at different storage temperature: (a) size evolution; (b)

Ostwald ripening rates extracted over 90 h experiment (Delmas et al., 2011).
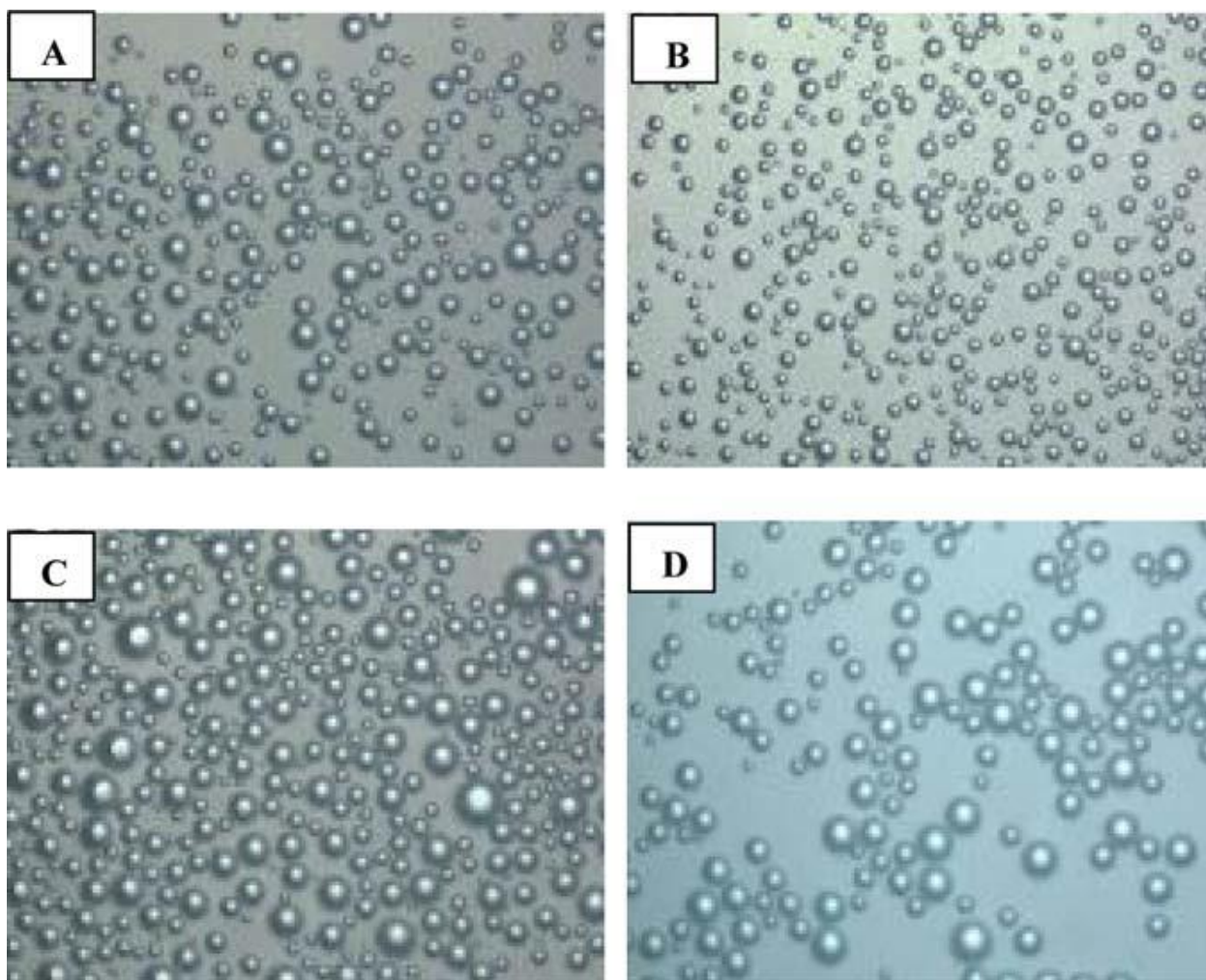
Figure 4 Optical Microscopic view (×100) of droplet coalescence in emulsion at different pressure: [A (21 MPa), B (42 MPa), C (63 MPa) and D (84 MPa)] (Jafari et al., 2007).

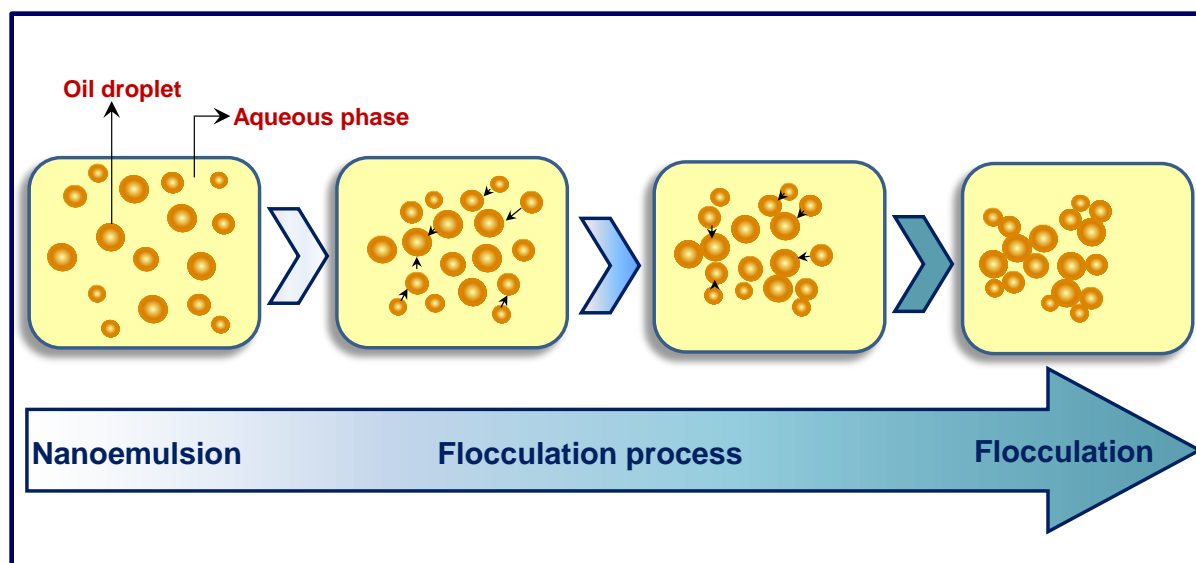**Figure 5** Graphical representation of flocculation process in nanoemulsion (oil-in- water).
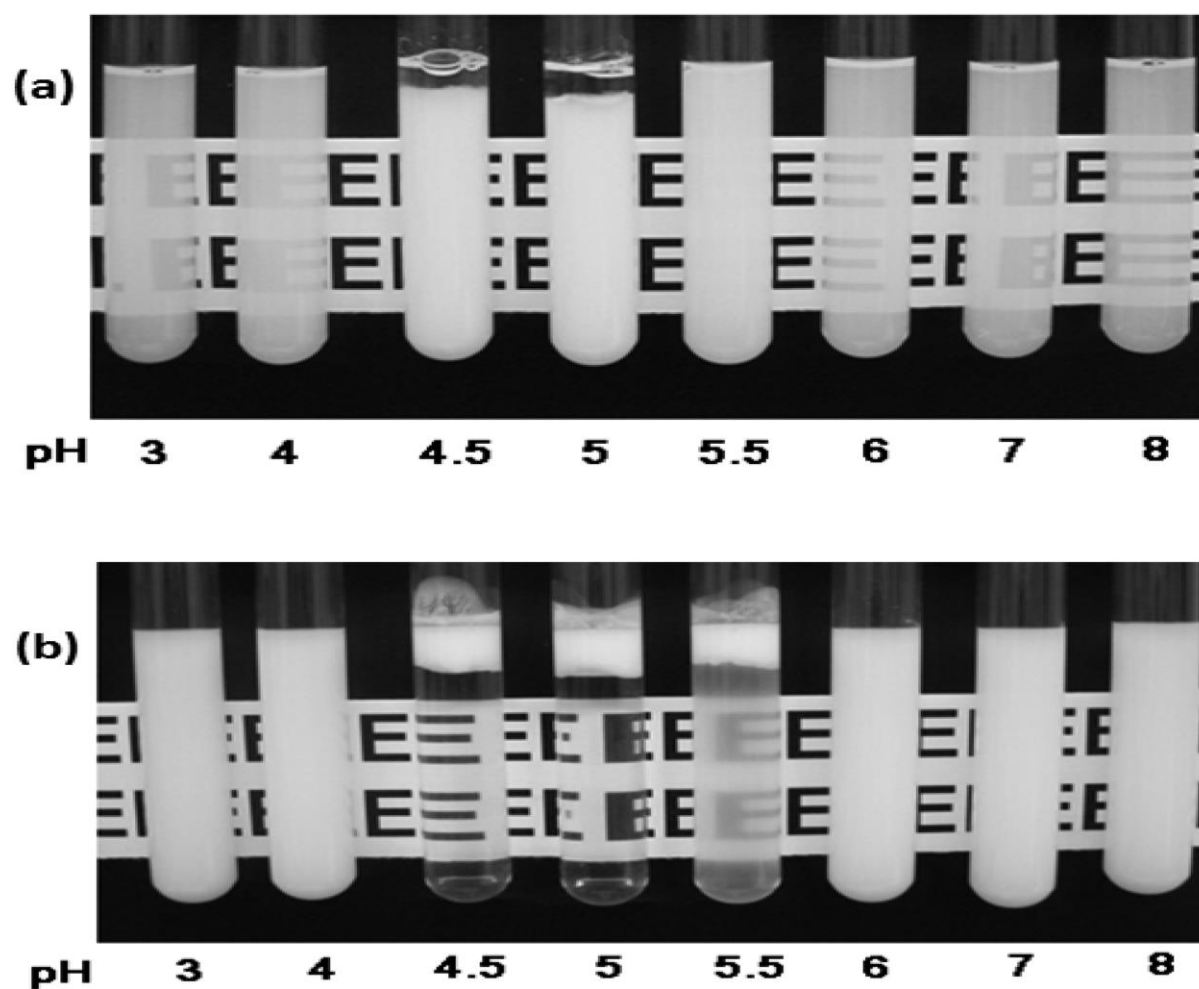
Figure 6 Photographs of corn oil emulsions: (a) nanoemulsions and (b) conventional emulsions at different pH levels (Lee et al., 2011).
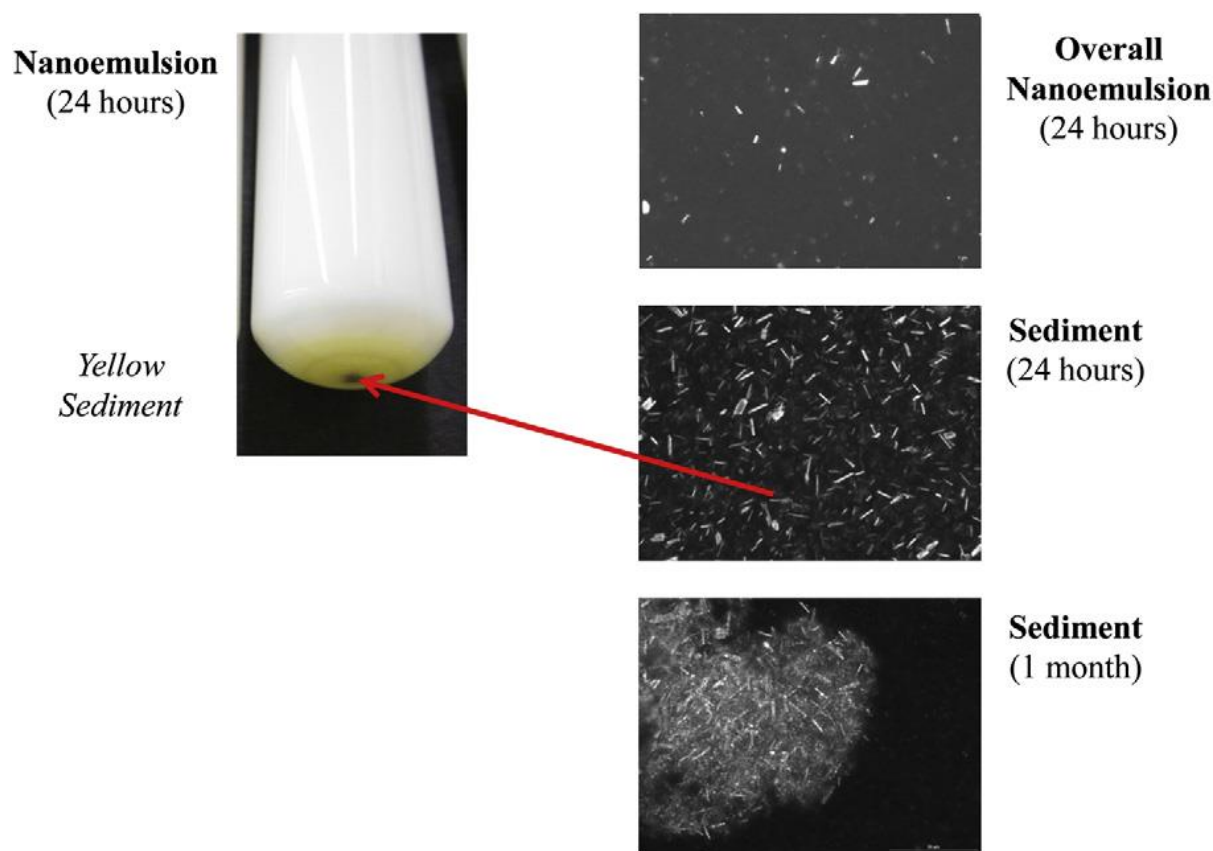
Figure 7 Polarized microscopic images of corn oil-in-water emulsions in the presence of polymethoxyflavone (PMF) measured after 1 day storage (Li et al., 2012).