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Research Article

Encapsulation of Vitamin A Palmitate in Nanostructured Lipid Carrier (NLC)-Effect of Surfactant Concentration on the Formulation Properties

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Abstract

Purpose: Nanostructured lipid carrier (NLC) is a useful delivery system with high capacity for bioactive loading, and suitable stability for fortification of foods and beverages. The objective of the present work is to prepare vitamin A palmitate (vitA)-loaded NLC and to investigate surfactant concentration effect on physicochemical properties of prepared formulation.

Methods: VitA-loaded NLC was prepared by hot homogenization method. Preciol as solid lipid, Octyloctanoate as liquid oil, and Poloxamer as surfactant were used to prepare the formulation. The effect of different concentrations of Poloxamer on particle size, particle size distribution, encapsulation efficiency and stability of vitA during storage were investigated. Fourier transform infrared spectra (FTIR) was exploited to study the possible bioactive-lipid complex formation.

Results: NLCs stabilized with 6% Poloxamer showed significantly lower particle size and particle size distribution. The encapsulation efficiency of this formulation was 98.5% and it was stable during the storage at 25°C for two months.

***Conclusion*:** This study suggests that surfactant significantly influences the final product properties. Our findings may pave the way of researchers to focus on fortifying the beverages with various lipophilic nutraceuticals.

Introduction

Vitamin A palmitate (vit A) is a fat-soluble vitamin, which plays a vital biological role in human body. Daily requirement of this vitamin in adults is 900 µg in average. Vit A is liable to degradation by ambient condition like proxidant agents, heavy metals, free radical-producing agents, and light. Bioactive materials such as vitA are regularly supplemented in functional food formulations to provide desirable health benefits. However, fortification is not straightforward, since these compounds are barely soluble in aqueous systems such as beverages, because of low polarity.[1-3] The dispersibility and stability of vitamin A can be improved by incorporating colloidal nano lipid carriers.[4,5] Lipid-based nanocarriers in food industry include nanospheres, nanoliposomes, nanoemulsions, niosomes and lipid nanoparticles with solid structures comprising Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC).[6,7] In the last decade, SLNs developed as an alternative system to the existing traditional carriers (emulsions, liposomes and polymeric nanoparticles) which have advantages including good stability, biocompatibility, precise targeting, good preservation of encapsulated bioactive molecules, sustained bioactive release, ease of scale-up methods and low cost. However, there are several problems for SLNs, such as limitation in bioactive loading capacity and bioactive expulsion during storage. Therefore, in order to solve these limitations, nanostructured lipid carriers (NLCs) have been developed in recent years. NLCs are created by controlled mixing of solid lipids with incompatible liquid oil.[3,7,8] Thus, NLCs are a promising delivery system for food application of lipophilic nutraceuticals, which may increase their stability, bioavailability and dispersibility in aqueous media e.g. beverages.[9,10] In recent years, numerous studies have been carried out to enrich foods with vitamins and nutricitical compounds using lipid nano dispersions.[11,12] The principal aim of this study was to prepare vitA-loaded NLC formulation to improve aqueous dispersibility of vitA. Additionally, the effect of surfactant concentrations on particle size, particle size distribution, encapsulation efficiency (EE) and physical stability of vitA loaded NLCs were investigated to get the optimized formulation.

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Materials and Methods

Materials

Precirol ATO 5 (Glyceryl distearate) was kindly donated from Gattefossè (Saint Periest Cedex, France). Miglyol 812 (caprylic/capric triglycerides) was provided from Sasol (Witten, Germany). VitA, Poloxamer 407 and Octyloctanoat were provided from Sigma Aldrich (Steinheim, Germany).

Preparation of NLCs containing vit A

VitA loaded NLCs were prepared by hot hemogenation method in which particle size is reduced by cavitation, high shear forces and particle collision in and after leaving the homogenizing gap (Figure 1).[13] For this purpose, vitA was dissolved in liquid oil (Octyloctanoat) and the mixture was added into melted solid lipid (Precirol). Then, the hot aqueous surfactant solution (at the same temperature with melted lipids) was added gradually into the lipid phase under homogenization (Silent crusher M, Heidolph, Nuremberg, Germany) at 20000 rpm for 45 minutes. 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 was formed. In this study, optimal formulation for vitA loaded NLC was investigated by changing concentrations of the aqueous surfactant as listed in Table 1.

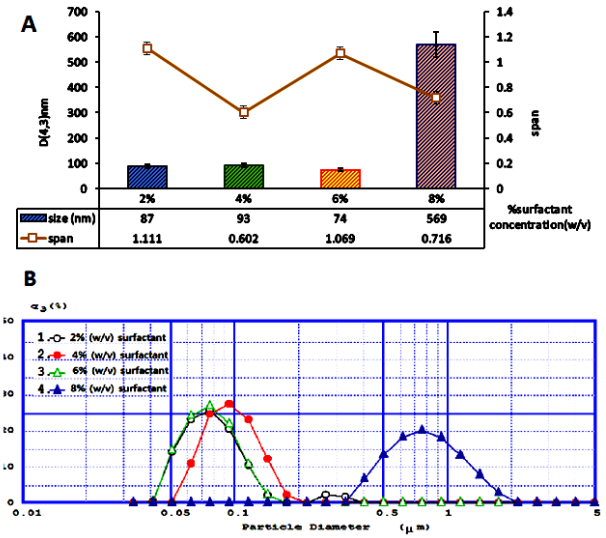

Figure 1. Effect of surfactant concentrations on the particle size (A) and particles size distribution (B) of vitA loaded NLC

Table 1. Composition of VitA palmitate-loaded nanostructured lipid carriers

Formulation	Solid Lipid (w/v)	Liquid Lipid (w/v)	Surfactant (w/v)
F1	Precirol (6%)	Octyl (0.6%)	Poloxamer (2%)
F2	Precirol (6%)	Octyl (0.6%)	Poloxamer (4%)
F3	Precirol (6%)	Octyl (0.6%)	Poloxamer (6%)
F4	Precirol (6%)	Octyl (0.6%)	Poloxamer (8%)

Particle size analysis

The average diameter and Span value of the formulations were determined using particle size analyzer (Wing SALD 2101, Shimadzo, Japan), at 25°C. The NLC dispersion was diluted with distilled water until suitable obscuration to prevent multiple scattering phenomena because of inter particle interactions. The average particle size was calculated according to the average volume diameter or DeBroukere mean in the equation below:

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

The Span value is an index helpful to evaluate the particle size distribution and is calculated applying the following equation:[14]

$$Span = \frac{D_{90\%} - D_{10\%}}{D_{50\%}}$$

Encapsulation Efficiency (EE)

Estimation of the loaded amounts of VitA into NLCs was carried out using HPLC (Knauer, Germany) equipped with a UV detector. Detection was carried out at 325 nm. Column used was C-18 (10µm 25mm×4.6 mm). The mobile phase consisted of Acetonitrile-methanol (70:30%, v/v) and the flow rate was set at 2.0 ml/min. HPLC linearity was calculated using various concentrations of vitA (0.5, 1, 2, 3, 4 µl) in each ml of the mobile phase. To extract lipid nanodispersion solution for injecting into the HPLC machine, first the samples were gently centrifuged (600 rpm, 10min) and then 0.5 ml of chloroform was added to one ml of transparent lipid nanodispersion, and finally the product was shaken for 15 min. Then the chloroform phase including encapsulated vitamin was extracted and dried using nitrogen gas. In the last stage, 0.5 ml of the mobile phase was added and the EE was calculated using the following formula:

$$\text{EE}\,(\%) = \frac{\text{Amount of vitA incorporated into NLC}}{\text{Amount of total vitA}} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

The infrared spectra were scanned on a FTIR spectrophotometer (Shimadzo, Japan), at 4 cm^{-1} resolution in frequency range between 4000 and 400 cm^{-1} using KBr Pellet method with the sample:KBr ratio of 1:10.

Determination of the physical stability of lipid nanodispersion

To determine the physical stability of colloidal nano carriers during storage, formulations with various concentrations of aqueous surfactant (2, 4 and 6% w/v) were studied. The stability of VitA loaded-NLC was assessed by determining the particle mean diameter and studying the leak out of the vitamin from

the NLC during two months (1, 15, 30 and 60 days) of storage at ambient temperature (25°C) by the follow:[15]

$$Stability = \frac{\text{Remaining amount of vitA incorporated in NLC}}{\text{Initial amount of vitA incorporated into NLC}} \times 100$$

Statistical analysis

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

Results and Discussion

The effect of surfactant concentrations on the particle size and particle size distribution

The crucial role of the surfactant is stabilization of the nanoparticles in the colloidal systems and avoidance of particle size growth during storage.[16] Poloxamer 407 is a hydrophilic nonionic surfactant with HLB number 22 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, producing a stabilizer layer.[17] The effects of increasing Poloxamer concentrations ranging from 2% (w/v) to 8% (w/v) on the particle size and particles size distribution (span value) of NLCs based on 10: 1 solid lipid (Precirol) to liquid oil (Octyloctanoate) ratio are shown in Figure 1. The average particle size and particles size distribution (span) of NLC formulations were in the range of 74-779 nm and 0.60-1.06, respectively. Increasing surfactant concentration from 2% to 6% resulted in a decrease in the size of particles, but only the formulation prepared with 6% Poloxamer was stable over theobservation period of 2 months, whereas other formulations that stabilized with 2% and 4% Poloxamer concentrations showed significant increases in particle size and span value during storage. Further increase in Poloxamer concentration from 6% to 8% (w/v) showed significant increase in particle size and span value and the particle size increased significantly after storage for 2 months at 25°C. As the surfactant decreases the interfacial tension among the lipid matrix and the aqueous phase of the lipid nanoparticles, the surfactant nature (Poloxamer is a nonionic surfactant and causes further steric stabilization effect, preventing aggregation of the particles in the colloidal system) and concentration of the surfactant is the main key for the production of smaller particles, covering the tiny lipid droplets' surfaces, and consequently preventing the coalescence of the droplets and preserving lipid nanoparticles' physical stability.[18] Generally, nanoparticles prepared at low surfactant concentrations, owing to insufficient surfactants to cover the new hydrophobic surfaces generated upon solidification, causes inadequate repulsion between particles and consequently causes instability during storage.[19] On the other hand, increasing surfactant concentration resulted in a significant increase in the particle size and span value. Increasing the surfactant concentration caused an increase in size report due to the probably depletion flocculation of micelles which have been formed by further molecules of surfactant remained in contentious phase.[20] Therefore, a certain level of surfactant concentration is necessary to yield a stable system. However, after a certain amount of surfactant concentration, particle size and stability did not improve. Other researches also observed the reduction in particle size at higher surfactant concentrations.[21,22] These results clearly suggest that an optimal concentration of 6% (w/v) of Poloxamer was effective in producing smaller particle sizes, covering the surface of nanoparticles efficiently, and consequently avoiding collection during storage.

Fourier transform infrared spectroscopy (FTIR)

In this study, we used FTIR spectroscopy to determine any active material-excipient interaction at the level of functional groups. In order to estimate any possible chemical interaction among the vitamin and the carriers, FTIR spectra of Poloxamer, Precirol, Octyloctanoate, vitA and NLCs formulations were studied as shown in Figure 2. The FTIR spectrum of the pure vitA showed the absorption bands at 2800-3200 cm^{-1} (free CH3 stretching), 1700 cm^{-1} (ester stretching), 1600-1620 cm^{-1} (aromatic cyclohexene stretching) and 1100 cm^{-1} (carbonyl stretching). All the peaks related to functional groups (hydroxyl, methyl, ester) of vitA present in FTIR spectra of blank NLC and vitA loaded NLC, except the peak related to aromatic cyclohexene stretching that only existed in the FTIR spectrum of vitA loaded NLC ,indicating vitA entrapment in lipid matrix. As all the characteristic peaks of the vitamin and the carriers were present in the vitamin loaded NLC spectrum and no predominate shifting of existing peaks or creation of new peaks were found, this suggests that there were physical interactions only among the vitamin and excipients and no chemical interaction took place among them. Similar results were also observed for other drugs. Fathi and Varshosaz[9] studied on Hesperetin loaded NLCs, which were coated with different biopolymers (chitosan, alginate, and low methoxypectin).Their FTIR analysis revealed encapsulation of hesperetin in used encapsulating compounds and no chemical reaction between encapsulating compound and hesperetin.

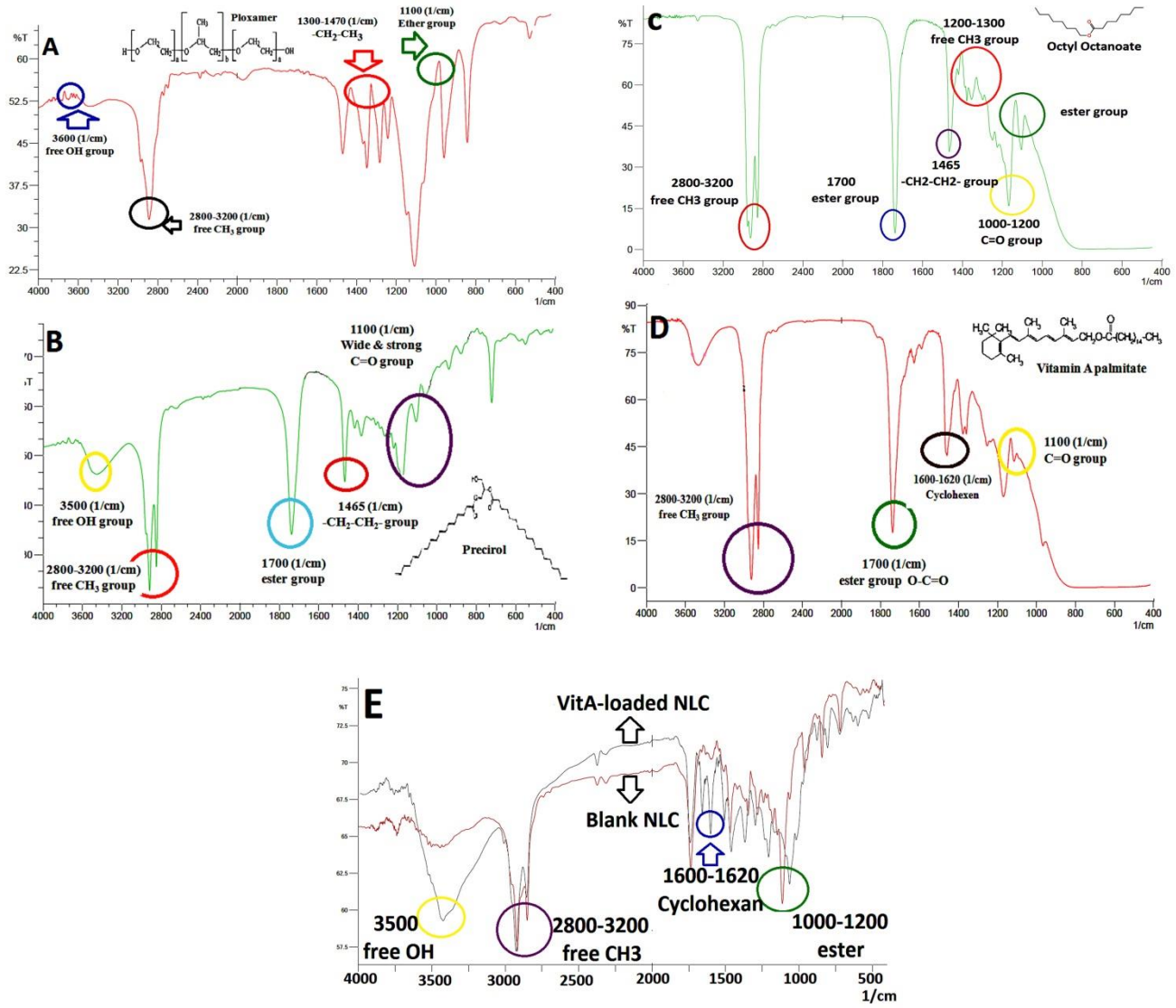

Figure 2. FTIR spectra of Poloxamer (A), Precirol (B), Octyloctanoate (C), VitA (D), blank NLC & vitA loaded NLC (E)

The effect of surfactant concentration on EE and stability of vitA loaded NLC

The EE is associated with the specification of encapsulated material (lipophilic or hydrophilic active agent and tendency for interaction with nanocarrier structure), the nanocarrier constituents, and the environmental factors (pH and temperature). The EE of various NLCs prepared by different surfactant concentrations (2-6% w/v) was measured by HPLC (Figure 3). As shown in the Figure 3, NLCs prepared at 6% Poloxamer concentration exhibited highest EE of 98.6% and other NLCs exhibited EEs higher than 80%. This is due to the high solubility of vitA in the oil (Octyloctanoate) and in the solid lipid (Precirol) and low solubility in water, as well. Figure 4, shows the percentage of vitA remaining in the NLCs, which was stabilized using different concentrations of Poloxamer (2-6%w/v) at room temperature (25 °C). After 60 days of storage, the highest percentage of vitA, was observed in NLCs stabilized with 6% Poloxamer and this amount did not cause a significant change within the initial amount of encapsulated vitA during storage time, whereas other formulations that stabilized with 2% and 4% Poloxamer, had significant leakages of vitA over the period of storage ($p < 0.05$). Using one way ANOVA at the significant level of 0.05, it was found that the stability of vitA Palmitate loaded NLC depended significantly on the concentration of the surfactant ($p < 0.05$). This effect can be attributed to the fact that the solid particles are non-spherical (e.g., disc-like or needle-like) and require higher amounts of surfactant to cover the new surfaces of solid particles. Indeed, at higher surfactant concentrations, enough surfactant was present to cover the tiny lipid, which resulted in stabilization of the particles and prevention from their coalescence, thus the encapsulated material was maintained in the nano lipid carrier. As a result of previous studies, leakage of lipophilic active agent from its nano dispersion structure is prohibited. Das et al[7] and Leo et al[21] showed that

increasing surfactant concentrations were accompanied with higher EEs of nano carrier systems.

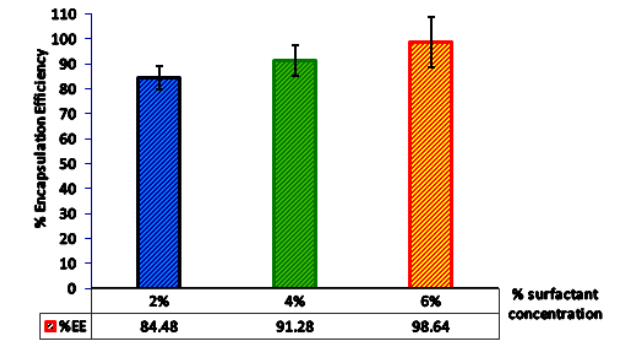

Figure 3. The effect of surfactant concentrations on encapsulation efficiency of Vitamin A Palmitate loaded NLC

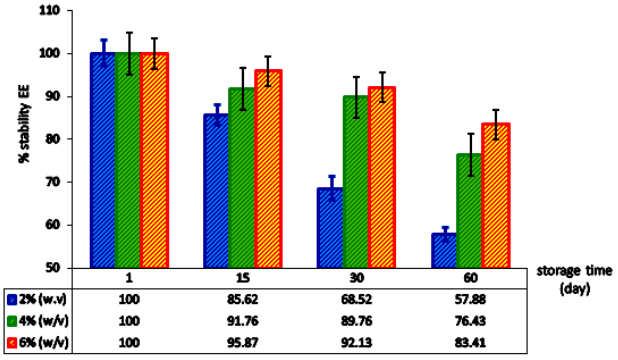

Figure 4. The effect of surfactant concentrations on stability of vitA loaded NLCs during storage at 25°C

Conclusion

In this study, we prepared a stable NLC and studied the effect of surfactant concentration on the final product and characterizations of the NLC. The study suggests that controlling the critical formulation and its processing factors, significantly influences the final product characteristics, like particle size, polydispersity index, and bioactive EE. Increasing the Poloxamer concentration up to 6% resulted in a decrease in particle sizes and particle size distributions and this formulation was stable over the period of storage, but further increases in concentration of Poloxamer had a negative effect on the particle size and the span value. According to obtained results, a certain surfactant concentration is required to produce stable nano structured lipid carriers. Investigations on the bioactive–lipid interactions using FTIR spectroscopy confirmed the incorporation of vitA in the applied encapsulating materials and the formation of physical interactions between vitA and encapsulating materials.

Conflict of Interest

There is no conflict of interest to be reported.

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