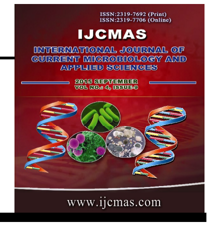

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Original Research Article

Effect of Surfactant and Oil Type on Size Droplets of Betacarotene-Bearing Nanoemulsions

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A B S T R A C T

Spontaneous emulsification is one of the low energy methods in the preparation of oil-in-water nanoemulsions (NEs). In this study the beta carotene loaded nanoemulsion was prepared by spontaneous method, then the influence of various types of surfactants (tween 21, 80, 85 and 1:1 ratio of 21:85), carrier oils (corn oil, octyl octanoate, miglyol 812) and different concentration of surfactant and oils (%SER) on the particle size and stability of the system were investigated to obtain optimized formulation of betacarotene nanoemulsion. The nanoemulsion containing tween 80 (as surfactant), miglyol 812 (as the carrier oil containing 3% beta carotene) with surfactant-to-emulsion ratio of SER= %17.5 was determined as optimum nanoemulsion with monomodalar particle size of 76 nm which was stable in 25 °C for 3 months. Droplet morphology using a scanning electron microscope without the occurrence of accumulation, aggregation, confirms the results of particle size. This study showed the spontaneous emulsification is a successful low-energy method for preparation of beta carotene nanoemulsion.

Keywords

Miglyol, Nanoemulsion, Non-ionic surfactant, Stability, Beta Carotene

Introduction

The use of nano carriers for hydrophobic nutricicticals such as carotenoieds, have multiple benefits e.g. protection of the degradation of active agent against oxidation, heat, light, heavy metals and enzymes. Also during the course of the digestive system, nano carries offer higher bioavailability and avoid the bad taste and color of the food (Lin *et al.*, 2009; Sagalowicz and Leser, 2010). Due to the low solubility of beta carotene in water generally its ability to confine to the nano lipid structures is high, thus its application into colloidal delivery systems that can help to spread it into aqueous-based beverage (De Vost *et al.*, 2010; Saberi *et al.*, 2013). Nanoemulsions are among the most important nano carrier systems, which are clear systems with droplet size in the range of 20–200 nm (Fathi *et al.*, 2012). They are good delivery systems for lipid-soluble nutriceticals which can be prepared by simple production methods and natural food ingredients. Formation and expansion of interfacial surface between the oil phase and the aqueous phase, requires energy input to the system and therefore these systems are thermodynamically unstable and due to various physicochemical phenomena over time they tend to separate to their constituent phases (Rao and McClements, 2012; Jafari *et al.*, 2008). This required energy could be provided by mechanical energy (high energy methods) or potential energy of its constituent components (low energy methods). Low energy methods generally depend on the interfacial phenomena at the boundary layer between oil and water phases and often it is more effective in small particles production, than high energy methods (Rao and McClements, 2012; Piorkowski and McClements, 2013; Sagalowicz and Leser, 2010).

Spontaneous emulsification is one of the low energy methods in the preparation of oil-in-water nanoemulsions. It relies on the formation of very fine oil droplets when an oil/hydrophilic surfactant mixture is added to the water. Due to the rapid diffusion of the surfactant /solvent from the oil phase into the aqueous phase, spontaneous formation of fine droplets at the oil-water boundary is done (Saberi *et al.*, 2013). In recent years many researches have been carried out to investigate the use of spontaneous emulsification: Encapsulation of retinyl palmitate in nanoemulsion based octyl octanoate as carrier oil and emulsifiers Montanov 68 EC (lactylate lactylate alcohol glucoside) (Carlotti *et al.*, 2005), spontaneous nanoemulsion containing vitamin E by various surfactants (Saberi *et al.*, 2013), encapsulation of resveratol in nanoemulsion based on peanut oil and different types of natural and synthetic surfactants (Pardo and McClements, 2014) and production of nanoemulsion by the application a model system (hexadecane as the oil phase, Brig 30 as non-ionic surfactant) (Komaiko and McClements, 2014). A variety of different oils and surfactants may be used to form nanoemulsions in the food and beverage industries. The aim of this study was to investigate the factors influencing the production and stability the droplets of betacarotene nanoemulsion for reaching to optimal size of droplets and stability of spontaneous betacarotene nanoemulsion for use in food and beverages fortification.

Materials and Methods

Materials

Miglyol-812 (caprylic/capric triglycerides) was provided from Sasol (Witten, Germany), beta carotene, octyl octanoate, corn oil, were provided from Sigma Aldrich (Steinheim, Germany). Nonionic poly sorbate surfactants tween 80, 21, 85 were provided from Merck (Hohenbrunn, Germany) and Scharlau (Sentmenat, Spain). Other chemicals were analytical grade and procured from Sigma (Merck Chemical Co. Darmstadt, Germany).

Methods

Preparation of beta carotene nanoemulsion

Nanoemulsion of beta carotene was produced using low energy spontaneous method by addition of the oil phase drops (solution of a hydrophilic nonionic surfactant with beta carotene in oil carrier) to the deionized water (Anton and Vandamme, 2009). During the formation of the emulsion the mixture is continuously stirred by the magnetic stirrer (500 rpm at 25° C). By the time when the pouring of the oil phase was completed, the systems were given stirred for 40 minutes to reach equilibrium. Considering the effect of temperature on the particle size of nanoemulsion, a magnetic stirrer equipped with a temperature sensor (Hiedolph, Germany) was used to maintain temperature during emulsion formation time.

Particle Size and Size Distribution

The average diameter and span value of the particles were determined using particle size analyzer (Wing SALD 2101, Shimadzo, Japan) at 25°C. The average particle size was calculated according to the average volume diameter or DeBroukere mean in the Equation (1):

$$\overline{D}[4,3] = \frac{\sum n_i d_i^{\,4}}{\sum n_i d_i^{\,3}} \qquad (1)$$

The span value is an index helpful to evaluate the particle size distribution and it is calculated applying the following Equation (2):

$$Span = \frac{D_{90\%} - D_{10\%}}{D_{50\%}} \qquad (2)$$

D (90%): describes diameter where 90% of the distribution has a smaller particle size and 10% percent has a larger particle size.

D (10%): describes diameter where 10% of the distribution has a smaller particle size and 90% percent has a larger particle size.

D (50%): describes diameter where 50% of the distribution has a smaller particle size and 50% percent has a larger particle size (Hamishehkar *et al.*, 2009).

Morphology characterization

Morphology of the nanoemulsions was observed using scanning electron microscopy (Tescan-MIRA3 FEG-SEM). Samples were dried on carbon-coated grids at room temperature (Klang *et al.*, 2012).

Surfactant concentration

The influence of surfactant concentration on the droplet size was studied by calculating (%SER). In this method surfactant-to-emulsion ratio was varied, while keeping the total oil content (MCT + betacarotene) constant (10 wt.%). Equation (3):

$$SER\% = \frac{M_s}{M_s + M_O + M_W} \times 100 \qquad (3)$$

Here, m_S, m_O and m_W are the masses of the surfactant, oil, and water respectively. This was attained by varying the relative amounts of surfactant and water present in the system. In generated nanoemulsions based on tween 80 and Mygliol 812 as a carrier oil, containing 0.03% beta carotene 6 different percentages of SER (7.5%, 10%, 12.5%, 15%, 17.5 %and 20%) were examined.

Influence of oil type

A series of emulsions were prepared with similar overall compositions (Tween 80 as surfactant, %SER= 17.5%), but using different oils including medium chain triglycerides, (MCT, Mygliol 812); octyl octanoate; olive oil; soybean oil; peanut oil; sesame oil and corn oil containing 2% beta carotene.

Effect of surfactant type

In this study, effect of arrange of food-grade non-ionic surfactants (Tween 21, 80 and 85 and 1:1 ratio of 21:85) on particle size of emulsion was examined. These surfactants are generally considered as the most suitable surfactants for the formation of emulsions by low energy methods.

Statistical analysis

Statistical analysis was designed based on a complete randomized optimization after 3 repetitions. One-way ANOVA and Duncken's mean comparison tests were used at 5% with SPSS version 16.0.

Results and Discussion

Effect of Surfactant Concentration on the Droplet Size

In low concentration of surfactant (SER = 7.5 %) the size of produced droplets was more than 300 nm and macro emulsion was produced. At a concentration of SER= 10%, size of produced droplets was 100 nm but the size distribution of droplets was biomodal (span= 3.03) (Figure 1b). By increasing the concentration of surfactant by SER= 15%, % 17.5, the size of obtained droplets decreased to less than 100 nm and these droplets almost had the same size (Figure 1a). In SER=15% the size distribution of droplets was bimodal, while the nanoemulsions with SER = 17.5% had monomodular size distribution. At low concentration of emulsifier in oil-water interfacial layer which not enough to completely cover the surface of drops, coalescence and folocation of droplets occurs and the size of droplets will be increased. Increasing the surfactant concentration would result in a greater number of surfactant molecules emigrating from the oil phase to the aqueous phase of the emulsion and nano droplets would be produced (Saberi *et al.*, 2013). By applying the shear force at the oil-water surface which is coated with emulsifiers, some of emulsifiers would be drown parallels to surface layer and some of them will be detached from the surface layer. These emulsifier molecules may be separated from a place where there are lots of emulsifiers or isolated from interfacial layer local where there is no emulsifier. When the droplet rolls collide each other, if these areas which have no emulsifier get close together, they can be merged together and this phenomenon occurs when the emulsifier absorption rate is less than the time that the stress is applied and also at the time the droplet collid velocity is greater than the rate of emulsifier absorption in interfacial layer (McClements, 2004). Unexpectedly by increasing surfactant concentration up to SER =20% droplet size increased and particles had broad, non-uniform size distribution (Figure 1b). Instability of nanoemulsions at high surfactant concentrations can be related to depletion-flocculation mechanism of adsorbed surfactant. By increasing the surfactant concentration, further molecules of surfactant form micelle in continuous phase, instead of orientation on particle surface, resulting to the increasing of the local osmotic pressure and so the continuous phase between the some droplets moves to them, which causes the depletion of the continuous phase between the drops. Consequently the aggregation takes place and the particle size increases (Wulff-Perez *et al.* 2009).

Droplet Morphology Using Scanning Electron Microscopy

Images obtained by Scanning electron microscopy (SEM), showed spherical droplets in nanoemulsions system, without the occurrence of phase separation, aggregation and also, the results of the size measurement of nano particles obtained from measurement devices, were confirmed (Figure 2). Cho *et al.* (2007) also investigated the microstructure and the size distribution of droplets containing beta carotene nanoemulsions using TEM, drops with an average diameter of 20 nm were observed in the DLS[1] and the particle size

[1] - Dynamic Light Scattering

was consistent with the measured results. Buchmal *et al.* (2004) studied the morphology and structure of food nanoemulsion using TEM. The nano droplets with spherical shape a smooth surface were observed

The effect of surfactant type on size and distribution of droplets

In figure 3, the effect of different types of nonionic poly sorbate surfactants (tweens) on the size of the droplets in nanoemulsions prepared with the optimized formulation (SER =17.5%, Mygliol 812 as carrier oil and the use of 0.02% beta carotene) is shown. This type of nonionic surfactants is suitable for the production of nano emulsions using low energy methods. Results showed significant differences between different surfactants and tween 80 produced the smallest droplets. Survey on the molecular structure of surfactants shows the presence of hydrophobic chain in their structure: A Monolurate tail in tween 21 (C12 = 0), a mono oleate tail in tween 80 (C18 = 1) and trioleate tail in tween 85 (3 * C18 =1) (Ostertag *et al.*, 2012). The smallest droplet size produced by tween 80 (HLB = 15), while the tween 21 with HLB equal to 7.16 (the higher rank shows it is more hydrophilic), produced larger droplets. Unsaturated surfactants have more mobility than saturated ones and it can be the cause of facilitation of spontaneous formation of emulsion. In tween 80, presence of double bond of the oleate ester in its hydrophobic chain, induced more mobility for it rather than other tweens (such as tween 21) with saturated chains. In addition, high molecular weight of some tweens (such as tween 85) reduces mobility of the surfactant from organic phase to aqueousso they show a poor emulsifier property (Wakerly *et al.*, 1986). Although hydrophilic surfactant (i.e. higher HLB) is a prerequisite for the production of oil in water nanoemulsion, and so tweens, which have more HLB are not able to produce nanoemulsions at low surfactant concentrations. It seems that the size of generated droplets is not relevant only on the HLB value and there are other effective factors.

The difference between tweens' chain and head groups influences the placement of surfactants in the water-oil surface and therefore the formation of tiny droplets. In a study done by li *et al.* (2012) on the production of nanoemulsions containing methoxy flavone extracted from citrus peel by high pressure homogenization, different types of corn oils, medium-chain triglyceride and also surfactants such as beta-lactoglobulin, lecithin, tween and dodecyl trimethyl ammonium bromide (DTAB) were used. The results showed that the use of all types of emulsifiers except DTAB, result in the production of nanoparticles with an average diameter of less than 100nm. It was reported that particle size distribution, morphology, and also precipitation of methoxy flavone, depends on the type of oil, surfactant and cosolvent addition (ethanol and glycerol).

Stability of optimized nanoemulsion

Long term stability of optimal nanoemulsion was checked out by measurement of changes in the size and also appearance of samples (see phase separation) during storage at room temperature (approximately 25 °C). Over a period of 3 months, significant changes were not observed between samples ($P<0.05$) and the size distribution for all samples were uniform and monomodular (Figure 4). Reduction in the size of the droplets overcome the Brownian motion to the gravitational force and also increases the viscosity. Then two important instability mechanisms, gravitational separation and aggregation (flocculation) are prevented.

Table.1 Composition of surfactant, oil and water and span value in beta carotene nanolemulsions

Furmulation	%SER	Surfactant (tween 80) (gr.)	Carrier oil (Mygliol 812) (gr.)	Water (gr.)	Span
F1	SER=7.5%,	0.75	1	8.25	**0.83**
F2	SER=10%	1	1	8	**3.09**
F3	SER=12.5%	1.25	1	7.75	**0.73**
F4	SER=15%	1.5	1	7.5	**0.92**
F5	SER=17.5%	1.75	1	7.25	**0.77**
F6	SER=20%	2	1	7	**0.5**

Figure.1 Effect of surfactant emulsion ratio (SER) on the (a) particle size and (b) particle size distribution of beta carotene nanoemulsion

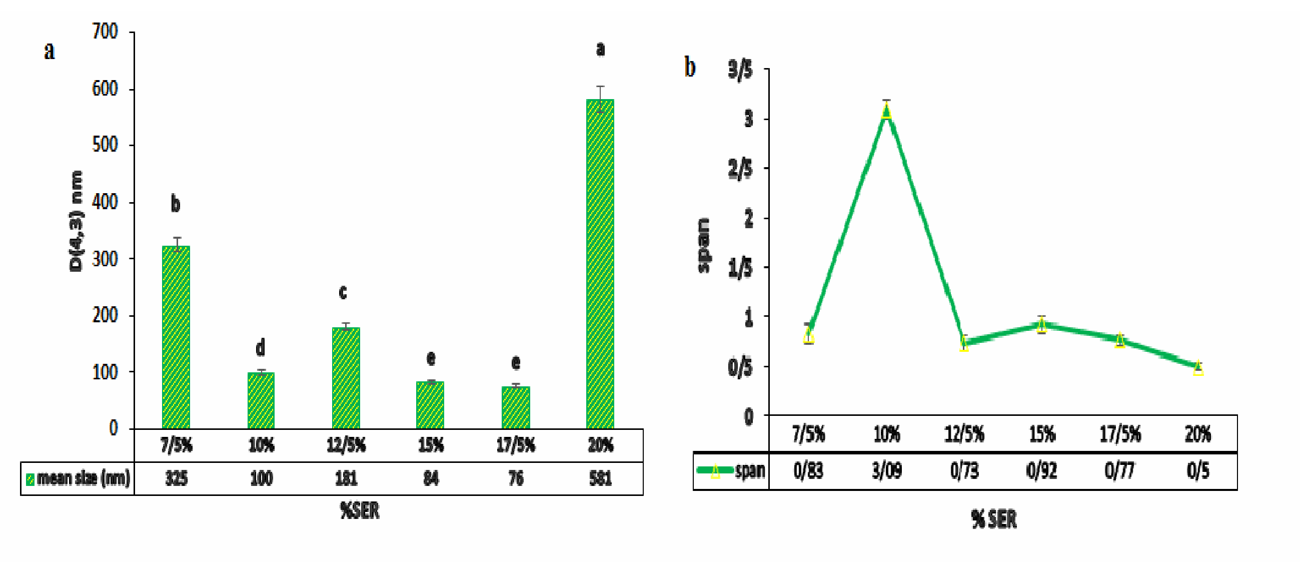


Figure.2 SEM morphology of beta carotene nanoemulsion

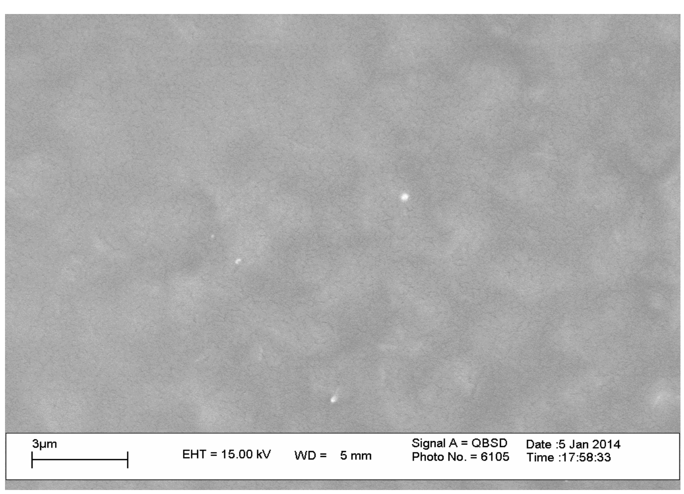


Figure.3 Effect of surfactant type on the (a) particle size and (b) particles size distribution of beta carotene nanoemulsion

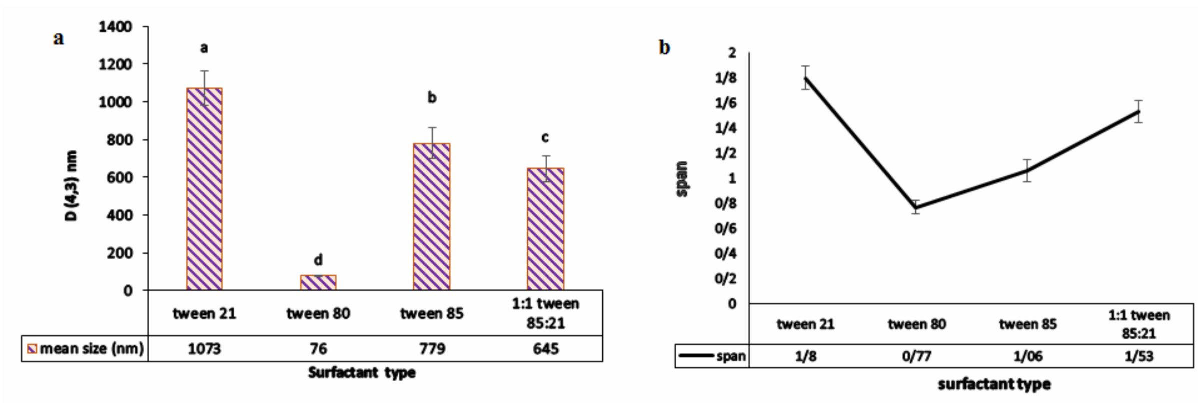


Figure.4 Stability of particle size of optimized formulation of beta carotene nanoemulsion. (a) particle size and (b) particles size distribution of beta carotene nanoemulsion

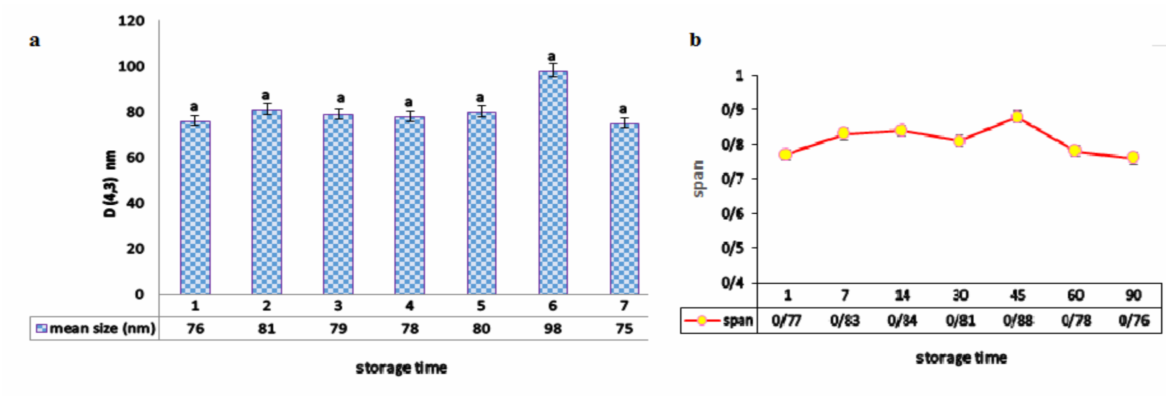
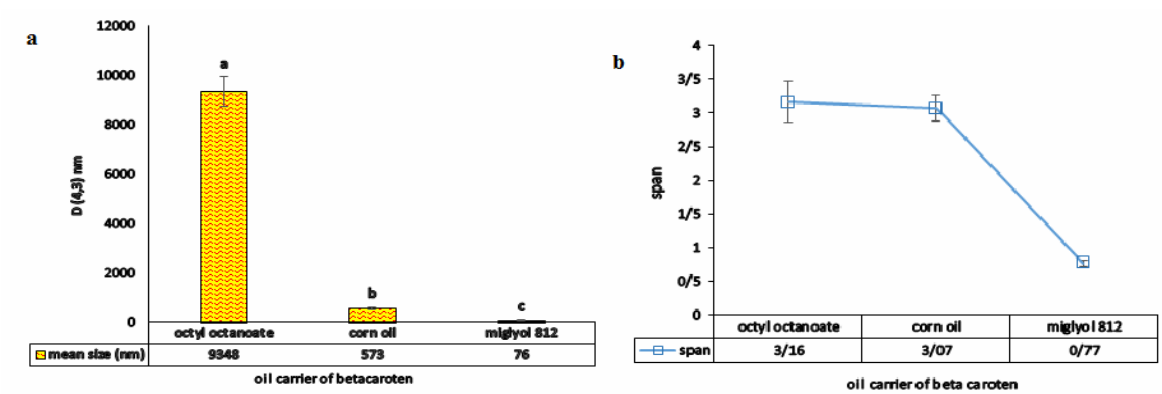


Figure.5 Effect of carrier oil type on the (a) particle size and (b) particles size distribution of beta carotene nanoemulsion



Generally, below the size of 300 nm, the rate of gravitational separation greatly reduces. However in the nanoemulsions, due to the increased surface contact of droplets and thus increased solubility in water, unsteady Ostwald ripening can occur more quickly. The density difference between aqueous and oily phase causes the sedimentation of droplets (McClements, 2004) which was reduced further by the addition of surfactants with higher densities. In addition the size of the droplets depends on interfacial tension. In low interfacial tension, smaller droplets are produced.

Influence of the Oil Carrier Phase

In the preparation of the emulsions of vitamin A derivatives, selection of the type of oil is very important because the oxidative stability of the encapsulated retinoids are affected by the physical properties (interfacial layer thickness, size and shape of the droplets of the dispersed phase) and chemical properties (the saturation degree, chain length and the presence of natural antioxidants) (Fathi *et al.,* 2012). As shown in figure 5, in all samples there is significant difference between size of droplets when different oil carriers are used, ($P<0.05$) and using Mygliol 812 as carrier oil compared to other oil phases, had significant reduction in the size of the nanoemulsion droplets. Mygliol 812 belongs to medium chain triglycerides (MCT) groups and it is a unique class of saturated lipids composed mainly of caprylic (C8:0; 50–80%) and capric (C10:0; 20–50%), fatty acids with a minor level of caproic (C6:0; $\leq$ 2%), lauric (C12:0; $\leq$ 3%) and myristic (C14:0; $\leq$ 1%) fatty acids; they are also considered as emulsifier and suspender rather than a solver (Rowe *et al.,* 2009). They are odourless. Viscosity of the oil is effective on the move of the organic phase to the aqueous phase, and with reducing the viscosity of oil, surfactant molecules move faster and therefore produce smaller droplets (Israelachivilbi, 2011).

Hu *et al.* (2005), produced nanoemulsion using a mixture of stearic acid and oleic acid and reported that using of the elevated levels of oleic acid, produces softer particles with low crystalline property. In some researches, no relation has been found between the average diameters of the generated droplets by the properties of the oil phase. Properties of the surfactant-oil-water system are more important than the properties of pure oil (Saberi *et al.,* 2013). Properties of the oil phase, aqueous phase (e.g. presence of salts and alcohols) and environmental factors (such as temperature) effect on the relative affinity of the surfactant molecules to the oil and water phase (Rao McClements, 2012).

Conclusion

The factors affecting droplet size of nanoemulsion contain betacarotene, include the type and concentration of surfactant and type of carrier oil. The using of tween 80 and mygliol 812 as surfactant and carrier oil, among nonionic surfactants and a variety of oil phases, produced the smallest droplet sizes in beta carotene nanoemulsion. Also, droplet size of nanoemulsion was affected by the surfactant concentration, and there was SER=17.5% as an optimum surfactant concentration. In nanoemulsion produced using nonionic surfactant (tween 80) and 812 Mygliol (oil carrier phase) initially by increment of the concentration up to SOR =175% and SER = 17.5%, the droplet size was decreased but after passing a special value, by increasing the surfactant concentration the average droplet diameter was increased. Optimal nanoemulsion had a high stability during 90 days of storage.

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