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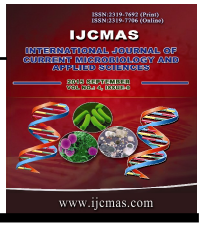

Original Research Article

Effect of Surfactant Concentration on the Particle Size, Stability and Potential Zeta of Beta carotene Nano Lipid Carrier

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Zeta potential,
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Stability,
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A B S T R A C T

Nutricitical compounds encapsulation by lipid nano dispersions (NLCs) is an effective way to prevent their dissolution and inactivation during production and storage. The objectives of the present study are to prepare and produce beta carotene -contained NLCs, and survey the effect of surfactant concentration on the particle size, stability and potential zeta of beta carotene NLC. Colloidal nano carriers of beta carotene were produced using an optimal method of Hot-High shear Homogenizer (Hot-HSH) method, which was developed after testing various conditions. Octyl octanoate as liquid oil-phase carrier, compritol ATO888 as solid lipid and 10:1 solid lipid/oil phase ratio were used. The results showed the least particle size in a formulation containing 3% Poloxamer 407 solution, and proposed that an optimum concentration of 3% of Poloxamer 407 was sufficient to cover the surface of nanoparticles efficiently. The optimal sample remained stable after 60 days of storage at 25°C. Zeta potential analysis confirmed no significant difference among samples with different concentration of surfactant. The production of beta carotene-contained NLCs was an efficacious method in terms of encapsulation, nanoscale particle size decrease, stability within time, and other expected specifications.

Introduction

There are different strategies for prevention of degradation of vitamin A and its procure i.e. beta carotene from light, oxygen and environment conditions, so it is of interest highly lipophilic compounds that must be integrated into a suitable colloidal delivery system before it can be distributed into an aqueous-based beverage (Hentschel-Test, 2008). The encapsulation of lipophilic nutraceutical compounds like beta carotene in lipid-based carrier systems has different advantage. For example, controlled release to its target site at the right period of time, prevention of degradation and inactivation during process, storage, higher bioavailability due to higher ratio of surface area to volume and no decrease in transparency because of little size of

particle. Beta carotene would therefore benefit from an encapsulation procedure that increases its solubility in an aqueous medium such as beverages and skim milk, slows down the degradation processes and /or prevents its degradation until the product is delivered at the sites where absorption is desired. Technologies that improve the stability of vitamin A and its procure i.e. beta carotene in foods are vital for ensuring the safety and efficiency of the vitamin A fortification of foods (Loveday and Singh, 2008). In parallel, attention must be paid to the encapsulation efficiency i.e. the amount of compound really incorporated into the structure vs the initial amount of it. There is therefore a need to understand how to formulate effective colloidal delivery systems from food-grade constituents using economic processing conditions. These delivery systems must have right physicochemical and sensory properties for the product they are incorporated in to (e.g., optical clarity, flavor profile, rheology), and they must remain stable throughout the shelf-life of the product (Mcclements *et al.*, 2012; Ziani *et al.,* 2012).

NLC are derived from o/w emulsions by replacing the liquid lipid (oil) by a solid lipid. The particles have a controlled nanostructure that offers enough space to accommodate the bioactive compounds (Das and Tan, 2012; Tamjidi *et al.,* 2013). Thus, NLC are a promising delivery system for food application of a lipophilic nutraceutical, may increase their stability, bioavailability and dispersibility in aqueous media for fortifying food products especially aqueous ones (Tamjidi *et al.,* 2013). In recent years many researches have been carried out to investigate the use of NLC variety of different oils and surfactants may be used to form nano lipid carrier in the food and beverage industries (Tamjidi *et al.,* 2013; Lacatusu *et al.,* 2012; Jenning and Gohla, 2001). The aim of this study was to investigate the factors influencing the production and stability the particles of beta carotene nanolipid carrier for reaching to optimal size of droplets and stability of beta carotene NLC for use in food and beverages fortification.

Experimental section

Material

Compritol -ATO888 (Glyceryl behenate) was kindly donated from Gattefossè (Saint Periest Cedex, France). Beta carotene, Poloxamer 407 and octyl octanoat was provided from Sigma Aldrich (Steinheim, Germany).

Methods

Preparation of nano dispersions containing beta carotenee (NLC)

Initially NLC containing beta carotene was prepared by Hot-High shear Homogenizer (Hot-HSH) method in which particle size is reduced by cavitation, high shear forces and particle collision in and after leaving the homogenizing gap (=high energy technique) (Shegokar and Muller, 2010). For this purpose, beta carotene was liquefied in liquid oil (octyl octanoat) and then the mixture was added into melted solid lipid (Compritol) and was heated inside a hot water bath to a temperature above the melting point of the solid lipid (85°C). Then, the hot aqueous surfactant (ploxamer 407) solution (in the same temperature of melted mixture of lipids) was added gradually into the lipid phase under homogenization (Silent crusher M, Heidolph, Nuremberg, Germany) at 20000 rpm for 30 minutes. The initial product of the hot homogenation was an o/w nanoemulsion due to using liquid oil. The produced hot o/w nanoemulsion was cold

down in the ambient or lower temperature resulting in the lipid phase recrystallization and finally the NLC were formed. Keeping temperature stable and concordant during preparation of the two phases, as well as at the time of NLC production is extremely critical. In this study, optimal formulation for producing formulation of this colloidal nano carrier was investigated by changing concentration of the aqueous surfactant three times as listed in table 1.

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).

Determination of lipid nanodispersion stability

Long-term stability of the optimal lipid nano dispersion was investigated through tracing particle size change and the product physical appearance during preserving at ambient temperature (25°C) for a period of 60 days (Yang *et al.,* 2014).

The employed tests included measuring of the stability of laboratory Nano Lipid Carriers through determining average particle size, span value and the endurance of lipid nano dispersions in keeping the beta carotene inside within 2 months after production (Gonnet *et al.*, 2010).

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

The effect of aqueous-phase surfactant concentration on the production of lipid nanodispersion particles

The effect of aqueous-phase surfactant concentration on the average size of the particles, and particles size distribution (span) of lipid nanodispersions are showed in figure 1a and shown in figure 1b. The particle size and particles size distribution (polydispercity) of colloidal systems such as lipid nanodispersions play important role in determining their specifications. Stability of these two parameters in a long period of time indicates the stability of a system. In this study various concentrations of aqueous-phase surfactant (ploamer 407: 2, 3, 4 and 6) were used to produce beta carotene

contained lipid nanodispersions with appropriate particle size in nanoscale. The average size of the particles and particles size distribution (span) of lipid nanodispersions were in the range of 79–115 nm and 0.71–1.01, respectively. In addition, the effect of independent variable (different concentration of aqueous-phase surfactant) on the lipid size was investigated and showed significant difference between the size of particles ($p<0.05$). In conventional emulsions, the type of used emulsifier determine the final size of particles and the stability of dispersion through providing enough repelling forces that prohibit aggregation and mixing of the particles. In addition, the used surfactant in lipid nanocarriers plays a more important role in controlling the process of crystallization. The choice of stabilizers is a key subject to be considered in preparation of any nanoparticle formulation to control the particle size and stabilization of the dispersions (Hu *et al.,* 2005). Poloxamer is a hydrophilic non ionic surfactant and block copolymer of polyethylene oxide (PEO) and polypropylene oxide (PPO). The hydrophobic PPO chains adsorb on the particle surfaces as the "anchor chain", while the hydrophilic PEO chains pull out from the surface to the aqueous medium, creating a stabilizer layer (Shegokar and Muller, 2010). If particle–particle collisions happen faster than surfactant molecules absorption rate to cover the exposed hydrophobic patches, then widespread particle accumulation will happen due to hydrophobic attraction between the particles (Das and Tan, 2012; Tamjidi *et al.,* 2013). Perhaps, Poloxamer could not sharply cover the newly formed lipid particles and may not adsorb quickly enough to the surface of lipids particles relative to the rate of particle–particle collisions due to its huge structure.

The surfactant concentration also affects the final size of the particles. High surfactant concentration decreases surface tension and stabilizes newly developed surfaces during homogenation and production of smaller particles (Mcclements, 2012). Likewise, low and insufficient amount of surfactant in lipid nanocarrier structures cause instability and recrystallization (Helgason *et al.,* 2009). For example, alpha crystalline structures, which are thermodynamically unstable, transform to beta forms. This transformation is along with morphological alterations; i.e. beta crystals may undergo direct growth and forming needleshape structures, with increasing surfaces in contact. If these newly developed surfaces are not stabilized by surfactant molecules, hydrophobic interactions may lead to aggregation and instability of lipid suspensions (Weiss *et al.,* 2008). The influence of an increasing Poloxamer concentrations ranging from 2% (w/w) to 6% (w/w) on the particle size and physical stability was studied.

The Effect of Surfactant concentration on stability of particles

In figure 3, the effect of different concentration of ploxamer on the size of the particles in NLCs prepared with the optimized formulation (formulation containing 3% w/v poloxamer 407 solution, compritrol 3% (w/v) and beta carotene - contained oil-phase octyl octanoate (0.03%w/v)) is shown. The range of particles size in colloidal systems should be narrow and under 1 micron. Narrower range of particles size minimizes the gradient between active agent concentration and the surrounding environment. As a result, the Ostwald ripening phenomenon, which describes the redeposit of small crystals onto larger crystals, is inhibited (Wu *et al.*, 2011). In the present study all the preparations had narrow and homogenous range of particle

size after 60 days of storage, except for the first preparation which had inhomogeneous particle size distribution after 30 days of storage and showed biggest increase in particle size comparing with other preparations. Considering the small size of produced particles, the number of lipid molecules that interact with the hydrophobic group of the emulsifier to balance and regulate the process of crystallization is sufficient. In addition, the surfactant is effective in synthetic stability of the crystalline structure and prevents recrystallization during storage (Bunjes *et al.*, 2002) Usually a combination of two groups of nonionic surfactants are required to regulate and balance crystallization and ionic surfactants in providing repelling forces and concomitant maintenance of particle stabilization and crystallization balance (Jenning and Gohla, 2001).

Table.1 Composition of beta carotenee-loaded nanostructured lipid carriers

Formulation	Solid Lipid (w/v)	Liquid Lipid (w/v)	Surfactant (w/v)
F1	Compritol (3%)	Octyl Octanoate (0.3%)	Poloxamer (2%)
F2	Compritol (3%)	Octyl Octanoate (0.3%)	Poloxamer (3%)
F3	Compritol (3%)	Octyl Octanoate (0.3%)	Poloxamer (4%)
F4	Compritol (3%)	Octyl Octanoate (0.3%)	Poloxamer (6%)

Table.2 Comparison of the stability mechanism of electrostatic and steric repulsion in stabilizing food emulsions and NLCs

parameters	***Repulsion***	
	Electrostatic repulsion	***Steric repulsion***
1	pH sensitive tend to aggrigation near the IP point of biopolymer	Insensitive to the pH
2	Tend to aggregation in high concentration of the electrolyte	Insensitive telectrolyteo
3	Low concentration of emulsifier requires to drop coverage	Droplet surface coverage with a high concentration of emulsifier
4	Strong aggregation	Weak aggregation

Table.3 Zeta potential of NLCs without and with beta carotene

Concentration of water surfactant (w/v%)	**Zeta potential (mv)**	
	Without beta carotene	**With beta carotene**
2	0.568	0.29
3	-0.342	0.276
4	0.42	0.38

Figure.1 Effect of surfactant concentration on the (a) particle size and (b) particle size distribution of beta carotene NLC

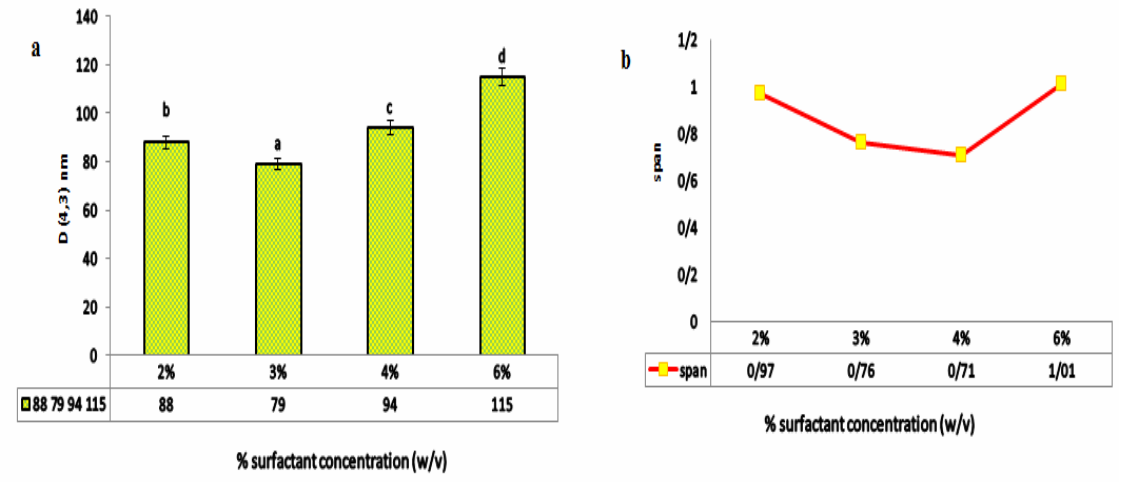


Figure.2 stability of particle size of (a) particle size and (b) particles size distribution optimized formulation of beta carotene NLC (formulation containing 3% w/v poloxamer 407 solution, compritol 3% (w/v) and beta carotene -contained oil-phase octyl octanoate (0.03%w/v))

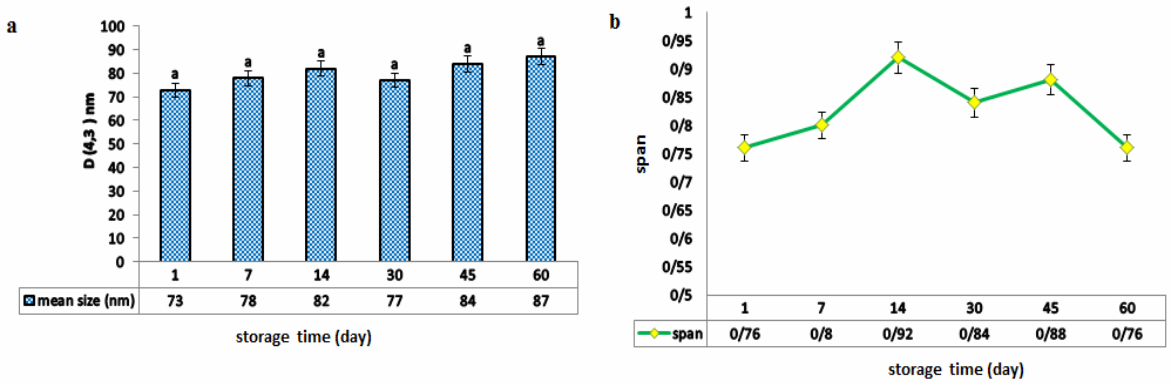
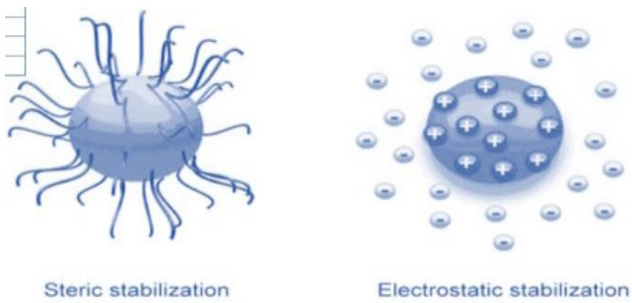


Figure.3 Steric and electrostatic repulsion around particles in a colloidal system



Zeta Potential

Colloidal systems such as NLC can be stable by creating electrostatic and steric repulsion between particles (Figure 3). In table 2 some properties of both electrostatic and steric repulsion is mentioned. The steric interaction is dependent on the separation distance between the internal aqueous droplets and the external aqueous phase, the thicknesses of the two adsorbed surfactant layers, the size of the internal aqueous droplets and the oil globules, all of which determine the extent of the compression of the adsorbed surfactant molecules. The thickness of each of the two surfactant layers have the same effect on the steric repulsion, and stronger steric interaction can be achieved with thicker adsorbed layers, which can effectively prevent coalescence between the internal aqueous droplets and the external aqueous phase. Increasing the internal aqueous droplet size can produce stronger steric repulsion; however, larger oil globules will weaken the steric repulsion, indicating that a more stable double-emulsion system can be achieved by preparing the system with smaller oil globules and larger internal aqueous droplets.

In this study, to determine how electrostatic repulsion forces within nanostructured lipid carrier particles act and the stability of the resulting system, the results of electrophoretic mobility and zeta potential of formulations with different concentrations of surfactants are presented in table 3. As it is identified in the table, with respect to the nonionic nature of aqueous phase surfactant the (poloxamer 407) as well as other constituents of the system, the zeta potential of samples were very small (close to zero) and increased concentration of surfactant did not have significant effect on increasing or decreasing the potential zeta of different samples. Having in mind the polymeric and bulky structure of poloxamer 407, the stability of the nanoparticles within the storage time could be due to the steric repulsion of the surfactant molecules in this system and in this system the electrostatic repulsion of the particles did not play a significant role in sustainability. Polyhydroxy surfactants, stabilize systems by creating spatial exclusion and due to their non-ionic nature, low and zero zeta potential would be obtained (Kovacevic *et al.,* 2011; Kovacevic *et al.,* 2014; Ghosh *et al.,* 2011). Torres *et al.* (2003) Stated that the stability of nano lipid carrier against aggregation is influenced by the ionic strength of the continuous phase and the charge density on the surface of the water and fat. High zeta potential along with the non-electrostatic agents such as steric forces has also an important impact on the stability.

In this study, we prepared a stable beta carotene bearing NLC and studied the effect of surfactant concentration on the final product and characterizations of the NLC (particle size, stability and potential zeta). According to obtained results, a certain surfactant concentration is required to produce stable nano structured lipid carriers during time. The results propose that an optimum concentration of 3% of Poloxamer was sufficient to cover the surface of nanoparticles efficiently and avoid collection during the homogenization process while there was statistically difference among the results of 2, 3, 4 and 6 % Poloxamer. Adequate concentration of surfactant induced surfaces well covered and aggregation among particles reduces. Also lipophilic nature of beta carotene preventing removal of active substance from lipid matrix of nano particle.

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