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A REVIEW ON NANOEMULSIONS: PREPARATION METHODS AND STABILITY

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Abstract: There is a growing interest for using of nano/sub-micron particles in the technology of pharmaceutical, cosmetic and also food. Especially, this interest has been increasing parallel with better emulsification techniques and stabilization mechanisms. There are two main groups of nanoemulsion preparation methods, namely high-energy and low-energy spontaneous emulsification methods. Preparation processes and components used are significant parameters that affect stability from few hours to years. Problems such as creaming, coalescence sedimentation and flocculation are not concern for nanoemulsions due to their small droplet size. However, the main destabilization mechanism is Ostwald ripening for them. In this paper, a comprehensive review is presented to give basic ideas about nanoemulsions, their preparation methods, and stability aspects.

Keywords: Nanoemulsion; preparation methods; stability; Ostwald ripening

NANOEMÜLSİYONLAR ÜZERİNE BİR DERLEME: HAZIRLAMA METOTLARI VE STABİLİTELERİ

Özet: İlaç, kozmetik ve gıda teknolojilerinde nano partiküllerin kullanılmasına yönelik gittikçe artan bir ilgi mevcuttur. Bilhassa, bu ilgi emülsifikasyon tekniklerinin ve stabilizasyon mekanizmalarının iyileşmesine paralel şekilde artmaktadır. Nanoemülsiyonların hazırlanmasına yönelik yüksek enerjili ve düşük enerjili-spontane olmak üzere farklı tipte metotlar vardır. Hazırlık süreçleri ve kullanılan bileşenler, stabilitenin birkaç saatten yıllara kadar sürmesini etkileyen önemli parametrelerdir. Nanoemülsiyonlar küçük parçacık boyutuna sahip olduklarından kremleşme, koalesans, sedimantasyon ve flokülasyon gibi problemlere maruz kalmazlar. Fakat Ostwald olgunlaşması nanoemülsiyonların destabilizasyonuna neden olan temel mekanizmadır. Bu derleme çalışmada, nanoemülsiyonlar hakkında temel bilgiler verilmiş ve nanoemülsiyonların hazırlama metotları ve stabilite durumları literatür incelenerek kapsamlı bir biçimde sunulmuştur.

Anahtar Kelimeler: Nanoemülsiyon; hazırlama metodları; stabilite; Ostwald olgunlaşması

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INTRODUCTION

Emulsions which have droplet sizes between 5-200 nm are named as nanoemulsions, ultrafine emulsions, submicron emulsions, translucent emulsions and miniemulsions (Solans et al., 2005; Caldero et al., 2011). Nanoemulsions are developed systems for the delivery of biologically active agents for controlled release and drug delivery. They are promising systems for the fields of cosmetics, diagnostics, drug therapy and biotechnology (Sukanya et al., 2013). Moreover, they possess great potential as a novel delivery system in food industry for fatty acids, polyphenols, natural colors, and flavors especially for producing functional foods (Silva et al., 2012). Lipophilic active compounds have poor water solubility and thus introducing them into food and beverages is a big challenge for food industry. Using nanoemulsions as a carrier system solve the solubility problem and also increase bioavailability of lipophilic active compounds such as vitamins and carotenoids (Chu et al., 2007; Sagis, 2015).

Emulsions, also called as macroemulsions, are generally described as two immiscible phases dispersed within another (Becher, 2001). There are two main differences between conventional emulsions and nanoemulsions which results from size and shape of the particles in the continuous phase. Firstly, particle sizes in nanoemulsions (5-200 nm) are very smaller than conventional emulsions (0.1-100 μm). Secondly, in emulsions there are roughly spherical droplets of one phase dispersed into another. However, nanoemulsions consist of various structures such as droplet like swollen micelles and bicontinuous structures (Fernandez et al., 2004; Deverajan & Ravichandran, 2011).

Table 1: Properties of emulsions (Fernandez et al., 2004; Zhang, 2011; Thakur et al., 2013).

Emulsion	Droplet Size	Thermodynamic Stability	Appearance
Macroemulsion	0.1-100 μm	Unstable	Turbid
Microemulsion	5-100 nm	Stable	Transparent
Nanoemulsion	5-200 nm	Unstable	Transparent

Both microemulsions and nanoemulsions are transparent or translucent systems. Although they have almost similar average droplet size as shown in Table 1, they are different due to their preparation methods. Both of them require energy input for preparation in such a way that mechanical shear is used for nanoemulsions and spontaneous emulsification methods are used for microemulsions. As compared with nanoemulsions (5-10 %, w/w), formation of microemulsions (>20 %, w/w) need high surfactant concentration (Tadros et al., 2004; Setya et al., 2014). However, differently from microemulsions, which are thermodynamically stable, nanoemulsions have only kinetic stability (Korelova & Yurtov, 2012). For this principal difference between them, nanoemulsions may separate into the constituent phases since they are not in equilibrium. In addition to these, comparing with microemulsions, nanoemulsions draw interest for utilizing in, pharmaceutical, cosmetic, chemical and food industry because moderate surfactant concentrations are sufficient to form them (Mei et al., 2011). Moreover, there are significant differences between their preparation methods, since nanoemulsions need a large input of energy.

METHODS OF PREPARATION

Nanoemulsions can be prepared by using high and low energy methods. In high energy methods, mechanical devices deliver required large disruptive forces. On the other hand, in low energy methods, there is no need for an external force. Production of nanoemulsions is achieved by using the intrinsic physiological properties of the system. In this nanoemulsion preparation method, stored energy of the system is utilized by alteration of parameters such as temperature, composition of the system (Setya et al., 2014). At the initial studies of nanoemulsions, the high energy methods were only choice for researches and thus high-energy stirring and ultrasonic emulsification were the most widely used methods (Korelova & Yurtov, 2012). Nowadays, low-energy methods have drawn considerable attention since they are 'soft', non-destructive and cause no damage to encapsulated molecules (Anton et al., 2008).

High-Energy Emulsification Methods

Nanoemulsions are non-equilibrium systems which cannot be formed spontaneously. For this reason, mechanical or chemical energy input is necessary to form them. Nanoemulsions are generally prepared by using high energy methods in which mechanical energy input is applied by high pressure homogenizers, high-shear stirring, and ultrasound generators (Sole et al., 2012). These mechanical devices provide strong forces that disrupt oil and water phases to form nanoemulsions. In high energy methods, input energy density is about 10^8-10^{10} W kg^{-1} (Gupta et al., 2016). Required energy is supplied in a shortest time to the system in order to obtain homogeneous small sized particles. High-pressure homogenizers are capable of doing this and therefore they are the most widely used devices for preparing nanoemulsions (Solans et al, 2005). Moreover, producing emulsions using ultrasound is a cost-effective process which needs less surfactant use (Kaltsa et al., 2013). Therefore, considering conventional mechanical processes more homogeneous batches are achieved (Tadros et al., 2004).

High Pressure Homogenization

It is the most popular method used for the production of nanoemulsions. This method benefits from the high-pressure homogenizer or the piston homogenizer (Figure 1) to manufacture nanoemulsions that particle sizes are up to 1 nm. During the method, the macroemulsion is forced to pass through in a small orifice at an operating pressure between 500 to 5000 psi (Chime et al., 2014). Extremely small droplet sized nanoemulsions are achieved because during the process several forces like hydraulic shear, intense turbulence and cavitation act together.

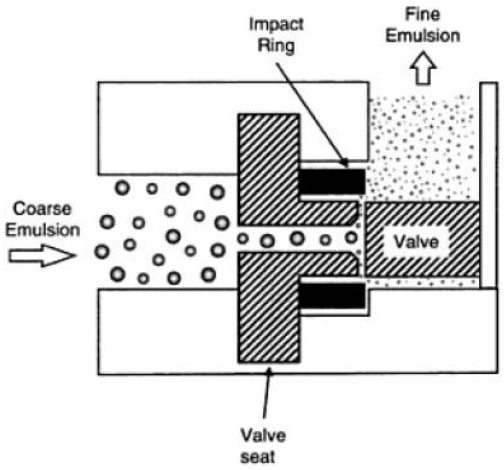

Figure 1. Schematic representation of high pressure valve homogenizer (McClements, 2005.)

This process can be repeated until the final product reaches the desired droplet size and polydispersity index (PDI). The uniformity of droplet size in nanoemulsions is specified by PDI (Jaiswal et al., 2015). Higher PDI means lower uniformity of droplet size in nanoemulsions. Monodisperse samples have PDI lower than 0.08, PDI between 0.08 and 0.3 states a narrow size distribution, whereas PDI greater than 0.3 indicates broad size distribution (Zhang, 2011). However, obtaining of small droplets that are in

submicron levels requires large amount of energy (Lovelyn & Attama, 2010). This amount of energy and increasing temperatures during high pressure homogenization process might cause deterioration of the components (Setya et al., 2014). Thermolabile compounds such as proteins, enzymes and nucleic acids may be damaged (Floury et al., 2000; Chime et al., 2014)

High-Shear Stirring

In this method, high-energy mixers and rotor-stator systems are used for the preparation of nanoemulsions. Droplet sizes of the internal phase can be significantly decreased by increasing the mixing intensity of these devices. However, obtaining emulsions with the average droplet size less than 200-300 nm is rather difficult (Korelova & Yurtov, 2012).

Ultrasonic Emulsification

There are two mechanisms which take part in ultrasonic emulsification. Firstly, acoustic field creates interfacial waves that makes oil phase to disperse in the continuous phase as droplets. Secondly, ultrasound provokes acoustic cavitation which provides formation and collapse of microbubbles respectively due to pressure fluctuations of a single sound wave. In this way, enormous levels of highly localized turbulence is generated and this causes micro implosions which disrupt large droplets into sub-micron size (Zhang, 2011).

In this method, premixed macroemulsion is agitated by vibrating solid surface at 29 kHz or larger frequencies. High-power ultrasonic devices such as focusing horns and pointed tips cause extreme shear and cavitation that result in breaking up of droplets. It has been observed that in most of the ultrasonic systems emitted sound field is inhomogeneous. For this reason, in order to have all droplets to experience highest shear rate, recirculation of the emulsion through the region of high power must be provided. Moreover, by doing this type of recirculation many times it is possible to obtain emulsions with uniform droplet size at dilute concentrations (Mason et al., 2006). Emulsifier type, the amount emulsifier, and viscosity of phases are the most critical parameters that affect homogenization efficiency (Maa & Tsu, 1999; Leong et al., 2009). Thus, optimization of these parameters is necessary to prepare nanoemulsions having fine droplets. However, there are some concerns about sonication methods due to fact that they have possibility to induce protein denaturation, polysaccharide depolymerization and lipid oxidation (Jafari et al., 2006; McClements & Rao, 2011).

Microfluidization

It is most widely employed in the pharmaceutical industry in order to acquire fine emulsions. In this method, a device called microfluidizer is used which provides high pressures (Figure 2). During the process, high pressure forces the macroemulsion to go through to the interaction chamber and thus nanoemulsions with submicron ranged particles can be produced. Uniform nanoemulsion production can be achieved by repeating the process many times and varying the operating pressure in order to get desired particle size (Chime et al., 2014; Jaiswal et al., 2015).

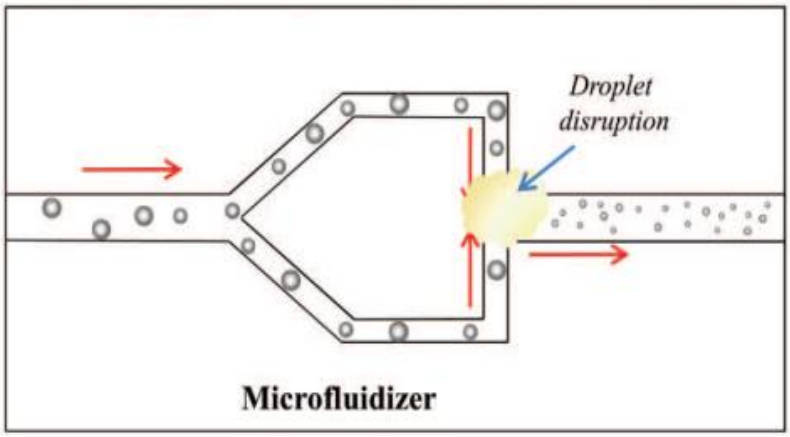

Figure 2. Schematic representation of microfluidizer (McClements & Rao, 2011).

There is a collision between crude emulsion jets from two opposite channels in the nozzle of microfludizer which is also called as the interaction chamber. The mobility of crude emulsion is provided by a pneumatically powered pump that has capability of compressing air up to pressures between 150 to 650 MPa. This high pressure forces the crude emulsion stream to go through microchannels and after the collison of two opposite channels enormous level of shearing force is obtained. Therefore, by the help of this force fine emulsions are produced (Gupta et al., 2010).

Low-Energy Emulsification Methods

Nanomulsification can also be achieved with low-energy methods which provides small size and more uniform droplets (Solans et al., 2005; Sole et al., 2012). These methods such as phase inversion temperature and phase inversion component provide smaller and more uniform droplets by using physicochemical properties of the system (Caldero et al., 2011). Although low energy procedures are generally more effective to produce small droplet sizes than high energy procedures, there are some limitations for them about the using of some types of oils and emulsifiers like proteins and polysaccharides. In order to overcome this problem high level of synthetic surfactant concentrations are used to produce nanoemulsions in low energy techniques but this narrows down their application area, especially for many food process (McClements & Rao, 2011).

Spontaneous Nanoemulsification

It benefits from the chemical energy releasement based upon dilution process with the continuous phase which occurs usually at constant temperature without any phase transitions in the system during the emulsification process (Solans & Sole, 2012). This method can produce nanoemulsions at room temperatures and no special devices are required. It basically subjected to interfacial tension, viscosity of interfacial and bulk, phase transition region, surfactant structure, and surfactant concentration (Setya et al., 2014). In the pharmaceutical industry, systems prepared by using this method are usually called as self-emulsifying drug-delivery systems (SEDDS) or self-nano-emulsifying drug-delivery systems (SNEDDS). When an oil phase with a water soluble substance is mixed with water, oil droplets spontaneously forms. The mechanism depends on the movement of water dispersible substance from the oil phase to the water phase, indicated as red arrows in Figure 3. This leads to interfacial turbulence and thus formation of spontaneous oil droplets (McClements & Rao, 2011).

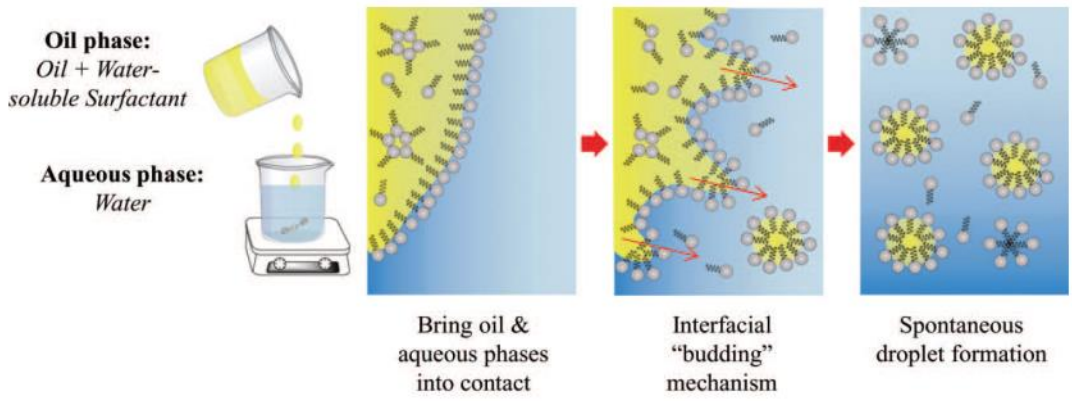

Figure 3. Schematic representation for spontaneous emulsification (McClements & Rao, 2011).

Phase Inversion Methods

These methods utilize the chemical energy that is released because of the phase transitions during emulsification process (Anandharamakrishnan, 2014). Required amount of phase transitions are achieved by changing the composition at constant temperature or by changing the temperature at constant composition (Thakur et al., 2013).

Phase Inversion Temperature (PIT)

In this method, temperature is changed at constant composition. Non-ionic surfactants which have temperature dependent solubility like polyethoxylated surfactants play important role. Emulsification is achieved by modifying affinities of surfactants for water and oil as a function of temperature (Lovelyn & Attama, 2010; Chime et al., 2014). During heating of polyethoxylated surfactants they become lipophilic due to dehydration of polyoxyethylene groups. Therefore, this circumstance establishes the principle of producing nanaoemulsions by PIT method. In order to prepare nanoemulsions by using PIT method, it is necessary to bring sample temperature to its PIT level or hydrophile–lipophile balance (HLB) level (Anandharamakrishnan, 2014). In the PIT method, the droplet sizes and the interfacial tensions reach their minimum value. This method promotes emulsification by benefiting from the extremely low interfacial tensions at the HLB temperature. Nevertheless, it has been observed that although emulsification is spontaneous at the HLB temperature, coalescence rate is greatly fast and emulsions are highly unstable (Ee et al., 2008). It has been reported that stable and fine emulsion droplets can be produced by rapid cooling of the emulsion near the temperature of PIT (Tadros et al., 2004; Rajalakshmi et al., 2011).

Phase Inversion Composition (PIC)

In this method, composition is changed at constant temperature. Nanoemulsions are obtained by consistently adding water or oil to the mixture of oil-surfactant or water-surfactant. The PIC method is more suitable for a large scale production than the PIT method since adding one component to an emulsion is easier than to generate abrupt change in temperature (Solans & Sole, 2012). By adding water to the system, volume of water increases and this result to reach a transition composition. In other words, the level of hydration of the the polyoxyethylene chains of the surfactant increases and thus spontaneous curvature of the surfactant goes to a change from negative to zero. As in the HLB temperature, in the transition composition a balance is obtained for the surfactant hydrophilic–lipophilic properties. When this transition composition is exceeded, small sized metastable oil in water droplet are composed due to the separation of the structures that have zero curvature (Anandharamakrishnan, 2014).

STABILITY OF NANOEMULSIONS

Emulsion stability is dependent on role of surfactants, its composition and the droplet size distribution. Surfactants have important role in nanoemulsion preparation methods by lowering the interfacial tension between two phases in order to obtain small sized droplets (Tadros et al., 2004). Emulsifier type influences the nanoemulsion stability against heating, cooling, pH, ionic strength and long-term storage (McClements & Rao, 2011). Surfactants promotes stability in different ways such as ionic surfactants provide electrical charge whereas non-ionic surfactants create a steric barrier with bulky molecular groups (Silva et al., 2012). In addition, effect of gravity on larger particles are much more than small ones (Fernandez et al., 2004).

Nanoemulsions possess great stability against coalescence, flocculation, sedimentation or creaming due to their characteristic particle size (Solans et al., 2005). Owing to the fact that nanoemulsions are subjected to Brownian motion rather than gravitational forces because of their very small droplet size, creaming or coalescence do not generally pose problem (Klang et al., 2012). Also, smaller droplet size provides less adhesion and higher stability against flocculation accompanied by steric stabilization which is a natural prevention for nanoemulsions (Anton et al, 2008; Delmas et al., 2011). On the other hand, Ostwald ripening is the main destabilization mechanism for them due to their nature of droplet sizes. Therefore, Ostwald ripening causes great limitation for their utilization for developing applications (Gutierrez et al., 2008).

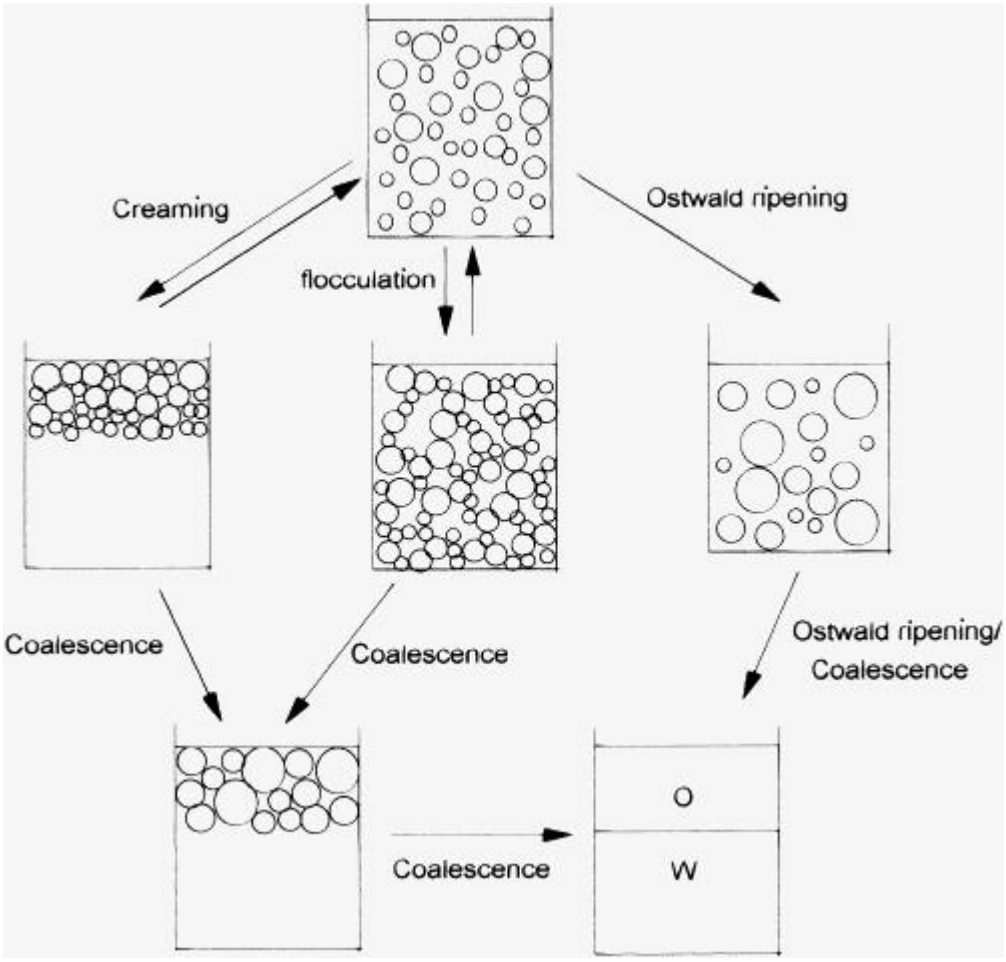

Figure 4: Physicochemical mechanisms cause instability (Taylor, 1998).

Ostwald ripening results from polydispersity of emulsions and the solubility difference between small and large droplets (Rajalakshmi et al., 2011). It is actually a mass transfer phenomenon which takes place between the droplets through the bulk phase (Figure 4). Due to the differences between droplet radiuses, chemical potential differences of the materials occurs inside of the droplets. The free energy in the emulsion begin to reduce and this give rise to decrease of the interfacial area. Thus, smaller sized droplets start to combine with bigger ones (Delmas et al., 2011). The reason for this is migration of the dispersed phase through bulk from small ones to bigger ones, because small droplets have higher solubility in the bulk than bigger droplets. Therefore, Ostwald ripening starts and accelerates during the process (Solans et al., 2005; McClements & Rao, 2011).

The Ostwald ripening rate for nanoemulsions can be decreased by some methods. For example, using a compound less soluble in the dispersion medium as the internal phase can be effective. It has been shown that the stability of the emulsions against the Ostwald ripening significantly increases with decreasing the hydrocarbon solubility in water as dispersion medium (Setya et al., 2014). Moreover, adding less polar lipid like long chain triglycerides can also reduce Ostwald ripening rate. Addition of this compound causes insolubility in water and therefore provides a kinetic barrier for Ostwald ripening (Wooster et al., 2008). Molecular weight of oil phase is another important factor since diffusion coefficient is correlated with the molecular weight. Therefore, the rate of Ostwald ripening can be diminished by using high molecular weighted oils (Zhang, 2011).

Some Studies on the Stability of Nanoemulsions

Ee et al. (2008) studied the formation and stability of nanoemulsions by using PIT emulsification method. According to their results, heating and cooling methods and the final temperature obtained after phase inversion have great influence on the size distributions. They found out that at the optimum storage temperature (20°C below PIT) which was dependent on surfactant concentration, nanoemulsions were most stable and most smallest-sized (25 nm to 54 nm) with low polydispersity indices (~0.2). The droplet sizes of nanoemulsions grow faster at room temperatures due to the Ostwald ripening. However, nanoemulsions stored at optimum temperatures retained their smallest droplet sizes, lowest polydispersity indices and superior stability. For this reason, they concluded that destabilizing effect of Ostwald ripening may be retarded by keeping nanoemulsions at the optimum storage temperatures.

A study about the influence of different kinds of inorganic salts on the PIT, electrophoteric properties and long term stability of nanoemulsions was conducted by Mei et al. (2011). They found that salts caused to growth of the emulsion droplets with time due to decreasing level of zeta potential and the PIT. The reason of that was explained by the countering and the salting-out effect of the inorganic salts. On the other hand, nanoemulsions containing emulsifiers with high PITs, could be formed more easily by adding salting-out salts in water which provides to obtain an optimum temperature by decreaing the PIT of the systems. They indicated that Ostwald ripening was responsible for the instability of nanoemulsions.

In another study, Yang et al. (2009) investigated stability of isopropyl mysristate (IPM) in water nonoemulsions (stabilized by PEG-60 hydrogenated caster oil varied ethanol concentration) which was prepared by PIC method at room temperature. Ostwald ripening was the main instability mechanism in their work. They assessed long term stability and found that flocculation may not occur due to the higher negative zeta potential of nanoemulsion. Addition of ethanol to the stock nanoemulsions (0.007 wt% IPM, 0.3 wt% ethanol, 0.007 wt% surfactant and water at 23°C) showed increasing rate of Ostwald ripening which was

explained by the increasing solubility of isoproplymysristate in the system. Moreover, increasing ethanol concentration in the continuous phase caused much larger droplet sizes for nanoemulsions. For this reason, they indicated that the difference between densities of continuous phases might be the main factor for controlling droplet sizes in nanoemulsions.

Delmas et al. (2011) applied ultrasonication method in order to obtain nanoemulsions which have very small sized droplets and high stability. They determined that the characteristic decay time for the mean droplet sizes had direct relationship with frequency of bubble collapse and thus sonication power. They showed also that Ostwald ripening was still the main destabilization mechanism but coalescence could be readily hindered because of the very small size of droplets.

CONCLUSION

This paper provides general information about nanoemulsions and presents the current studies about nanoemulsion stability. All of the studies point out that main destabilization mechanism for nanoemulsion stability is Ostwald ripening. Although small droplet sizes provide natural defense system in nanoemulsions for flocculation, Ostwald ripening occurs whatever the preparation method is. However, this does not change the truth of long-term stability of nanoemulsions if they are stored at proper conditions. There is increasing use of nanoemulsions in many practical applications primarily in pharmacy as a drug delivery system since they are capable of solubilized non polar active compounds. However, a deeper understanding is necessary in order to develop nanoemulsions in food processing applications. Having elucidative mechanisms that rule the preparation and stability of food nanoemulsions can be beneficial for better formulation and application for nanoemulsions to use in food industry. In the light of these information, further work is needed to be done about nanoemulsion formulations and their stability.

REFERENCES

1. Anandharamakrishnan, C., Techniques for Nanoencapsulation of Food Ingredients, *Springer*, 2014.
2. Anton, N., Benoit, J.P., and Saulnier, P., Design and production of nanoparticles formulated from nano-emulsion templates-A review, *Journal of Controlled Release*, 128, 185-199, 2008.
3. Becher, P., Emulsions: Theory and Practice, Oxford University Press, New York, 2001.
4. Caldero, G., Maria, J.G.C. and Solans, C., Formation of polymeric nano-emulsions by a low-energy method and their use for nanoparticle preparation, *Journal of Colloid and Interface Science*, 353, 406–411, 2011.
5. Chime, S.A., Kenechukwu, F.C., and Attama, A.A., Nanoemulsions-Advances in Formulation, Characterization and Applications in Drug Delivery, Ali DS, *Application of Nanotechnology in Drug Delivery, Crotia: InTech*, 77-111, 2014.
6. Chu, B.S., Ichikawa, S., Kanafusa, S., and Nakajima, M., Preparation of protein-stabilized β-carotene nanodispersions by emulsification–evaporation method, *Journal of the American Oil Chemists' Society*, 84(11), 1053-1062, 2007.
7. Delmas, T., Piraux, H., Couffin, A.C., Texier, I., Vinet, F., Poulin, P., Cate, M.E. and Bibette J., How To Prepare and Stabilize Very Small Nanoemulsions, *Langmuir*, 27(5), 1683–1692, 2010.
8. Devarajan, V. and Ravichandran, V., Nanoemulsions: As Modified Drug Delivery Tool, *Devarajan V / Pharmacie Globale (IJCP)*, 4 (1), 2011.
9. Ee, L.S., Duan, X., Liew, J. and Nyugen, Q.D., Droplet size and stability of nano-emulsions produced by the temperature phase inversion method, *Chemical Engineering Journal*, 140, 626–631, 2008.
10. Fernandez, P., Andre, V., Rieger, J. and Kühnle A., Nano-emulsion formation by emulsion phase inversion, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 251, 53–58, 2004.
11. Floury, J., Desrumaux, A., and Lardieres, J., Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions, *Innovative Food Science & Emerging Technologies*, 1(2), 127-134, 2000.

12. Gupta, P.K., Pandit, J.K., Kumar, A., Swaroop, P., and Gupta, S., Pharmaceutical Nanotechnology Novel Nanoemulsion–High Energy Emulsification Preparation, Evaluation and Application, *The Pharma Research*, 3, 117-138, 2010.

13. Gutierrez, J.M., Gonzalez, C., Maestro, A., Sole, I., Pey, C.M. and Nolla J., Nano-emulsions: New applications and optimization of their preparation, *Current Opinion in Colloid & Interface Science*, 13, 245–251, 2008.

14. Jafari, S.M., He, Y.H., and Bhandari, B., Nano-emulsion production by sonication and microfluidization: A comparison, *International Journal of Food Properties*, 9, 475–485, 2006.

15. Jaiswal, M., Dudhe, R., and Sharma, P.K., Nanoemulsion: an advanced mode of drug delivery system, *3 Biotech*, 5, 123–127, 2015.

16. Kaltsa, O., Michon, C., Yanniotis, S., and Mandala, I., Ultrasonic energy input influence on the production of sub-micron o/w emulsions containing whey protein and common stabilizers, *Ultrasonics sonochemistry*, 20(3), 881-891, 2013.

17. Klang, V., Matsko, N., Valenta, C. & Hofer F., Electron microscopy of nanoemulsions: An essential tool for characterisation and stability assessment, *Micron*, 43, 85–103, 2012.

18. Koroleva, M.Y., and Yurtov, E.V., Nanoemulsions: the properties, methods of preparation and promising applications, *Russian Chemical Reviews*, 81(1), 21-43, 2012.

19. Leong, T.S.H., Wooster, T.J., Kentish, S.E., and Ashokkumar, M., Minimising oil droplet size using ultrasonic emulsification, *Ultrasonics Sono-chemistry*, 16(6), 721-727, 2009.

20. Lovelyn, C., and Attama, A.A., Current State of Nanoemulsions in Drug Delivery, *Journal of Biomaterials and Nanobiotechnology*, 2, 626-639, 2011.

21. Maa, Y.F., and Hsu, C.C., Performance of sonication and microfluidization for liquid-liquid emulsification, *Pharmaceutical Development and Technology*, 4(2), 233–240, 1999.

22. Mason, T.G., Wilking, J.N., Meleson, K., Chang, C.B., and Graves, S.M., Nanoemulsions: formation, structure, and physical properties, *Journal of Physics: Condensed Matter*, 18, 635-666, 2006.

23. McClements, D.J., Food Emulsions, principles, practice, and techniques, CRC Press, Boca Raton, FL, 2005.

24. McClements, D.J., and Rao, J., Food-Grade Nanoemulsions: Formulation, Fabrication, Properties, Performance, Biological Fate, and Potential Toxicity, *Critical Reviews in Food Science and Nutrition*, 51, 285–330, 2011.

25. Mei, Z., Xu J., and Sun, D., O/W nano-emulsions with tunable PIT induced by inorganic salts, *Colloids and Surfaces A: Physicochem. Eng. Aspects*, 375, 102–108, 2011.

26. Rajalakshmi, R., Mahesh, K., and Kumar, C.K.A., A Critical Review on Nano Emulsions, *International Journal of Innovative Drug Discovery*, 1, 1-8, 2011.

27. Sagis, L.M. (Ed.), Microencapsulation and microspheres for food applications, Academic Press, 2015.

28. Setya, S., Talegaonkar, S., and Razdan, B.K., Nanoemulsions: Formulation Methods and Stability Aspects, *World Journal of Pharmacy and Pharmaceutical Sciences*, 3, 2214-2228, 2014.

29. Sole, I., Solans, C., Maestro, A., Gonzalez, C. and Gutierrez J.M., Study of nano-emulsion formation by dilution of microemulsions, *Journal of Colloid and Interface Science*, 376, 133–139, 2012.

30. Solans, C., Izquerdo, P., Nolla, J., Azemar, N. and Garcia-Celma, M.J., Nano-emulsions, *Current Opinion in Colloid & Interface Science*, 10, 102-110, 2005.

31. Solans, C., and Sole, I., Nano-emulsions: Formation by low-energy methods, *Current Opinion in Colloid & Interface Science*, 17, 246–254, 2012.

32. Silva, H. D., Cerqueira, M. Â., & Vicente, A.A., Nanoemulsions for food applications: development and characterization, *Food and Bioprocess Technology*, 5(3), 854-867, 2012.

33. Sukanya, G., Mantry, S., and Anjum, S., Review on Nanoemulsions, *International Journal of Innovative Pharmaceutical Sciences and Research*, 1(2), 192-205, 2013.

34. Tadros, T., Izquierdo, P., Esquena, J. and Solans, C., Formation and stability of nano-emulsions, *Advances in Colloid and Interface Science*, 108, 303–318, 2004.

35. Taylor, P., Ostwald ripening in emulsions, *Advances in colloid and interface science*, *75*(2), 107-16, 1998.

36. Thakur, N., Walia, M.K., and Kumar, S.L.H., Nanoemulsion in Enhancement of Bioavailability of Poorly Soluble Drugs: A Review, *Pharmacophore*, 4(1), 15-25, 2013.

37. Yang, H.J., Cho, W.G. and Park, S.N., Stability of oil-in-water nano-emulsions prepared using the phase inversion composition method, *Journal of Industrial and Engineering Chemistry*, 15, 331–335, 2009.

38. Wooster, T.J., Golding, M. and Sanguansari, P., Impact of oil type on nanoemulsion formation and Ostwald ripening stability, *Langmuir*, 24 (22):12758-12765, 2008.

39. Zhang, J., Novel Emulsion-Based Delivery Systems, Faculty of The Graduate School of the University of Minnesota, Master Thesis, 2011.