Preparation and Characterization of α-Tocopherol-Loaded Nano-Lipid Carriers: Effect of Lipid Type and Carrier Oil Content

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ABSTRACT: *Nano lipid carriers for encapsulating α-tocopherol (α-TC) were successfully fabricated using the hot homogenization method. The effect of oil content and type of lipid on the physicochemical properties of different formulations were investigated. The results showed that α-TC nanocarriers exhibited a small particle size (259.2-379 nm), narrow PolyDispersity Index (PDI) (0.178-0.432), and high Entrapment Efficiency (EE) (95.73-99.22%). Nanostructured Lipid Carrier (NLC) of Compritol containing 45% corn oil (α-TC-NC45) was selected as the optimum formulation due to its smaller particle size as well as PDI and higher EE. The Differential Scanning Calorimetry (DSC) and X-Ray Diffractometry (XRD) data revealed a low degree of crystallinity for α-TC-NC45 with two crystal forms of α and ß′. Transmission Electron Microscopy (TEM) images also revealed an almost spherical morphology for the particles. The α-TC-NC45 was stable concerning particle size, PDI, and EE during a 3-month storage at both 4 and 25 °C. The results suggested that nanostructured lipid carrier of Compritol containing 45% corn oil may be an appropriate delivery system for food and beverage fortification with α-tocopherol.*

KEYWORDS: *Nanostructured lipid particles, α-tocopherol, encapsulation, Compritol.*

INTRODUCTION

α-Tocopherol (α-TC) is the most important biological active form of vitamin E. It suppresses lipid peroxidation and free radical formation thereby inhibiting cell damages, impeding cardiac disease, and improving blood circulation as well as tissues repair [1]. However, α-TC is very sensitive to oxidation and is easily decomposed during food processing and storage. Further, as it is a lipophilic compound, it is impossible to directly incorporate it into aqueous media [2,3]. Considering these problems efficient methods to protect and solubilize α-TC have always been of interest.

Recently, there has been a growing interest in encapsulation techniques owing to their ability to preserve bioactive compounds against harsh physicochemical conditions. These techniques can be used for fortification of daily food and drinks by nutrients [4]. One of the most recently introduced encapsulation systems for lipophilic nutrients is Solid Lipid Nanoparticles (SLN). SLNs are crystalline lipid networks in which encapsulated bioactive

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compounds are entrapped. These systems may consist of one or more solid lipids developing a network. However, the loading capacity of SLNs is low and burst release often occurs following polymorphism of lipid crystals. Given these technical issues and the fact that most lipophilic bioactive compounds have higher solubility in liquid lipids than in solid fats, modified SLN systems known as nanostructured lipid carriers (NLCs) have been introduced. These new structures contain both solid and liquid lipids simultaneously at room temperature [5-6].

NLCs include almost all the advantages of liposomes, microemulsions, nanoemulsions, and SLNs, while being free from their constraints. Hot homogenization is the most common technique for production of SLN and NLC thanks to its advantages [7].

The physicochemical characteristics and release properties of NLCs are affected by various factors including the type of lipid and carrier oil as well as their ratio in the formulation [8]. The type of lipid has a considerable impact on the size of nanoparticles [9]. This suggests that a properly designed formulation is of a great importance to the fulfillment of the predetermined goals [8].

Accordingly, the main objective of this work was developing and selecting the best formulation of SLNs or NLCs with the greatest features for encapsulating α-TC to promote the fortification ability of foods and beverages in industrial processes with this vitamin. To this end, α-TC-loaded SLNs and NLCs have been prepared *via* hot homogenization method with different types of lipids and various oil contents. Subsequently, the influence of lipid type and carrier oil content was investigated on the nanoparticle's physicochemical properties. The selected formulation was further evaluated by DSC, XRD, TEM and stability tests.

EXPERIMENTAL SECTION

Materials and Methods

The solid lipids glycerol distearate (Precirol ATO 5) and glycerol dibehenate (Compritol 888 ATO) were generously gifted by Gattefossé (France). Corn oil (Mazola, Manufactured by BFSA) was purchased from a local supermarket. α-Tocopherol (≥98% purity, HPLC grade) was provided from Sigma-Aldrich (Germeny). Polysorbate 80 (Tween 80) was supplied by Scharlau (Spain). All other chemical reagents and solvents were of analytical grade.

Preparation of α-TC-loaded nanocarriers (SLNs and NLCs)

α-TC-loaded SLNs (α-TC SLNs) and α-TC-loaded NLCs (α-TC NLCs) were prepared by hot homogenization method. SLNs and NLCs were comprised of 10% (w/w) lipid phase. The formulations of SLNs and NLCs, their names and codes are reported in Table 1.

For preparing α-TC SLNs, a certain amount of α-TC was dispersed in molten lipid (80-85°C) and then mixed with an aqueous phase containing Tween 80 at 85°C at a speed of 16000 rpm for 3 minutes using an Ultra-Turrax T25 (IKA, T25, Germany). The resulting coarse emulsion was further homogenized for 3 minutes by a probe sonicator (HD 3200 Bandelin, Germany) at 40% amplitude and a fixed temperature of 85°C at 200W. The ultrasound applicator was a cylindrical titanium alloy probe of 13 mm in diameter.

α-TC NLCs were prepared in the same way as described for SLNs except that the molten lipid (80-85°C) was first mixed at different ratios with corn oil containing a certain amount of α-TC. Finally, the nanoemulsions obtained were cooled down to room temperature which resulted in solidification of the lipid phase and formation of suspended nanoparticles [8-10].

Particle size and size distribution measurement (PDI)

The mean particle size (Z-average) and PDI of the nanoparticles diluted with deionized water were determined using a dynamic light scattering (DLS) technique (Malvern Instruments, Ltd, United Kingdom) [11].

α-TC assay

α-TC was spectrophotometrically measured by a UV-Visible spectrophotometer (Shimadzu, UV-160A, Japan) [12].

A total of 2 ml of specimen containing α-TC was dissolved in 2 mL of ethanol, stirred well and then centrifuged (Hettich Tuttlingen Zentrifugen, model EBA 20, Germany) at 6000 rpm for 10 minutes. The absorbance of the supernatant was recorded at 292 nm against ethanol as blank. α-TC content was calculated using the calibration curve multiplied by the dilution factor.

Determination of encapsulation efficiency and loading capacity

According to Eq. (1), the encapsulated α-TC was calculated as the difference between Total α-TC content

Table 1: The names and codes, formulations, particle size (nm), polydispersity index and entrapment efficiency of nanoparticles.

Name of formulation	Code of formulation	Lipid phase composition			Particle size (nm)	PDI	EE (%)
		Solid lipid (%)		Liquid oil (%)			
		Compritol	Precirol	Corn oil			
SLN Compritol	SC	10		0	352.4^{g} ±2.4	0.398^{e} ±0.014	98.46^{cde} ±0.19
NLC Compritol with 15% corn oil	NC15	8.5		1.5	334.2^{f} ±1.9	0.234^{abc} ±0.017	96.22^{ab} ±0.23
NLC Compritol with 30% corn oil	NC30	7		3	281.3^{c} ±3.8	0.249^{c} ±0.018	99.19^{e} ±0.69
NLC Compritol with 45% corn oil	NC45	5.5		4.5	259.2^{a} ±5.4	0.178^{a} ±0.021	99.27^{e} ±0.48
SLN Precirol	SP		10	0	267.8^{b} ±2.4	0.432^{e} ±0.023	98.84^{de} ±0.66
NLC Precirol with 15% corn oil	NP15		8.5	1.5	317.3^{e} ±2.9	0.424^{e} ±0.026	98.72^{de} ±0.78
NLC Precirol with 30% corn oil	NP30		7	3	285.8^{c} ±2.7	0.245^{bc} ±0.025	98.2^{cde} ±0.9
NLC Precirol with 45% corn oil	NP45		5.5	4.5	268.3^{b} ±2.5	0.213^{abc} ±0.037	97.57^{bcd} ±1.33
SLN Compritol and Precirol	SPC	5	5	0	379.1^{h} ±4.9	0.326^{d} ±0.026	95.73^{a} ±0.57
NLC Compritol and Precirol with 15% corn oil	NCP15	4.25	4.25	1.5	307.2^{d} ±3.1	0.252^{c} ±0.024	97.05^{abc} ±1.65
NLC Compritol and Precirol with 30% corn oil	NCP30	3.5	3.5	3	377.8^{h} ±2.9	0.188^{ab} ±0.062	96.95^{abe} ±0.35
NLC Compritol and Precirol with 45% corn oil	NCP45	2.75	2.75	4.5	317.1^{e} ±3.8	0.207^{abc} ±0.034	98.51^{cde} ±0.39

(A_1) and the free α-TC content (A_2) obtained after separating the particles from the aqueous medium by dispersion–ultrafiltration/centrifugation method using 100k Amicon Ultra Centrifugal Filters (Millipore, Ireland) [8]:

$$EE\% = \frac{(A1 - A2)}{A1} \times 100 \quad (1)$$

Loading capacity (LC) is defined as the amount of encapsulated α-TC per unit mass of the lipid phase (L) as shown by Eq. (2) [13]:

$$LC = \frac{A1 - A2}{L} \times 100 \quad (2)$$

Differential scanning calorimetry

A differential scanning calorimeter (Mettler Toledo DSC 822, Switzerland) was used to study the thermal properties of particles. NLCs, with or without α-TC, were first lyophilized and were then placed in individual aluminum pans and hermetically sealed under nitrogen atmosphere at a flow rate of 50 mL/min. An empty pan was used as a reference. All specimens were heated from 5 to 80 °C and cooled back down to 5°C at a heating rate of 5°C/min. The resulting thermograms were analyzed by STAR^{e} Software with the thermal properties of particles including melting point and enthalpy of phase transition being determined. The Crystallinity Degree (CD) was calculated by Eq. (3):

$$CD\% = \frac{\Delta Hm}{\Delta H^{*}(\text{concentration of lipid phase})} \times 100 \quad (3)$$

where, ΔH_m is the enthalpy of fusion of NLCs and ΔH is the enthalpy of fusion of bulk lipid [13-14].

X-ray diffractometry

The X-ray diffraction patterns of lyophilized NLC particles were obtained by an X-ray diffractometer with Cu K-α radiation generated at 40kV and 0.7mA (XRD, PHILIPS X-PERT PRO, Netherland). Diffractograms were collected as 2θ versus absolute intensity in the continuous mode with a step size of 0.02° and a scan speed of 1s per step over an angular range of 5-100° 2θ [8]. The recorded x-ray diffractograms were analyzed by XQFUITE software.

Transmission electron microscopy (TEM)

Aqueous dispersions of NLC particles were 10-fold diluted with deionized water and slowly dropped onto the surface of a copper grid coated with carbon film (200 mesh). After holding for 2 minutes the excess dispersion was removed and the specimen was air-dried. It was then negatively stained with uranyl acetate 2% (w/v) and air-dried for 3 minutes at room temperature followed by examination by TEM (Leo 912 AB OMEGA, Germany) [10].

Stability Study

α-TC-NLCs were stored in sealed amber dark glass vials at 4°C and 25°C for 90 days with the changes in particle size, PDI and EE monitored at predetermined time intervals [8].

Statistical analysis

The experimental data were analyzed using full factorial design with type of lipid and carrier oil content as the factors at a significance level of $p < 0.05$. The means were compared by Duncan's multiple range test at a confidence level of $\alpha < 0.05$. SPSS 16 software was used for statistical analysis. All experiments were carried out in triplicates.

RESULTS AND DISCUSSION

Particle size and polydispersity index

The Z-average and PDI of the formulations are reported in Table 1. Z-average is expressed as the mean diameter based on the intensity of scattered light [6]. All formulations showed significant changes in Z-average as a function of carrier oil ratio and the type of solid lipid. The size of SLNs increased in the order of SLN Precirol (SP) < SLN Compritol (SC) < SLN Precirol and Compritol (SPC). Compritol is a solid lipid composed of 85 % C22 fatty acid glycerides. Owing to its high molecular weight, it has a high melting point around 65-77°C thus creating a high viscosity which significantly reduces the energy dissipation during ultrasonic homogenization. Hence, Compritol produces SLN with a larger particle size. Also, the long bulky hydrocarbon chain of behenic acid in Compritol prevents efficient packaging of molecules in the nanoparticles that increases the size. Unlike Compritol, Precirol is a mixture of mono-, di-, and triglycerides of C16 and C18 fatty acids which are smaller in the molecular size, and therefore can more densely be packed in particles thereby reducing the mean diameter. On the other hand, blending Compritol with Precirol increases the inhomogeneity of lipid phase and enhances the interaction with water which can lead to the swelling of particles. Elevation of the ratio of corn oil in both SC and SP nanoparticles, except at 15% in Precirol-based SLNs (NP15), resulted in a decrease in their size. This can be attributed to the reduction of viscosity and possibly the interfacial tension in the presence of liquid oil which enhances the mobility of molecules and the distortion efficiency of solid lipid matrix. Further, the incorporation of liquid oil can interfere with crystallization of solid lipid. Note that this lattice imperfection also reduces the size of particles.

Tiwari and Pathak (2011), Mehnert and Mäder (2012) and Das *et al.* (2012) also reported the significant contribution of liquid oil content and type of lipid to the size of particles [9, 13, 14]. Rosli *et al.* (2018) confirmed that particle size was affected by the liquid lipid content [10]. Also, Souza *et al.* (2019) observed that particle size diminished by raising the liquid oil content [15]. Note that NLC Compritol with 45% corn oil (NC45) showed the smallest particle size among all formulations. This indicates that the mean diameter of SLNs is more strongly affected by the liquid oil content than by the type of solid lipid.

Matos de carvalho *et al.* (2013) reached a smaller size for α-TC-loaded nanoparticles of Compritol (214.5 nm), which is supposed to be due to the type of surfactants they used [8].

Here, the size of NC45 particles fabricated in this study was smaller than that of other α-TC delivery systems that have been developed so far such as poly ε-caprolacton (PLC) nanoparticles (368.03 nm), liposomes (0.5-400 μm) and zein/chitosan coacervates (364.1 nm) [12, 16, 17].

Incorporation of corn oil into Compritol and Precirol nanoparticles markedly reduced the PDI values (Table 1). This was more pronounced for the former in response to more efficient ultrasonic homogenization possibly owing to the stronger reduction of viscosity in the presence of liquid oil. A similar trend was also observed for Compritol-Precirol blend nanoparticles.

The data in Table 1 reveal that Precirol-based SLN (SP), despite their smaller average size, had a wider particle size distribution compared with the other two formulations.

Since Precirol has a smaller molecular size, it can produce SLN with a smaller particle size. Thus, SLN Precirol has a larger number of nanoparticles in the same lipid phase volume of the SC and SPC. More nanoparticles of SLN Precirol led to uneven distribution of the input ultrasound energy by shielding and scattering of the waves, resulting in a wider particle size distribution. *Yu et al.* (2018) also expressed that liquid oil concentration can affect the PDI [18].

α-TC entrapment efficiency (EE)

EE is strongly dependent on the lipid matrix of particles [16]. α-TC nanocarriers exhibited high EEs for all formulations (Table 1). This might be due to the high solubility of α-TC in the lipid phase [2]. The EEs determined for Compritol- and Precirol-based SLNs were comparable and greater than 98%, and at the same time higher than those for the SLNs containing the mixture of both (SPC). The similarity of the EEs for single Compritol and Precirol nanoparticles denotes that the solubility of α-TC in both molten lipids was the same. On the other hand, the lower EE for the blend of them implies that the lipid matrix likely lacked enough space to accommodate high amounts of α-TC likely because of variations in the polymorphic state of the network. The addition of liquid oil to Compritol and Compritol-Precirol blend nanoparticles increased the EE of α-TC, while leaving it almost unchanged in the case of Precirol. Lipophilic compounds including α-TC have higher solubility in liquid oils than in solid lipids. It is not, therefore, surprising that EE is enhanced by adding corn oil to SLNs. Further, the incorporation of liquid oil can interfere with the organization of the solid lipid crystal lattice. This imperfection increases the intermolecular voids and thus higher amounts of the compound can be contained in the network. Rosli *et al*. (2018) confirmed that liquid lipid and solid lipid had substantial effects on the EE [10]. Zoghi *et al*. (2016), also reported that process variables such as composition and concentration of lipid had an important effect on the EE [19].

The EE achieved for the lipid nanoparticles in this study was higher than that reported for other systems such as poly ε-caprolactone (PCL) nanoparticles (90.95%) and niosomes (61-98%) [16-20].

Note that the α-TC loading capacity (%) of lipid nanoparticles insignificantly changed from 1.44 ± 0.16 to 1.49 ± 0.09 which could be related to the high solubility of α-TC in the lipid matrix of particles.

Selection of the optimum formulation

Based on the results of the preliminary measurements the optimum formulation leading to the nanoparticles with the smallest size and the highest EE was selected for further studies. Of the twelve samples examined, NC45 proved to fulfill the above criteria and thus was chosen for the next evaluations. Note that among the many attributes contributing to the physical stability and release behavior of lipid nanoparticles their size and EE seem to be more substantial [6]. It has been shown that a smaller particle size had higher stability against gravity owing to the Brownian motion of the particles [21]. Further, the bioavailability of the compound is enhanced by reducing the size of particles. On the other hand, the release behavior and the bioavailability improve upon EE elevation [16].

Differential scanning calorimetry

Thermal behavior was studied to characterize the physical state of nanoparticles during a heat-cool cycle (Fig.1). Upon heating to 80 °C, a single sharp endothermic peak at 70.93 °C was observed for bulk Compritol corresponding to the melting point of stable ß´ polymorphic form. The melting point of 71.1 °C for glycerol behenate is related to the ß´-form of the crystals [8]. This endothermic peak shifted to 67.91 °C and 67.98 °C in the thermograms of α-TC-free and α-TC-loaded NLCs, respectively. Upon cooling from 80°C, a single sharp exothermic peak at 66.49 °C was recorded for bulk Compritol which shifted to 63.39 °C and 63.11 °C for α-TC-free and α-TC-loaded NLCs, respectively. This peak is probably due to crystallization of Compritol into α-form. These results clearly indicate that the incorporation of corn oil reduces the melting and crystallization temperatures of NLCs which can be explained based on Hildebrand solubility law [22]. Indeed, Compritol is partially dissolved in corn oil which leads to lowered melting point. Further, the interaction between Tween 80 and Compritol could prevent the polymorphic transition by inhibiting the reorientation of the less-ordered configurations into a more ordered lattice.

The addition of corn oil and Tween 80 also reduced the enthalpy of melting and hence the Crystallinity Degree (CD)

Table 2: DSC parameters of bulk Compritol 888 ATO, alphatocopherol-free (α-TC-free NLC) and alphatocopherol -loaded nanostructured lipid carriers with 45% cornoil (α-TC-loaded NLC),

Sample	Melting point (°C)	Enthalpy (J/g)	CD (%)
Compritol 888 ATO	70.93	115.90	100
α-TC-free NLC	67.91	48.34	41.71
α-TC-loaded NLC	67.98	45.94	39.6

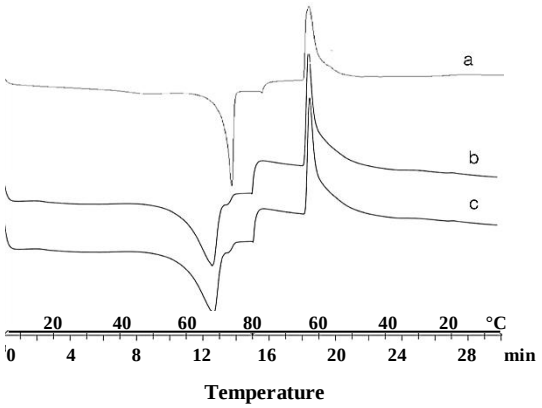

Fig. 1: DSC thermograms of bulk Compritol (a), alphatocopherol-free (b) and alphatocopherol -loaded nanostructured lipid carriers with 45% corn oil (c) during a heat-cool cycle.

of NLCs (Table 2). These changes in the physical state of nanoparticles are expected to enhance their stability and prevent the expulsion of α-TC during storage [23].

X-ray diffraction analysis

The polymorphic state of the lipid matrix has a great impact on the long-term stability of the dispersion and the entrapment efficiency of nanoparticles. Polymorphic transition from unstable α- to stable β-form leads to aggregation of particles due to the morphological changes from spherical to disk-like shape and expulsion of the encapsulated compound as a consequence of the internal organization of the matrix [23-24].

X-ray diffraction was used to study the polymorphic transition of NLCs.

As can be seen in the XRD pattern of Compritol (Fig. 2), there was a sharp high intensity peak at a diffraction angle (2θ) of 21.3° and another one with lower intensity at 23.31°. These peaks correspond to the short spacings of chains at 0.42 and 0.38 nm (calculated based on the Bragg law), respectively, which is attributed to the β form of Compritol with an orthorhombic subcell structure [8]. In contrast to bulk Compritol, the diffractogram of α-TC-free NLCs revealed three peaks with lower intensities at 2Θ of 19.44°, 21.45°, and 23.32° associated with the short spacings of chains at 0.46, 0.41, and 0.38 nm, respectively. Similarly, for α-TC-loaded NLCs, three peaks were observed at 2Θ of 19.89°, 21.92°, and 23.62° which can be related to the short spacings of chains at 0.45, 0.40, and 0.38 nm, respectively. These lines are characteristics of α and β polymorphic forms [8]. Peak shifting and lower intensity of the signals in the diffractograms of NLCs compared to that of Compritol can be attributed to the presence of the other components in the formulation (i.e., corn oil, Tween 80, and α-TC) causing more imperfections in the crystal lattice of the solid lipid and thus a reduction in the crystallinity degree [25].

Transmission electron microscopy

In order to gain a better insight into the structures and morphology of NLCs, they were studied by TEM. NLCs had an elliptical or ovoidal shape (Fig. 3) and a size range fairly consistent with that measured by DLS (Table 1). Furthermore, no sign of aggregation was observed, which along with the shape of particles, implicitly confirm the β′ polymorphic form of the lipid lattice. The shape of NLCs can be related to the crystal structure of the lipid matrix. Polymorphic transitions from α to β result transformation from spherical to elongated needle-shaped crystals [8]. The TEM micrographs clearly revealed that the presence of carrier oil and α-TC strongly averted the polymorphic transition to the β form, which is in line with DSC results.

Physical stability

The size of particles did not increase significantly at 4°C and 25°C after 90 days (Fig. 4a). The dispersions did not show any sign of visual instability or sedimentation either during storage at both temperatures. The high stability against gravity is due to the type of polymorphic transition of the lipid matrix which greatly reduced

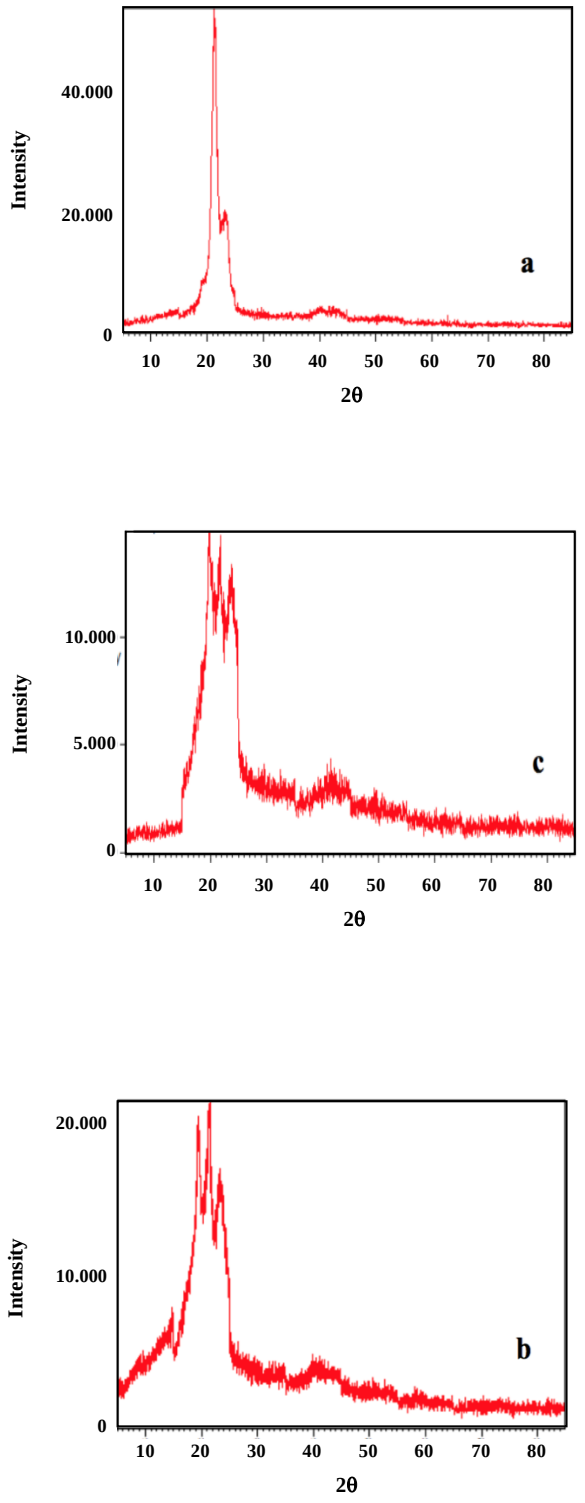

Fig. 2: X-ray diffractograms of bulk Compritol 888 ATO (a),), alphatocopherol-free (b) and alphatocopherol -loaded nanostructured lipid carriers with 45% corn oil (c).

the hydrophobic interactions between particles and thus their aggregation. The EE diminished by about 3% and 4% at 4°C and 25°C, respectively, during the first 45 days and remained constant afterwards until the end of the storage period at both temperatures (Fig. 4b). The slight reduction of EE can be attributed to the high miscibility of α-TC in corn oil and Compritol. As demonstrated earlier, the low crystallinity degree of NLCs and the presence of α and β′ crystal forms in their lipid matrix can considerably prevent perfect solidification and thus expulsion of α-TC from the particles [22].

Considering the results, it seems 4°C was more appropriate for storage of α-TC-loaded NLCs dispersion. Rincon *et al.* (2018) also expressed that pranoprofen-NLCs were more stable at 4°C in 180 days than at 25°C [11]. Similarly, Sun *et al.* (2014) reported that quercetin-loaded NLCs were more stable at 4°C compared to 25°C [26].

CONCLUSIONS

This study examined the influence of the type of solid lipid and the ratio of liquid oil on the quality attributes of α-TC-loaded NLCs prepared by hot homogenization method. The results indicated that increasing the corn oil content of Compritol-based formulations up to 45% led to the smallest particle size and PDI as well as highest EE. DSC and XRD studies on the optimized formulation (NC45) revealed that the incorporation of corn oil reduced the crystallinity degree of the lipid matrix and changed the polymorphic transition to α and β́ forms. These imperfections in the crystal lattice resulted in ovoid-shaped particles and prevented their aggregation as confirmed by TEM micrographs. The α-TC-loaded NLCs were also found to be stable during 90 days of storage at both temperatures of 4°C and 25°C. The findings of the present study clearly suggest that the addition of liquid oil can significantly improve the structure of lipid nanoparticles thus enhancing the retention of the encapsulated compound. Further, it seems that the contribution of the liquid oil to the physical and structural properties of NLCs is more significant than that of the solid lipid, which facilitates tailoring the particles' characteristics. Considering these results, these NLCs seem to be a useful delivery system for α-tocopherol in food and beverages. In future studies, it would be useful to investigate the release behavior of α-tocopherol from NLCs in food systems and its bioavailability.

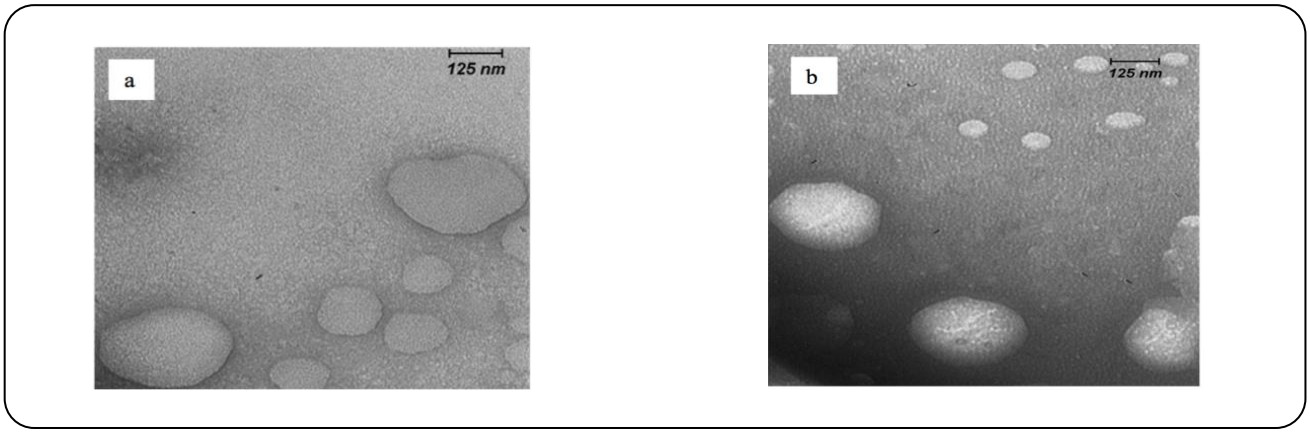

Fig. 3: Transmission electron microscopy images of alphatocopherol-free (a) and alphatocopherol -loaded nanostructured lipid carriers with 45% corn oil (b). Magnification: (×16,000).

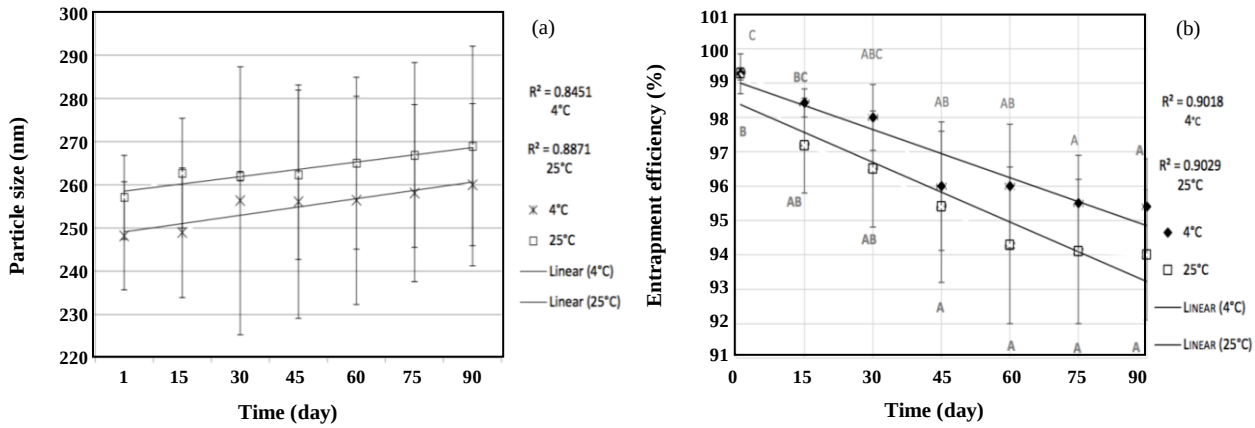

Fig. 4: X- Changes in particle size (nm) (a) and entrapment efficiency (b) of alphatocopherol -loaded nanostructured lipidcarriers with 45% corn oil during 90 days storage at 4 and 25 ◦C.

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REFERENCES

[1] Waniek S., Giuseppe R., Esatbeyoglu T., Plachta-Danielzik S., Ratjen I., Jacobs G., No¨thlings U., Koch M., Schlesinger S., Rimbach G. Lieb W., Vitamin E (α- and γ-Tocopherol) Levels in the Community: Distribution, Clinical and Biochemical Correlates, and Association with Dietary Patterns, *Journal of Nutrients,* **10:** 3 (2017).

[2] Yang Y., McClements D.J., Encapsulation of Vitamin E in Edible Emulsions Fabricated Using a Natural Surfactant, *Food Hydrocolloids,* **30(2)**: 712-720 (2013).

[3] Basiri L., Rajabzadeh G., Bostan A., Physicochemical properties and release behavior of Span 60/Tween 60 niosomes as Vehicle for a-Tocopherol Delivery, *LWT - Food Science and Technology*, **84**: 471-478 (2017).

[4] Drosou C.G., Krokida M.K., Biliaderis C.G., Encapsulation of Bioactive Compounds Through Electrospinning/Electrospraying and Spray Drying: a Comparative Assessment of Food-Related Applications, *Drying Technology*, **35:** 139-162 (2017).

[5] Chanburee S., Tiyaboonchai W., Enhanced Intestinal Absorption of Curcumin in Caco-2 Cell Monolayer Using Mucoadhesive Nanostructured Lipid Carriers, *J Biomed Mater Res B Appl Biomater.* **106(2)**: 734-741 (2018).

[6] Tamjidi F., Shahedi M., Varshosaz J., Nasirpour A., Nanostructured Lipid Carriers (NLC): A Potential Delivery System for Bioactive Food Molecules, *Innovative Food Science and Emerging Technologies*, **19**: 29–43 (2013).

[7] Zhang M., Falkeborgm M., Zheng Y., Yang T., Xu X., Formulation and Characterization of Nanostructured Lipid Carriers Containing a Mixed Lipids Core, *Colloids and Surfaces A: Physicochemical Engineering Aspects*, **430**: 76– 84 (2013).

[8] Matos de Carvalho S., Montanheiro Noronha C., Floriani C.L., Calegari Lino R., Rocha G., Casagrande Bellettini I., José Ogliar P., Manique Barreto P.L., Optimization of α-Tocopherol Loaded Solid Lipid Nanoparticles by Central Composite Design, *Industrial Crops and Products*, **49:** 278-285 (2013).

[9] Mehnert W., Mäder K., Solid Lipid Nanoparticles Production, Characterization and Applications, *Advanced Drug Delivery Reviews*, **47(2-3)**: 165–196 (2012).

[10] Rosli N.A., Hasham R., Abdul Aziz A., Design and Physicochemical Evaluation of Nanostructured Lipid Carrier Encapsulated *zingiber zerumbet* Oil by d-Optimal Mixture Design, *Journal of Technology*, **80(3)**: 105-113 (2018).

[11] Rincón M., Calpena A.C., Fabrega M., Garduño-Ramírez M.L., Espinam M., Rodríguez-Lagunas M.J., García M.L., Abrego G., Development of Pranoprofen Loaded Nanostructured Lipid Carriers to Improve Its Release and Therapeutic Efficacy in Skin Inflammatory Disorders, *Journal of nanomaterials*, **8:** 1322 (2018).

[12] Marsanasco M., Márquez A.L., Wagner J.R., Alonso S.V., Chiaramoni N.S., Liposomes as Vehicles for Vitamins E and C: An Alternative to Fortify Orange Juice and Offer Vitamin C Protection After Heat Treatment, *Food Research International*, **44(9)**: 3039–3046 (2011).

[13] Das S., Kiong Ng W., Tan R.B.H., Are Nanostructured Lipid Carriers (NLCs) Better Than Solid Lipid Nanoparticles (SLNs): Development, Characterizations and Comparative Evaluations of Clotrimazole-Loaded SLNs and NLCs, *European Journal of Pharmaceutical Science*, **47(1)**: 139–151 (2012).

[14] Tiwari R., Pathak K., Nanostructured Lipid Carrier Versus Solid Lipid Nanoparticles of Simvastatin: Comparative Analysis of Characteristics, Pharmacokinetics and Tissue Uptake, *International Journal of Pharmaceutics*, **415:** 232–243 (2011).

[15] Souza I.D.L., Saez V., Campos V.E.B., Mansur C.R.E., Size and Vitamin E Release of Nanostructured Lipid Carriers with Different Liquid Lipids, Surfactants and Preparation Methods, *Journal of Macromolecular Symposia*, **383(1)**:1-7 (2019).

[16] Byun Y., Hwang J.B., Bang S.H., Darby D., Cooksey K., Dawson P.L., Park H.J., Whiteside S., Formulation and Characterization of α-Tocopherol Loaded Poly 3-Caprolactone (PCL) Nanoparticles**,** *LWT - Food Science and Technology*, **44(1)**: 24-28 (2011).

[17] Luo Y., Zhang B., Whent M., Yu L., Wang Q., Preparation and Characterization of Zein/Chitosan Complex for Encapsulation of α-Tocopherol, and Its in vitro Controlled Release Study, *Colloids and Surfaces B: Biointerfaces*, **85(2)**: 145–152 (2011).

[18] Yu Y., Feng R., Yu S., Li J., Wang Y., Song Y., Yang X., Pan W., Li S., Nanostructured Lipid Carrier-Based pH and Temperature Dual-Responsive Hydrogel Composed of Carboxymethyl Chitosan and Poloxamer for Drug Delivery, *International Journal of Biological Macromolecules*, **15(114)**: 462-469 (2018).

[19] Zoghi A., Khosravi-Darani K., Omri A., Process Variables and Design of Experiments In Liposome and Nanoliposome Research, *Mini-review in medical chemistry*, **18(4)**: 324-344 (2016).

[20] Basiri L., Rajabzadeh G., Bostan A., α-Tocopherol-Loaded Noisome Prepared by Heating Method and Its Release Behavior, *Food chemistry*, **221**: 620-628 (2017).

[21] Fathi M., Varshosaz J., Mohebbi, Shahidi F., Hesperetin-Loaded Solid Lipid Nanoparticles and Nanostructure Lipid Carriers for Food Fortification: Preparation, Characterization, and Modeling, *Food Bioprocess Technology*, **6(6)**: 1464–1475 (2013).

[22] Carvalho S.P., Lucas E.F., Gonzálezb G., Spinelli L.S., Determining Hildebrand Solubility Parameter by Ultraviolet Spectroscopy and Microcalorimetry, *J. Braz. Chem. Soc.*, **24(12)**: 1998-2007 (2013).

[23] Qian C., Decker E. A., Xiao H., McClemen D.J., Solid Lipid Nanoparticles: Effect of Carrier Oil and Emulsifier Type on Phase Behavior and Physical Stability**,** *Journal of the American Oil Chemists Society,* **89(1)**: 17-28 (2012).

[24] Gamboa O.D., Guaraldo Gonçalves L., Ferreira Grosso C., Microencapsulation of Tocopherols in Lipid Matrix by Spray Chilling Method, *Procedia Food Science,* **1**: 1732-1739 (2011).

[25] Muller R.H., Radtke M., Wissing S.A., Nanostructured Lipid Matrices for Improved Microencapsulation of Drugs, *International Journal of Pharmaceutics,* **242(1-2)**: 121-128 (2002).

[26] Sun M., Nie S., Pan X., Zhang R., Fan Z., Wang S., Quercetin-Nanostructured Lipid Carriers: Characteristics Andanti-Breast Cancer Activities in Vitro, *Colloids and Surfaces B: Biointerfaces,* **113**: 15– 24 (2014).