Production of Novel Vitamin E Loaded Nanostructure Lipid Carriers and Lipid Nanocapsules for Milk Fortification

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ABSTRACT

In this research, novel vitamin E loaded Nanostructured Lipid Carriers (NLCs) and Lipid Nanocapsules (LNCs) were produced and their physicochemical properties were characterized. The optimum ratio of liquid to solid lipid and vitamin to total formulations were determined. Particle size, polydispersity index, zeta potential, encapsulation efficiency and encapsulation load of optimum formulations were evaluated. Optimized formulations had encapsulation efficiencies of 95 and 99% for NLC and LNC, respectively. X-ray diffraction results indicated a new crystalline lattice with lower degree of crystallinity for vitamin E nanocarriers in comparison to bulk fats because of curvature effects. Fourier transforms infrared spectroscopy showed that there were no adverse reactions between vitamin E and lipids. Release profile and kinetic modeling were investigated in gastrointestinal media that showed developed nanocarriers can protect vitamin E against acidic condition by decreasing its release in gastric media (release percentage of 29 and 4% for NLC and LNC in gastric media, respectively). Milk was fortified with vitamin E loaded nanocarriers and its sensory evaluation indicated their potential application for production of functional food.

Keywords: Encapsulation efficiency, Functional food, Polydispersity index, Zeta potential.

INTRODUCTION

The term vitamin E refers to a group of fat soluble vitamins (four tocopherols and four tocotrienols) that, due to its promising therapeutic potential and nutritional roles, has been widely used as a functional ingredient in food, pharmaceutical and cosmetic products. Alpha-tocopherol molecule can trap two peroxyl radicals responsible for lipid oxidation initiation. In addition, there are evidences that show vitamin E has positive effects on cardiovascular disease (Pham and Plakogiannis, 2005), cancer prevention (Yang and McClements, 2013a), the immune system (Maggini, Wintergerst *et al.*, 2007) and slowing down the aging process by protection of membrane lipids against oxidation (Khayata *et al.*, 2012). Despite numerous benefits, α-tocopherol is highly unstable to unfavorable environmental conditions in many food products (i.e. oxidation, high temperatures, pH and UV-light) and may therefore be lost during processing, storage and utilization (Yang and McClements, 2013b). Moreover, poor water solubility of vitamin E has limited its food applications. Therefore, it must be incorporated into an appropriate colloidal delivery system. Some studies reported that bioavailability of vitamin E may be increased when it is delivered by a colloidal nano range system (Ziani *et al.*, 2012). Recently, increasing attentions have been dedicated toward nanoencapsulation technology, which enables introduction of active ingredients in foods for their protection or controlled release (Fathi *et al.*, 2014; Hani *et al.*, 2016; Sahari *et al.*, 2016).

Typically, food applicable nanocarrier systems can be carbohydrate, protein or

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lipid based. In comparison to carbohydrate and protein based nanocapsules, lipid based nanocarriers have some advantages such as the possibility of industrial production, more encapsulation efficiency and low toxicity (Fathi *et al.*, 2012). Furthermore, lipid-based nanocarriers are more beneficial for the delivery of poorly water soluble compounds. Examples for such lipid nanocarriers are nanoemulsions, Solid Lipid Nanoparticles (SLNs), Nanostructure Lipid Carriers (NLCs), and Lipid Nanocapsules (LNCs) (Kiani *et al.*, 2017).

Nanostructure Lipid Carriers (NLCs) are second generation of lipid based nanoparticles developed to overcome limitations of Solid Lipid Nanoparticles (SLNs) (e.g. burst release and low encapsulation efficiency). NLC comprises blend of solid and spatially incompatible liquid lipids as a core matrix wherein the incorporation of liquid lipid in solid lipid leads to massive crystal order disturbance (Zhang *et al*., 2008). Lipid Nanocapsules (LNCs) are new generation of lipid nanocarriers and are characterized by a hybrid structure between polymer nanocapsules and liposomes. LNC has an oily core that is composed of medium-chain triglycerides surrounded by a membrane made from a mixture of lecithin and a pegylated surfactant. This nanocarrier is formulated with Generally Recognized As Safe (GRAS) ingredients. It should be noted that there are few reports on application of LNC for encapsulation of food bioactives (Kiani *et al*., 2017).

There is not any report on application of NLC and LNC for encapsulation of vitamin E for fortification of milk. Therefore, the objectives of this research were to develop and characterize nanostructure lipid carriers and lipid nanocapsules for entrapment of vitamin E, study physicochemical features, release profile and analyze their potential application for milk fortification.

MATERIALS AND METHODS

Alpha-Tocopherol acetate (α-Tac) was purchased from DSM Nutritional Products Ltd Company (Farace). Glyceryl Behenate (GB, Compritol® 888) and Labrafac (capric and caprylic acid triglyceride), were provied by Gattefossé (France) and Glyserol Monostearate (GMS) by Condea (Germany). Solutol HS15 (PEG 660 12-hydroxystearate) was obtained from BASF (The Chemical Company, Ludwigshafen, Germany). Tween 80, soybean lecithin and methanol (HPLC grade) were purchased from Merck, Germany. Absolute ethanol 99.8% (Kimia Alcohol Zanjan, Iran) and acetone (Pars Shimi, Iran) were supplied at least of analytical grade. Milk (1.5% fat sterilized; Pegah Company) was purchased daily.

Preparation of Vitamin E Loaded NLC

NLC were prepared by emulsification-solvent diffusion method. Briefly, lipid phase was prepared by mixing Glyceryl Behenate (GB) and Glycerol Monostearate (GMS) as solid lipids (GB to GMS ratio of 1:1), olive oil (solid to liquid weight ratios of 90:10 and 80:20) as a liquid lipid, soy lecithin as emulsifier (the same weight of lipids) and vitamin E in two weight ratios of 10 and 20 of total lipid (total weight ratio of lipid to aqueous phase was 5:95); and subsequently, this phase was completely dissolved in a mixture of acetone and absolute ethanol (volume ratio 1:1), in a water bath at 70°C. The resulting organic phase was quickly dispersed in the aqueous phase containing 1% tween 80 (w/v) using a syringe, at 65°C under magnetic stirring for 15 minutes at 1,000 rpm. Rapid diffusion of the organic phase into aqueous phase resulted in formation of an O/W (oil in water) nanoemulsion. The solvent was evaporated by string and under high temperature. The obtained nanoemulsion was then placed in an ice bath at 0°C in order to obtain NLC (Figure 1). The compositions of all developed formulations are shown in Table 1.
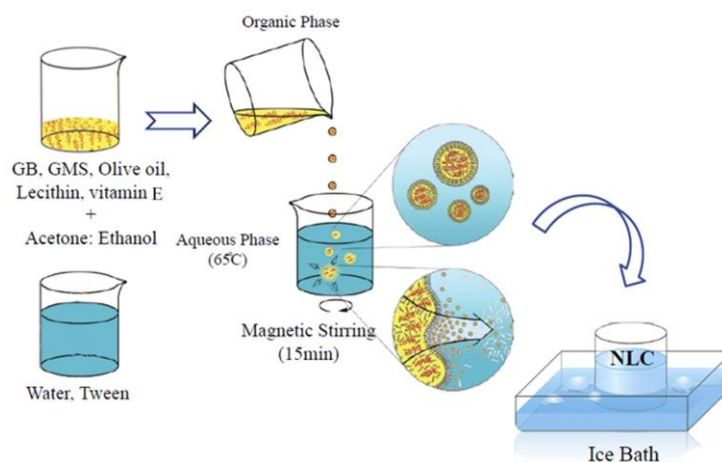
Figure 1. Schematic of production method of Nanostructured Lipid Carrier (NLC) by emulsification-solvent diffusion technique**.**

Preparation of Vitamin E Loaded LNC

LNCs were prepared according to Phase-Inversion Temperature (PIT) process. Production method was based on a previous paper (Kiani *et al.*, 2017). Briefly, preparation method involved two steps: (i) W/O (water in oil) emulsion was formed by mixing all components (i.e. labrafac, lecithin, solutol HS15, NaCl, water and vitamin E) under magnetic stirring and heating from room temperature to about 85 ℃ (above the PIT) (Anton *et al.*, 2007). Then, the solution was cooled to 60°C (below the PIT) leading to formation of an O/W emulsion. Several temperature cycles crossing the Phase-Inversion Zone (PIZ) between 85 and 60°C were then carried out (85-60, 85-60, and 85-60°C). (ii) Sudden dilution with cold water (0°C) which led to irreversible shock caused breaking of the micro emulsion system and formation of stable nanocapsules. The formulation codes are tabulated in Table 1.

Particle Size and Zeta Potential

The average diameter, polydispersity index and zeta potential of the nanoparticles were determined by Dynamic Light Scattering (DLS) based on Photon Correlation Spectroscopy (PCS) with a Zetasizer (Zetasizer 3000, Malvern, UK) (Maurya and Aggarwal, 2019a, b). Using this method, fluctuations were analyzed by means of the intensity or photon autocorrelation function.

Encapsulation Load and Efficiency

Encapsulation Efficiency (EE) and Encapsulation Load (EL) were determined using centrifugation method (Fathi *et al.*, 2012). A 500 μL NLC or LNC dispersion was placed in a Millipore tube with a cutoff of 10 kDa (Millipore, Bedford, MA, USA) and ultracentrifuged (Sigma; Germany) at 10,000 rpm for 10 minutes. The amount of unloaded vitamin E in the filtrate phase was determined by HPLC at wavelength of 290 nm (HPLC; Shimadzu, Japan, equipped with a C18-ODS column and UV (FPD-6AV) detector). Methanol by isocratic flow and the flow rate of 1.2 mL min^{-1} was considered as the mobile phase. EE and EL were calculated based on the following equations:

$$EE\% = \frac{(W_T - W_F)}{W_T} \times 100$$

(1)

Table 1. Formulation code of vitamin E loaded NLCs and LNCs.

NLC code	Liquid to solid lipid ratio	Vitamin E ratio to total lipid phase (%)
1N	20:80	10
2N	10:90	10
3N	20:80	20
4N	10:90	20
LNC code	Lipid percent	Vitamin E ratio to total lipid phase (%)
1L	16.8	4
2L	20.5	4
3L	16.8	8
4L	20.5	8

$$EL\% = \frac{(W_T - W_F)}{W_L} \times 100 \tag{2}$$

Where, W_T was the Total Weight of applied vitamin E in the formulation of nanocarrier, W_F was amount of Free vitamin in filtrate phase and W_L was Weight of the used Lipid in preparation of the nanocarriers.

X-Ray Diffraction

The XRD patterns were obtained by collecting intensity data measured by Philips MPD-X'PERT diffractometer (Amelo, The Netherlands) using Cu Kα radiation (λ= 1.540598 Å; voltage 40kV; current 40mA) with a step width of $0.05°$ s^{-1} over the 2θ range of 5-40°. The produced nanocarriers were lyophilized for X-ray study.

Fourier Transform Infrared Spectroscopy

The infrared spectra on FTIR spectrophotometer (FTIR, 8400S; Shimadzu, Japan) was detected at 4 cm^{-1} resolution in frequency range between 800 and 4,000 cm^{-1} using KBr Pellet method for freeze-dried samples and ingredients.

Release Study

Vitamin E release from the nanocarriers was studied in gastric (pH: 1.2) and intestinal (pH: 7.4) simulated solutions applying dialysis bag method at 37°C and 100 rpm. Three milliliters of vitamin E-loaded nanocarrier solution was sealed into dialysis bag (Sigma, Canada) with a 12-kDa cutoff. The bag was then placed into 50 mL gastric buffer for 2 hours. It was subsequently subjected to 60 mL intestinal buffer for 6 hours (Fathi and Varshosaz, 2013). At specific time intervals, the amount of released vitamin was determined by HPLC method. To study the release kinetic of vitamin E in the gastric and intestinal media, the release data were kinetically modeled by zero-order, first-order, Higuchi, Rigter-Peppas and Hixson-Crowell models (Equations 3-7) (Fathi *et al.*, 2012):

$$C = kt \tag{3}$$

$$C = [1 - \exp(-Kt)] \times 100 \tag{4}$$

$$C = kt^{0.5} \tag{5}$$

$$C = kt^n \tag{6}$$

$$1 - \sqrt[3]{1 - C} = k_1 t \tag{7}$$

Where, C is vitamin Concentration at time t, k is kinetic constant and n is release exponent.

Sensory Properties

To study the potential application of nanocarriers for food enrichment, milk was selected as a sample food system. Sensory evaluation of enriched milk samples was performed based on both oral and non-oral feature by 12 trained panelists. Three milk samples, i.e., blank, vitamin E loaded nanocarrier enriched milk (10% of daily requirement for vitamin E) and direct vitamin E enriched milk

were provided at temperature about 7°C and assessed using hedonic scale of 1–7. The parameters such as taste (creaminess, bitter tastes and after taste), color (yellowness), homogeneity, and total acceptance were used for descriptive analysis of milk samples (Fathi *et al*., 2012).

Statistical Analysis

Experiments were carried out at least in three replications. Analysis of variances was performed by MSTAT software (Version C) and means were compared by Duncan's multiple range test at 5% significant level.

RESULTS AND DISCUSSION

In order to improve the stability of vitamin E, NLC and LNC were successfully prepared by emulsification-solvent diffusion and phase inversion methods, respectively. The mean values of particles size (nanometer), zeta potential (millivolt), polydispersity index, percentage of encapsulation efficiency and encapsulation load are listed in Table 2.

Particle Size Analyses

Particle sizes for the different compositions of NLC were in the range of 18.20-22 nm. As Table 2 shows, particle size of NLC did not change significantly with varying contents of vitamin ($P > 0.05$).

However, particle size increased ($P < 0.05$) with raising liquid lipid ratio, which could be due to increase in interfacial tension with increase of olive oil (Tamjidi *et al*, 2014). GB mainly consists of diacylglycerols of behenic acid and may have more surface activity than olive oil (mainly is in the form of triglycerides) and, therefore, has less surface tension and thus it facilitated breaking of particles.

For LNC, the particle size values for the different compositions were in the range of 35.47-52.75 nm. The percentage of labrafac had a significant effect ($P < 0.05$) on the average size of LNC, such that the particle size increased with increase of oil proportion. The average size of LNC was mainly dependent on the ratio of surfactant to oil phase. It was reported that an increase of surfactant proportion led to an decrease of LNC particle size (Lamprecht *et al.*, 2002). This phenomenon could be explained by the stabilizing properties of solutol, which conferred great stability to the oil in water system, thus allowing formation of lower size particle. Higher emulsifier concentration led to exposure of more emulsifier molecules on the interface of oil-water and reduction in the interfacial tension. This phenomenon protects the droplets against aggregation and thus it causes further droplet size reduction (Heurtault *et al*., 2003).

The results showed that size of the LNC decreased by decreasing vitamin E concentration in the oil phase ($P < 0.05$).

Table 2. Particle size, Polydispersity Index (PDI), zeta potential, Encapsulation Efficiency (EE), and Encapsulation Load (EL) of produced formulations.[a]

Formulation code	Particle size (nm)	Zeta potential (mV)	PDI	EE (%)	EL (%)
1N	21.43±2.45	-2.91±0.20	0.197±0.010[c]	95.71±0.38[a]	9.57±0.03[c]
2N	18.20±0.31	-3.58±0.10	0.165±0.008[d]	90.71±0.80[c]	9.07±0.08[d]
3N	22.00±2.13	-2.74±0.52	0.271±0.010[a]	93.76±0.67[b]	18.75±0.13[a]
4N	20.69±1.77	-2.93±0.44	0.258±0.008[b]	90.20±0.65[c]	18.17±0.20[b]
1L	35.47±2.68[c]	-4.65±0.49	0.318±0.004[d]	99.71±0.015[b]	4.22±0.0006[d]
2L	43.15±0.96[b]	-4.45±0.42	0.324±0.053[c]	99.86±0.015[a]	4.23±0.0006[c]
3L	36.92±0.17c	-4.58±0.51	0.336±0.016[b]	89.55±0.007[d]	7.58±0.0006[b]
4L	52.75±1.88a	-3.27±1.28	0.410±0.005[a]	89.84±0.008[c]	7.61±0.0007[a]

[a] Values in each column for NLC or LNC followed by different letters are significantly different ($P < 0.05$).

These effects were attributed to changes in viscosity of oil phase and big structural volume of vitamin E, which is an oil component that is difficult to be homogenized due to its relatively high viscosity. Previous researches showed that droplet disruption decreased as the viscosity of the dispersed phase increased for various types of mechanical homogenizers. The interfacial tension increased with increase in vitamin E concentration and, therefore, the raising of average size of LNC was reasonable.

Zeta Potential

The result of zeta potential measurement showed that all NLC formulations with pH around 7 had small negative charges (in the range of -2.74 to -3.58 mv). Other studies also showed that oil droplets coated by non-ionic surfactants such as tween have a slight negative charge. This negative value can be attributed to polarization of the surfactant, followed by adsorption of polarized water molecules at the particle surface that offers a relatively lower zeta potential compared to the ionic surfactants (Mayer *et al.*, 2013). Zeta potential of NLC formulations did not change significantly at different vitamin E concentration as well as liquid to solid lipid ratio (P> 0.05). Zeta potential values of LNC formulations were also slightly negative in the range of -3.27 to -4.65mV (Table 2), that could be due to PEG groups at the surface forming dipoles able to interact with counter ions or water dipoles (Hirsjärvi *et al.*, 2012). Lipid percentage and vitamin concentration did not have any significant effect on the zeta potential of LNC formulations (P> 0.05). However, lecithin as an ionic emulsifier usually exhibits high negative charge, while the salt and pH in the system may affect the zeta potential values to be near zero.

Polydispersity Index

The results showed that all produced vitamin loaded NLC formulations had PDI value between 0.165-0.271 indicating their narrow size distribution. The same results were reported by Maurya and Aggarwal (2019b). PDI values increased by increasing of the liquid lipid ratio as well as vitamin E concentration (P< 0.05). It could be attributed to the presence of higher number of larger particles because of the insufficient shearing force that led to production of large and small particles together. Viscosity of the formulation increased in higher vitamin concentration and therefore size disruption became wider. The PDI values of LNC formulations were in the range of 0.31 to 0.41 (Table 2). The increase in vitamin E and lipid concentrations led to increase of PDI values (P< 0.05). Tamjidi *et al.* (2014) reported that increase in the PDI value by increasing lipid to emulsifier ratio as well as liquid lipid might be due to presence of large number of huge particles because of insufficient droplet disruption.

Encapsulation Efficiency and Encapsulation Load

The values of encapsulation efficiency showed high incorporation of vitamin E into the developed nanocarriers (Table 2). Generally, lipids with high order crystalline structure have lower load efficiency, which led to leakage of encapsulated bioactive during storage. The EE values of NLC were in the range of 90.20 to 95.71%. The results showed that the EE of NLC was significantly affected by different amounts of vitamin as well as liquid to solid lipid ratio (P< 0.05). Applying more liquid lipid in NLC mixture led to higher (P< 0.05) value of EE that was due to higher solubility of vitamin in liquid lipid (olive oil) compared to solid lipid (GB and GMS). Furthermore, increasing of liquid lipid in NLC formulation led to more restriction of recrystallization and forming less order crystalline state, which resulted in more

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imperfection and accommodation of higher vitamin entrapment.

The EE values of produced LNC were about 89.5 to 99.86% (Table 2) and significantly increased (P< 0.05) by increasing oil phase content in formulation, which was due to higher ability of oil core to accept higher amount of vitamin.

However, increasing of vitamin content led to a reduction of EE (P< 0.05). This phenomenon could be attributed to capacity constraints of developed nanocarriers to accepting of vitamin in their structure. Each nanocarrier has certain ability to incorporation of bioactive compound based on its structure, surfactant type, and applied lipids. The results also showed that EE of LNC was higher (P< 0.05) than NLC, which might be due to better solubility of vitamin in LNC oil core.

Encapsulation load is mainly influenced by the ratio of encapsulant to shell, polymorphic state of the lipid, solubility of the encapsulant in the melted lipid, types of lipids and chemical and physical structure of the solid lipid matrix (Tamjidi *et al.*, 2013). As shown in Table 2, EL values for NLC formulations were in the range of 9.07 to 18.75%. Increasing the liquid to solid lipid ratio led to an increase in EL (P< 0.05). This phenomenon is because of higher solubility of vitamin in liquid compared to solid lipid. On the other hand, it could be due to the relatively higher EE and higher amount of vitamin E (20%) encapsulated in the particle. Furthermore, higher liquid lipid content led to formation of less ordered crystalline or more amorphous solid structure and, therefore, higher space for entrapment of bioactive (McClements and Rao, 201).

Produced LNC had encapsulation load ranging from 4.22 to 7.61% (Table 2). Higher encapsulation load was related to the formulations containing more lipid and vitamin E that allowed greater amounts of vitamin to enter into the oil core. It should be noted that having a high encapsulation efficiency is always favorable, while encapsulation load more than 50% is not

proper due to increase of risk of bioactive leakage (Fathi *et al.*, 2012).

The NLC and LNC formulations that had the lowest size and polydispersity index as well as highest zeta potential, EE and EL, were selected as the optimal formulation for further analysis (1N and 1L).

X-Ray Diffraction Analysis

Triacylglycerides (TAGs) molecules might have α, β′, and β crystals with hexagonal, orthorhombic, and triclinic unit structures, respectively. The lattice spacing of crystals is different from 4.15 Å for the thermodynamically least stable and lowest melting α-form to 4.6 Å for the thermodynamically most stable and highest melting β-form (Weiss *et al.*, 2008). Lattice space of triglycerides could be described as follows: α: 0.42 nm; β′: 0.42–0.43 and 0.37–0.40 nm; β: 0.46 nm (Jenning *et al.*, 2000). Mainly, pure homogenous lipids tend to form perfect crystals with typical plated-like pattern of β-modification. Using heterogeneous lipid for encapsulation can help formation of spherical particles due to higher presence of α-crystals in the lipid phase (Weiss *et al.*, 2008). The polymorphic changes and the melting behavior of nanocarriers might be the major factors that affect release rate, loading capacity and thermal stability (Tamjidi *et al.,* 2014).

Diffractograms of bulk lipids (GB and GMS); blank NLC (without vitamin loading) as well as vitamin loaded NLC (1N) are shown in Figure 2. GB showed reflections at 21.18° and 23.37° that indicating short spacing of fatty acid chains at 0.38 and 0.42 nm, respectively, which are typically related to orthorhombic metastable polymorph (β′). XRD pattern of GMS also showed diffraction peaks at 19.34° and 23.38° which attributed to the short spacing of fatty acid chains at 0.45 (related to triclinic-orthorhombic polymorph (βi)) and 0.38 nm (related to orthorhombic metastable polymorph (β′)), respectively. NLC also revealed reflections at 21.25° and 23.49°
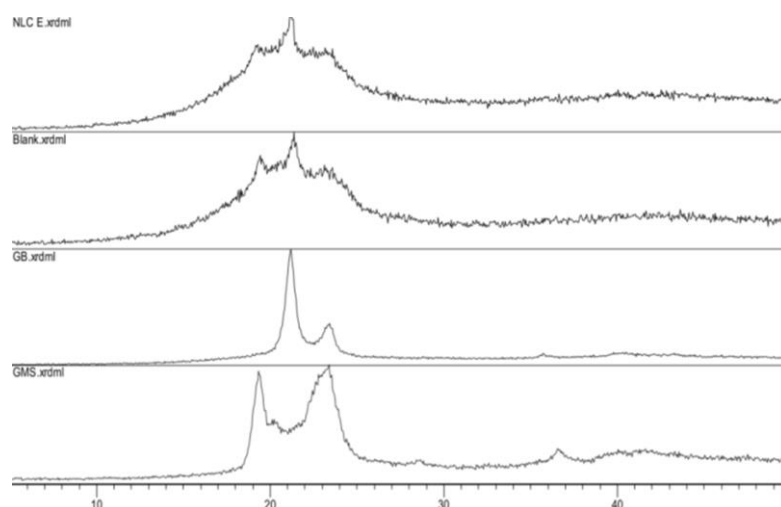
Figure 2. X-ray diffractograms of vitamin E loaded NLC, blank NLC, Glyceryl Behenate (GB) and Glycerol Monostearate (GMS).

showed short spacing of 0.418 and 0.378 nm, for formulation containing vitamin E and at 21.39° and 23.38° indicating short spacing of 0.415 and 0.380 nm, for blank formulation, all of which correspond to β′ polymorph (Jenning *et al*., 2000). The diffractograms of vitamin E loaded NLC showed wider peaks with less intensity compared to pure lipids. Wider peaks came from less crystalline order lattice and its defects, meanwhile, lower intensities of reflections also indicated decrease in crystallinity degree (Tamjidi *et al*., 2014). This result can be attributed to the presence of liquid oil within the lattice causing decrease in the crystallinity. As noted earlier, increase of amorphous state is favorable for incorporation of larger amounts of vitamin into nanocarriers and reduction of leakage rate during storage. Other peaks detected in diffractograms of vitamin loaded NLC and blank NLC at 19.14° and 19.39° correspond to short spacing at 0.460 and 0.450 nm, respectively, indicating the partial formation of triclinic-orthorhombic (βi) polymorph. It can be attributed to the presence of other ingredients in the nanocarrier formulations, and particle size effect. Usually, the crystalline structure formed in nanolipids is different from bulk fats because of curvature effects, the limited volume present for

crystal growth in droplets, and the lack of secondary nucleation sites. Furthermore, the first and third peaks in the vitamin loaded NLC diffractograms had lower intensities compared to those of blank NLC, indicating higher portion of β′ polymorph. This phenomenon might be due to incorporation of vitamin among parts of the crystal lattice of the lipid that led to more imperfections in crystalline structure and, consequently, lower encapsulant leakage during storage.

Fourier Transform Infrared Spectroscopy

FTIR spectrum of vitamin E showed characteristic bands at 921-1,077 cm^{-1} for C=O stretching vibration, 1,205 cm^{-1} of C-O formation, 1,758 cm^{-1} for C=C formation and 2,925 cm^{-1} which related to C-H alkanes group (Figure 3). Furthermore, FTIR peaks at 1,708 (C=O formation), 1,460 (C-O and C-O-C formation) and 1,367 cm^{-1} were also observed. Vitamin E loaded NLC and blank NLC showed absorption bands at 718-720 cm^{-1} related to aliphatic C–H bonds, C=O groups at 1,101 cm^{-1} and CH2 bonds at 1,461 cm^{-1}. Several peaks assigned to the stretching vibration of C=O at 1,737 cm^{-1} and also two peaks at 2,849 and 2,916 cm^{-1} related to symmetric and asymmetric
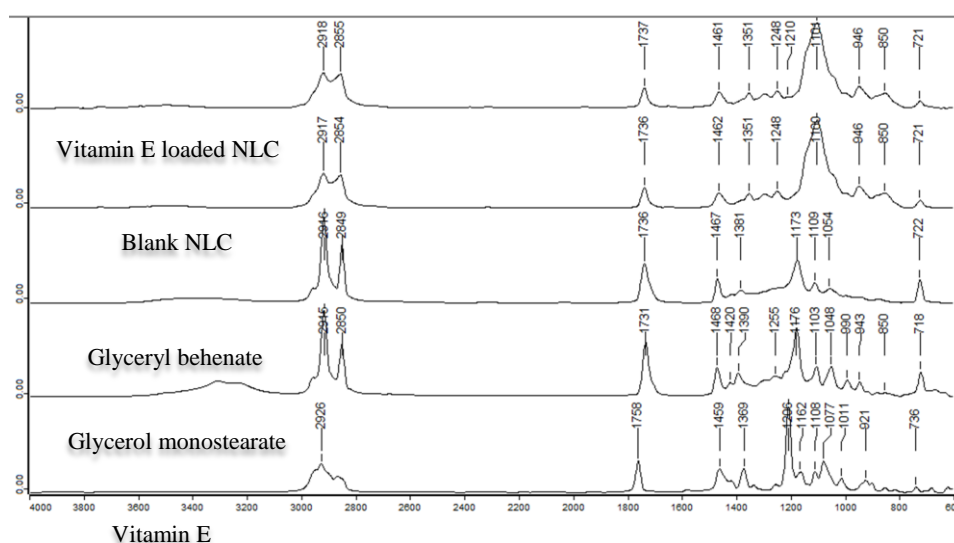
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Figure 3. FT-IR spectra of vitamin E loaded NLC, blank NLC, glyceryl behenate, glyserol monostearate and vitamin E.

stretching vibration of C-H alkanes were observed. Moreover, FTIR spectra showed a small shift in the peak related to the C-O group in the NLC (at 1,210 cm⁻¹) in comparison to pure vitamin E (at 1,205 cm⁻¹). Since this peak was not detected in blank NLC, it can be attributed to the presence of vitamin E in nanocarriers. Applied lipid showed similar peaks to produced NLC with a small shift at 718-720 cm⁻¹ corresponding to aliphatic C–H band, 1,054, 1,109 and 1,173 cm⁻¹ related to C=O vibration and also aliphatic C–H at 1,467 cm⁻¹.

Regarding LNC and its ingredients, labrafac showed peaks corresponding to aliphatic C–H bonds (around 724 cm⁻¹), C-O groups (at 1,104 and 1,104 cm⁻¹), symmetric stretching vibration of C=O (at 1,377 cm⁻¹), also stretching vibration of C=O ester groups and symmetric stretching vibration of C=H at 1,742, 2,855 and 2,925 cm⁻¹ (Figure 4). Vitamin E loaded LNC as well as blank LNC spectra revealed absorption bands of C-O groups at 1,102 cm⁻¹, stretching vibration of C=O ester groups at 1741 cm⁻¹, and symmetric stretching vibration of C=H at 2,857 and 2,923 cm⁻¹.

Considering that no significant shifts in the characteristic peaks of produced carriers (NLC and LNC) of pure vitamin E were observed, it could be presumed that no chemical interaction occurred, and vitamin is compatible with the applied lipids.

Release Study and Kinetic Modeling

Figure 5 shows release profiles of vitamin E from NLC and LNC of optimum samples.

The results showed that release profile in the first two hours in gastric media was about 28.73% and over the next 6 hours in intestine media was about 52.9% for NLC. Investigation of release profile showed that release rate in the first 2 h in the gastric condition followed the faster kinetics. This burst release in gastric media might be due to immediate dissolution of the adsorbed vitamin over the particles' surfaces. In emulsification-solvent diffusion method, the solubility of lipophilic bioactive component in the dispersed phase (solvent) increased and this phenomenon caused an increase in concentration of bioactive component on the surface of the nanocarriers. The burst release also could be attributed to the faster dissolution of applied lipid in acidic condition. Furthermore, liquid lipid could increase the bioactive diffusion rate during the release. Thus using liquid lipid in NLC formulations could cause increase of initial release rate (Fathi *et al.*, 2012).
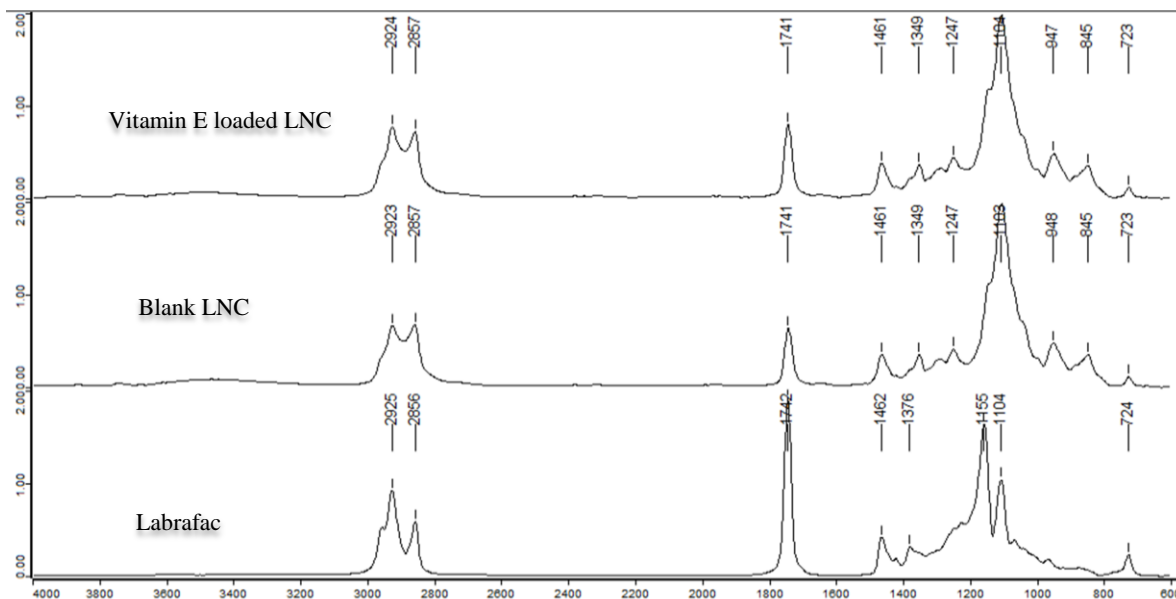
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Figure 4. FT-IR spectra of vitamin E loaded LNC, blank LNC and labrafac.
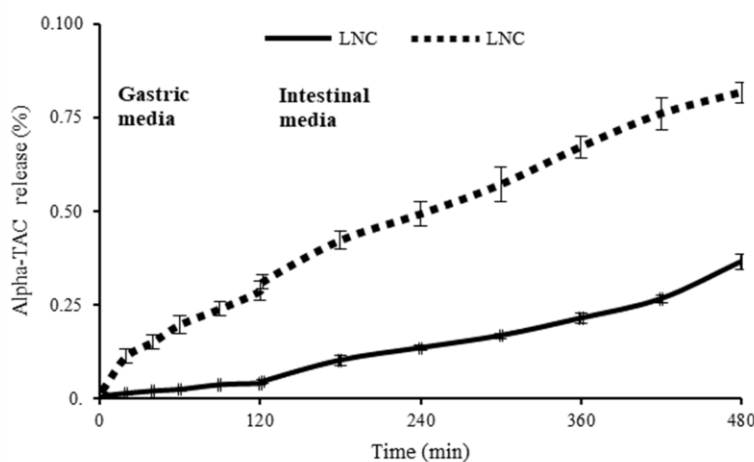

Figure 5. Release profile of vitamin E from NLC and LNC in gastrointestinal media.

The release percentages for LNCs were 4 and 32.5% in the gastric and intestine media, respectively (Figure 5). Lower release rate of LNC in comparison to NLC can be due to the thicker external shell of LNC, which one can understand based on their larger particle size in comparison to NLC, for the same EE and EL. Moreover, previous study showed that no degradation occurred during in vitro release experiments. Consequently, the release might mainly occur by diffusion throughout the lipid capsule matrix (Lamprecht *et al*., 2002). Moreover, higher encapsulation load and smaller size of NLC

in comparison to LNC caused faster release rate due to increase of driving force and surface to volume ratio (Fathi *et al*., 2012).

Vitamin release from produced nanocarriers was kinetically studied using zero-order, first-order, Higuchi, Rigter–Peppas and Hixson-Crowell models. Table 3 shows the model parameters and their corresponding correlation coefficients. As can be observed, for both types of nanocarriers (NLC and LNC) Rigter–Peppas showed the best fitness. In this model, n is an exponent parameter that is used to describe different release

Table 3. Model parameters of vitamin E release from nano carriers. [a]

Model		Whole release process		Intestinal media		Gastric media	
		LNC	NLC	LNC	NLC	LNC	NLC
Zero-order	k	0.001	0.002	0.001	0.002	9.5×10^{-5}	0.003
	R	0.950	0.934	0.966	0.982	0.845	0.813
First-order	k	6.35×10^{-6}	1.89×10^{-6}	8.08×10^{-6}	1.55×10^{-5}	9.5×10^{-5}	2.71×10^{-5}
	R	0.950	0.935	0.966	0.982	0.845	0.813
Higuchi	k	0.011	0.033	0.013	0.025	0.010	0.025
	R	0.711	0.949	0.814	0.923	0.959	0.992
	N	1.423	0.704	1.16	0.833	0.601	0.553
Rigter–Peppas	k	5.25×10^{-5}	0.011	0.001	0.004	0.001	0.020
	R	0.989	0.997	0.973	0.995	0.972	0.997
Hixsonñ-Crowel	k	0.001	0.001	0.001	0.001	3.17×10^{-5}	0.001
	R	0.934	0.914	0.956	0.992	0.846	0.857

[a] k: kinetic constant, R: Correlation coefficient, LNC: Lipid nanocapsule, NLC: Nano structured lipid carrier.

mechanisms of bioactive compound. The value of n< 0.45 corresponds to the bioactive compound diffusion control; n> 0.89 is attributed to the dissolution of particles and 0.45< n< 0.89 is due to the combination of diffusion and dissolution mechanisms. Therefore, the result indicated that release phenomenon in produced nanocarriers are mainly governed by combination of both Fickian diffusion and dissolution mechanism for gastric media and diffusion mechanism for intestinal media.

Sensory Properties

In order to investigate the potential application of developed nanocarriers for food enrichment, the lyophilized nanocarriers were added to milk sample as a food system. Table 4 shows sensory results of blank milk, fortified milk with vitamin E loaded nanocarriers (NLC and LNC) and fortified milk using direct vitamin addition. The allocated scores for fortified milk with vitamin E loaded NLC and LNC indicated that creamy flavor of these samples were significantly higher than blank milk (P< 0.05). Significant difference in after taste of fortified milk with vitamin E loaded NLC in comparison to blank milk was observed (P< 0.05). While LNC fortified milk did not show significant difference in after taste

with blank sample (P> 0.05). Total acceptance of direct vitamin fortified milk was significantly lower than blank milk (P> 0.05). While NLC and LNC fortified milk did not have significant difference with blank sample, there were no significant differences in other characteristics of fortified samples with both nanocarriers.

CONCLUSION

In this study, feasibility of production of Nanostructure Lipid Carrier (NLC) and Lipid Nanocapsule (LNC) for encapsulation of vitamin E was studied. XRD analyses showed that the crystalline states of the produced NLC were less ordered than pure lipids. Further increase in the amorphous state and more β' polymorph due to the presence of α-TAC in the NLC matrix were observed, which led to formation of more defects in the nanocarriers crystalline structure. FTIR results showed that no significant shift at the characteristic peaks of pure vitamin E in produced nanocarriers was detectable. LNC showed slower release profile in comparison to NLC and, therefore, could be suggested for encapsulation of acid sensitive compounds. Kinetic study in gastrointestinal conditions showed that the Rigter–Peppas was the best model to describe release behavior. Finally, sensory

Table 4. Sensory results of fortified milk with vitamin E loaded NLC, LNC, direct fortification and blank samples.

Sample	Properties				
	Creamy flavor	Yellowness	Creamy aroma	Homogeneity	Total acceptance
Vitamin loaded NLC	4.66±1.15[a]	3.25±1.60	3.91±1.67	5.66±1.37	5.00±1.27[a]
Vitamin loaded LNC	4.66±1.66[a]	2.33±1.07	2.33±1.56	6.00±1.34	5.66±1.23[a]
Direct vitamin fortification	3.75±1.48[ab]	2.25±0.96	2.25±1.72	6.16±1.19	4.50±1.31[b]
Blank milk	3.50±0.79[b]	2.75±1.60	2.75±1.55	6.25±1.05	5.16±1.11[a]
Sample	Creamy flavor	Yellowness	Creamy aroma	Homogeneity	Total acceptance

evaluation revealed the acceptability of fortified milk with vitamin loaded nanocarriers. The result of this study indicates that both developed nanocarriers are appropriate to be used in food and drinks and selection of the carriers depends upon application and release condition.

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تولید حامل‌های لیپیدی نانوساختار و نانوکپسول‌های لیپیدی ویتامین E به منظور غنی‌سازی شیر

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چکیده

هدف از این مطالعه تولید و بررسی خصوصیات فیزیکوشیمیایی حامل‌های لیپیدی نانوساختار (NLC) و نانوکپسول‌های لیپیدی (LNC) حاوی ویتامین E بود. مقادیر بهینه‌ی نسبت لیپید مایع به جامد و همچنین نسبت ویتامین به کل فرمولاسیون تعیین شد و پارامترهایی از قبیل اندازه‌ی ذرات، شاخص بس‌پاشیدگی، پتانسیل زتا، راندمان و بارگیری انکپسولاسیون برای فرمولاسیون بهینه مورد بررسی قرار گرفت. راندمان انکپسولاسیون برای فرمولاسیون‌های بهینه‌ی حامل‌های لیپیدی نانوساختار و نانوکپسول‌های لیپیدی به ترتیب ۹۵ و ۹۹٪ بود. نتایج بدست آمده از بررسی با پراش پرتوی ایکس نشان‌دهنده‌ی شبکه‌ی جدید با میزان بلورینگی کمتر در نانوحامل‌های حاوی ویتامین در مقایسه با نمونه‌ی شاهد بود. نتایج حاصل از مطالعات طیف‌سنج تبدیل فوریه مادون قرمز نیز بیانگر عدم رخ دادن واکنش نامطلوب بین ویتامین و نانوحامل‌ها بود. همچنین بررسی پروفایل رهایش و مدلسازی سینتیکی آن در محیط دستگاه گوارش نشان داد که نانوحامل‌های تولید شده قادر به محافظت از ویتامین E در شرایط اسیدی با کاهش رهایش آن در محیط معده می‌باشند (میزان رهایش برای حامل‌های لیپیدی نانوساختار و نانوکپسول‌های لیپیدی، به ترتیب ۲۹ و ۴٪ بود). در ادامه ارزیابی حسی نمونه‌ی شیر غنی‌شده با نانوحامل‌های حاوی ویتامین E نشان داد این نانوحامل‌ها دارای پذیرش قابل قبولی برای تولید غذاهای فراسودمند می‌باشند.