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Original Article

SOLID LIPID NANOPARTICLES AND NANOSTRUCTURED LIPID CARRIERS OF TOLNAFTATE: DESIGN, OPTIMIZATION AND IN-VITRO EVALUATION

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ABSTRACT

Objective: The target of our work is the preparation of *tolnaftate* (TOL) loaded solid lipid nanoparticles (SLNs) as well as nanostructured lipid carriers (NLCs).

Methods: The high shear homogenization method was chosen for the preparation of nanoparticles. The nanoparticle dispersions were prepared using Compritol 888ATO, Tefose 63, Miglyol® 812, Poloxamer188, and Tween80. Particle size (PS), zeta potential (ZP), polydispersity index (PDI), drug entrapment efficiency (EE) and *in vitro* release study were determined. Differential Scanning Calorimetry (DSC) analysis and morphological transmission electron microscopy (TEM) examination were conducted. A stability study for 3 mo was performed.

Results: The results revealed that NLC and SLN dispersions had spherical shapes with an average size between 41.10±1.92 nm and 98.85±1.01 nm. High entrapment efficiency was obtained with negatively charged zeta potential with PDI value ranging from 0.251±0.012 to 0.759±0.028. The release profiles of all formulations were characterized by a sustained release behavior over 24 h and the release rates increased as the amount of liquid lipid in lipid core increased. *Tolnaftate* loaded NLC showed more stability than its corresponding SLN.

Conclusion: It can be fulfilled from this work that NLCs may represent a promising carrier for *tolnaftate* delivery offering both sustained release and stability.

Keywords: *Tolnaftate*, Solid lipid nanoparticles, Nanostructured lipid carriers, Tefose 63.

INTRODUCTION

Topical drug delivery is one of the most exciting and challenging issues for the pharmaceutical scientist [1]. A long time ago, topical drug application was emerged to achieve several goals on different levels. However, several problems have appeared with the conventional topical preparations e. g. low uptake due to the barrier function of the stratum corneum leading to low absorption to the systemic circulation [2]. Recently, many studies have suggested novel drug delivery systems that are based on lipid nanoparticles. These lipid nanoparticles have the potential to increase cutaneous drug delivery of both hydrophilic and lipophilic drugs when compared to the conventional formulations [3,4]. Topical application of Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers(NLCs) have been developed as novel systems that are composed of physiological lipid materials suitable for topical, dermal and transdermal administration [5].

Nowadays, Solid lipid nanoparticles (SLNs) are attracting a lot of attention as new colloidal drug carriers for topical use [6]. Nanostructured lipid carriers (NLCs) have been developed to rise above the drawbacks of SLNs, for example, drug leakage during storage and inadequate total drug load [7, 8]. NLCs are considered as the second generation (Müller *et al.*, 2002). NLCs are based on a mixture of solid lipids with incompatible liquid lipids [5]. NLC shows many characteristics for application of pharmaceutics, e. g. controlled release of actives, targeting of drugs, and enhancing the amount of drug penetrating into the mucosa. SLN and NLC were investigated for several routes of administration, such as parenteral [6], oral [9] and topical routes [10] providing controlled release systems for many actives. SLN and NLC have been used in cosmetic and dermatological formulations [5]. Both SLN and NLC possess a lot of features that are advantageous for the topical route of administration [11, 12].

Tolnaftate (TOL) is a synthetic thio carbamate that is used as the topical antifungal agent. It inhibits the squalene epoxidase enzyme [13]. Squalene epoxidase is an essential enzyme in the biosynthetic pathway of ergosterol which is an important constituent of the fungal membrane. TOL is only active by topical application and inactive when administered via oral and intraperitoneal routes [14, 15]. Although TOL is present in the market in several topical dosage forms e. g. cream, powder, spray and liquid aerosol, each of these dosage forms has its own disadvantages. These disadvantages include mild temporary stinging caused by aerosols and poor penetration noticed by creams and gels. This poor penetration needs a long time of therapy and decreases the patient compliance [16].

The objective of the present work was to study the preparation, characterization and optimization of TOL loaded SLN and NLC in order to increase the release, stability and patient compliance of TOL dosage forms.

MATERIALS AND METHODS

Materials

Tolnaftate was kindly donated by Misr Company for drugs and pharmaceuticals, Cairo, Egypt. Glyceryl behenate (Compritol® ATO 888) and Glycol stearate (Tefose®63) was generously supplied by Gattefossée (Nanterre, France). Poloxamer 188 (Pluronic F68), Tween 80 and Miglyol® 812 were obtained from Sigma-Aldrich (St. Louis, USA). Dialysis tubing cellulose membrane (molecular weight cut-off 12,000-14,000 g/mole), was supplied by Sigma-Aldrich (St. Louis, USA). Methanol, Potassium dihydrogen orthophosphate, and citric acid were of analytical grade.

Preparation of TOL-loaded SLNs and NLCs

The high shear homogenization technique was used to prepare TOL-loaded SLNs and NLCs followed by ultrasonication [17-19]. The lipid phase containing Compritol ATO 888 was melted to approximately 5 °C above its melting point (74.09 °C), TOL was dispersed in the melted lipid. The aqueous phase was prepared by dissolving the surfactant in distilled water and heating up to the same temperature of the molten lipid. The aqueous phase was poured into the lipid phase, at a stirring speed of 25,000 rpm for 5 min using Heidolph homogenizer (Silent Crusher Homogenizer, Germany). The obtained

O/W emulsion was sonicated for 30 min and was cold down in the ambient resulting in the lipid phase recrystallization, and finally, the SLN was formed [20]. Different formulations have been studied by varying the concentration of both lipid and surfactant to study their effect on physical properties. After determination of the best concentration of lipid and surfactant, preparation of NLCs was carried out using the same method, by replacing 30% and 50% of the solid lipid with liquid Miglyol® 812.

Characterization of TOL-loaded nano particles

Drug entrapment efficiency

The entrapment efficiency (E. E. %) was determined indirectly by measuring the concentration of the drug in the supernatant after centrifugation [21]. The unentrapped TOL was determined by adding 1 ml of TOL loaded nanoparticles to 9 ml methanol and then this dispersion was centrifuged at 9000 rpm for 30 min at-4 ℃. The supernatant was collected, filtered through millipore membrane filter (0.2 µm) then diluted with methanol and measured spectrophotometrically at λ=256.8 nm. The entrapment efficiency was calculated using the following equation:

$$\text{E. E. \%} = \frac{\text{Winitial} - \text{Wfree}}{\text{Winitial}} \text{ x } 100$$

Measurement of particle size and poly dispersity index

Particle size (PS) and poly dispersity index (PDI) were measured by photon correlation spectroscopy (PCS) using a Zetasizer (Zetasizer Nano ZS; Malvern), at a fixed angle of 90 ° at 25 °C. The aqueous SLN and NLC dispersions were diluted with distilled water before analysis. Each sample was measured in triplicate.

Measurement of zeta potential

The zeta potential (ZP) of SLN and NLC dispersions was measured at 25 °C using a Zetasizer (Zetasizer Nano ZS; Malvern). The measurements were conducted in triplicate.

Differential scanning calorimetry

Thermal characteristics of selected SLN and NLC were determined by differential scanning calorimetry (DSC) (Shimadzu, Kyoto, Japan). SLN and NLC dispersions were lyophilized before DSC analysis using freeze dryer (VirTis Freeze Drying Equipment, Snijders Scientific). Samples containing 10 mg nanoparticle dispersions were weighed accurately into standard aluminum pans using an empty pan as a reference. DSC scans were recorded at a heating and cooling rate of 10 °C/min. The samples were heated from 30-300 °C and cooled from 300-30 °C under liquid nitrogen.

Transmission electron microscopy

The morphologies of the SLN and NLC were examined by transmission electron microscopy (TEM) (CM12 Philips, Amsterdam, Netherlands). One drop of the diluted sample was stained with 2 % (W/V) phosphotungstic acid for 30 seconds and placed on copper grids with films for examination.

***In-vitro* drug release of TOL from SLNs and NLCs**

The dialysis bag diffusion technique was chosen to perform the *in vitro* release studies [22]. The dialysis bag (molecular weight cut off 12000–14000) was soaked in deionized water for 12h before use [23]. The cellulose bag was filled with the SLN and NLC dispersions equivalent to 2 mg of drug and tied at both ends and placed in a beaker containing 50 mL of phosphate buffer (pH 5.5), temperature and speed were maintained at 32 ℃ and 100 rpm, respectively [24]. Samples were withdrawn at predetermined time intervals, and the same volume was replaced with fresh buffer to maintain the sink condition. Samples were analyzed at 256.2 nm UV spectrophotometrically. The cumulative percent of drug released was plotted against time. The order of the drug released from the different formulations was determined through analysis of the data using linear regression equations (zero order, first order or Higuchi diffusion model).

Stability test for the optimized TOL-SLNs and NLCs

The selected SLN and NLC formulations were stored in a sealed amber colored glass vials to be protected from light and water at refrigerator temperature (2-4 °C) in a dark environment. Physical appearance was assessed, and the formulations were analyzed with respect to drug entrapment efficiency, particle size and zeta potential after 1, 2 and 3 mo of storage and compared with fresh formulations.

Statistical analysis

Data was expressed as mean±SD. The results were statistically analyzed by analysis of variance (ANOVA) test using social package for statistical study software (SPSS 17®, SPSS Inc., Chicago, USA); P values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

Preparation of SLNs and NLCs

Ten different SLN and NLC formulations produced by high shear homogenization method are presented in table 1. Various parameters were optimized by varying one parameter while keeping others constant. It is recognized that the stabilization of the disperse system can be reached by the employment of two surfactants of lipophilic and hydrophilic nature [23]. Tefose 63 and Poloxamer 188 were chosen for the preparation of TOL loaded solid lipid nanoparticles as lipophilic and hydrophilic surfactants, respectively. The formulation F8 showing high entrapment efficiency was chosen for the production of NLC.

Characterization of TOL loaded nanoparticles

Entrapment efficiency

Entrapment efficiencies of all SLNs and NLCs are presented in table 2. Among the SLN formulations, increasing the concentration of Compritol ATO 888 from 3 % w/w to 5 % w/w at a constant amount of surfactant 2.5 % w/w results in an increase in entrapment efficiencies of TOL SLNs as shown in SLN2 and SLN4. This can be justified by increasing lipid concentration offer more space to encapsulate more drugs which decrease the drug partition in the outer phase [25]. As shown in SLN5 and SLN6, there is no significant increase in the entrapment efficiency after addition of Tefose 63 in the presence of Tween 80. However, a significant increase ($p<0.05$) in the entrapment efficiency was observed in SLN8 upon addition of Tefose 63 in the presence of Poloxamer 188. This may be due to high HLB value of Poloxamer 188 than Tween 80 [26]. The higher HLB values may enhance the encapsulation efficiency depending on the reduction of interfacial tension and enhancement of solubilization of drug [27]. High entrapment efficiency was recorded for SLN8 (80.15 %) compared to other dispersions. Depending on high entrapment efficiency of SLN8, NLC1 and NLC2 were prepared by replacing 30% and 50% w/w of Compritol ATO 888 by Miglyol 812. NLC2 formulation showed highest entrapment efficiency (86.20±0.36%). It was found that the solubility of the drug is higher in liquid lipid than in solid lipid, which in turn increases the entrapment efficiency [28]. The presence of liquid lipid with solid lipid cause a reduction in the crystallinity and increasing imperfections in the crystal lattice leaving enough space to accommodate drug molecules, which in turn, enhance drug loading capacity and drug entrapment efficiency [29].

Particle size, poly dispersity index and zeta potential

The mean particle size, poly polydispersity index and zeta potential of colloidal carriers are important characteristics of SLNs from which the stability of the compound loaded SLNs can be predicted. Average particles size, polydispersity, and zeta potential are shown in table 2.

The results revealed that all the sizes of the SLN and NLC are in the nano-size range. Sizes ranged from 41.10±1.92 nm and 98.85±1.01 nm. The results reveal that the particle size increase with increasing Compritol ATO 888 concentration from 2.5% w/w (SLN1 and SLN3) to 5%w/w (SLN2 and SLN4), this could be explained by the fact that homogenization efficiency decreases with increasing content of dispersed phase (lipid phase) [30]. When the concentration of the lipid exceeded 2.5% w/v with a fixed concentration of surfactants, there was insufficient surfactant available to coat the surface of all the lipid droplets, resulting in particle aggregation and increase in particle size [31]. The presence of Tefose 63 cause a decrease in the

particle size in the presence of Poloxamer 188 as shown in SLN7 and SLN8 compared to SLN4, however, his presence with Tween 80 show an increase in the size as shown in SLN5 and SLN6 compared to SLN2. As shown in table 2 there is no significant difference in the particle size of NLC1 and NLC2 and their corresponding SLN7 and SLN8 respectively.

The polydispersity index is a ratio providing information about the homogeneity of particle size distribution in the system. A PDI value lower than 0.3 indicates a homogeneous and monodisperse population [32]. For SLNs, PDI values ranged from 0.276±0.01 to 0.759±0.02 indicating wide particle size distribution while PDI results for NLC were somehow lesser, indicating homogenous population as presented in table 2. The higher the polydispersity, the lower the uniformity of the vesicle size in the formulation [33].

The stability of colloidal dispersion is highly related to the zeta potential value [23]. The zeta potential values of different TOL-SLN and NLC formulations are presented in table 2. The results showed a relatively good stability and dispersion quality. The nano particles are thermodynamically unstable systems and for stability, the zeta potential value should be above+30 mV or below-30 mV [34].

Usually, particle aggregation is less likely to occur for charged particles with high zeta potential (>30) due to electric repulsion which prevents aggregation of the particles [35]. For SLN3 and SLN4, the zeta potential data were-34.7±1.50 and-33.4±0.98, respectively. It was known that, The use of steric stabilizer favored the formation of stable nanoparticle dispersion [23]. Poloxamer 188 provides a steric stability for maintaining the stability of SLNs [36].

Table 1: Composition of TOL loaded SLNs and NLCs

Formulations	Compritol ATO 888 (w/w %)	Surfactant (w/w %)	Liquid lipid (w/w %)
SLN1	3	Tween 80(2.5%)	-
SLN2	5	Tween 80(2.5%)	-
SLN3	3	Poloxamer188 (2.5%)	-
SLN4	5	Poloxamer188 (2.5%)	-
SLN5	5	Tween 80(2.5%)+Tefose 63 (2.5%)	-
SLN6	5	Tween 80(2.5%)+Tefose 63 (5%)	-
SLN7	5	Poloxamer188 (2.5%)+Tefose 63 (2.5%)	-
SLN8	5	Poloxamer188 (2.5%)+Tefose 63 (5%)	-
NLC1	5	Poloxamer188 (2.5%)+Tefose 63 (5%)	Miglyol® 812(30%)
NLC2	5	Poloxamer188 (2.5%)+Tefose 63 (5%)	Miglyol® 812(50%)

Table 2: Particle size, Zeta potential, PDI and EE (%) of TOL loaded SLN and NLC (mean±SD, N=3)

Formulations	E. E. (%)	PS(nm)	ZP (mV)	PDI
SLN1	52.76±0.18	53.10±4.00	-22.5±1.06	0.519±0.06
SLN2	64.38±0.96	73.93±3.45	-21.9±1.11	0.477±0.04
SLN3	46.61±0.79	41.10±1.92	-34.7±1.50	0.759±0.02
SLN4	64.54±1.19	72.99±4.22	-33.4±0.98	0.411±0.02
SLN5	53.12±1.03	95.43±2.93	-22.6±0.98	0.276±0.03
SLN6	64.66±0.56	98.85±1.01	-21.8±1.27	0.404±0.07
SLN7	69.25±0.81	45.32±1.06	-22.1±1.23	0.697±0.06
SLN8	80.15±0.72	47.40±2.53	-18.5±2.50	0.404±0.03
NLC1	80.41±0.36	45.46±2.34	-22±1.01	0.271±0.02
NLC2	86.20±0.36	44.56±0.52	-19.2±1.97	0.251±0.01

Transmission electron microscopy

The results of TEM imaging of TOL loaded SLN and NLC, which are shown in fig. 1A and 1B, indicate that the particles had nanometer-size spherical shapes, and no drug crystal was noticed. fig. 1C represents the enriched shell model which might be explained by the significant difference between the melting points of the lipid and the drug. The drug-enriched shell model is characterized by the drug located at the interface of the lipid and the surfactants, either by fast solidification of the lipid matrix, or the successful competition of the drug for the interface due to solubility properties. According to this model of drug incorporation, a solid lipid core is formed when the recrystallization temperature of the lipid is reached [37].

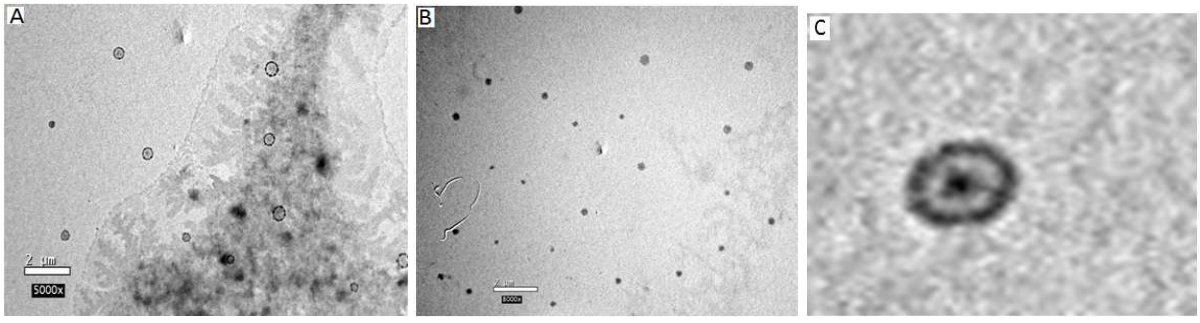

Fig. 1: Transmission electron micrograph of (A) TOL loaded NLC; (B) TOL loaded SLN and (C) NLC micrograph representing the core and shell theory

Differential scanning calorimetry (DSC)

DSC is used to investigate the melting and recrystallization behavior of crystalline material like SLNs [38]. The physical state for NLC/SLN lipid matrix should be in the form of solid. DSC studies had revealed that all formulations possess melting point over 40°C which indicate the solid state at room temperature [39]. Thermogram of the pure drug, as well as of Compritol ATO 888, Tefose 63, compared with the

thermograms of lyophilized TOL loaded SLN and NLC formulae in the range of 10-350 °C are shown in fig. 2. TOL thermogram demonstrates a sharp endothermic peak at 112.2 °C. A sharp endothermic peak at 75.71°C was observed for Compritol ATO 888. Three consecutive endothermic peaks were shown for Tefose 63 thermogram, namely at 35.43 °C, 46.43 °C and 58.29 °C. The endotherm of the drug was completely absent in the thermograms of TOL loaded SLN and NLC. The absence of the drug endotherm indicates either formation of an amorphous dispersion of TOL in the lipid matrix or that the drug was completely solubilized inside the lipid matrix of the SLN and NLC. For the less ordered crystal, the melting of the substance requires less energy than the perfect crystalline substance. It is reported that if a substance has high melting point value, this would suggest highly ordered lattice arrangement [40]. The amorphous form is thought to have high energy with an increase in the surface area leading to higher solubility, dissolution rates and bioavailability of the drug incorporated [41, 42]. Incorporation of TOL inside the lipid matrix led to more defects in the lipid crystal lattice. This caused a decrease in the melting point of the lipid, from 75.71 °C to 67.26°C and 61.87 °C in the final SLN and NLC formulations, respectively. These melting point depressions might be due to the small particle size (nanometer range), the high specific surface area, and the presence of a surfactant, i.e., these depressions may be due to the Kelvin effect [43]. Kelvin showed that small, isolated particles would show lower melting temperature than that of bulk materials.

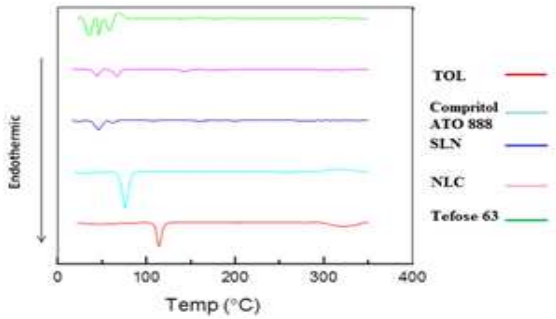

Fig. 2: DSC thermograms of pure TOL, bulk Compritol ATO 888, Tefose 63, SLN and NLC formulations

Release study

The *in vitro* drug release profiles of TOL loaded SLNs and TOL loaded NLCs are shown in fig. 3 and 4. In order to compare the drug release profile of the prepared SLN and NLC formulations, the release of TOL from the lipid particles was investigated for 24 h. The maximum amount of TOL released was found from the formulation SLN8 as shown in fig. 3. This increase may be attributed to the presence of Tefose 63 with a concentration 5%w/w compared to other formulations as increasing surfactant concentration leads to an increase in the percent of drug released. This can be explained by, as during particle production by the hot homogenization technique, drug partitions from the liquid oil phase to the aqueous water phase. As the solubility of the drug increase in water, the amount of drug partitioning to the water phase will increase. The higher the temperature and surfactant concentration, the greater is the solubility of the drug in the water phase, so the amount of drug in the outer shell increased and released in a relatively rapid way [44].

Due to the addition of liquid lipid in the NLC1 and NLC2 formulations, a significant increase in the amount of drug released in comparison to SLN8 as seen in Fig.4. This increase due to adherence of liquid lipid to the lipid matrix and decreases the diffusion path length of the lipid matrix [23]. In addition, the increase of Miglyol 812 from 30% to 50%w/w in NLC2 lead to an increase in the percent of drug released. This may be due to increasing of the percent of drug dissolved in the presence of liquid oil leading to increasing the amount of drug in the outer shell increased and released more. The release data are analyzed according to zero, first order and Higuchi equations which are widely used in determining the release kinetics of lipid nanoparticles. The amount of TOL released from both the SLN and NLC formulations studied shows a linear relationship with the square root of time. Therefore, the release rate of TOL is expressed following the theoretical model by Higuchi [45].

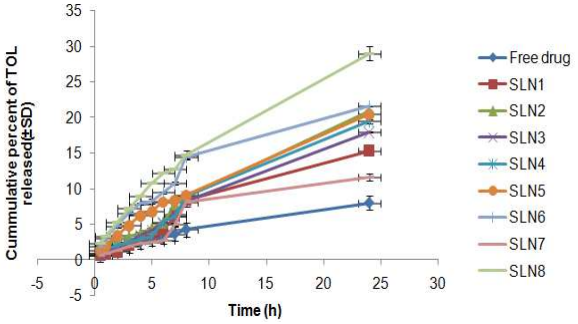

Fig. 3: *In-vitro* drug release profile of SLN formulations

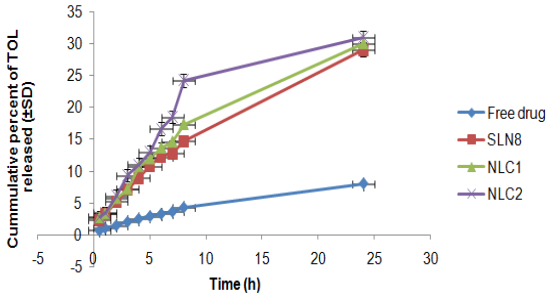

Fig. 4: *In-vitro* drug release profile of NLC formulations compared to SLN formulation

Table 3: Stability profiles of TOL SLN and NLC

Items for comparison	Formulations	
	SLN8	NLC2
Entrapment efficiency (%)		
Fresh	80.15±.72	86.20±0.30
1 mo	73.71±0.91	85.44±1.46
2 mo	64.17±0.75	83.67±0.50
3 mo	63.45±0.23	83.42±0.75
Particle size (nm)		
Fresh	44.23±2.53	44.56±0.52
1 mo	47.40±0.45	43.26±0.32
2 mo	50.33±0.22	43.41±0.21
3 mo	51.54±0.32	43.45±0.23
Zeta potential (mV)		
Fresh	-18.5±2.50	-19.2±1.97
1 mo	-17.3±0.54	-18.9±1.10
2 mo	-16.9±0.32	-18.3±0.35
3 mo	-16.9±0.45	-19.2±1.02

Stability test

The stability study was done for 2 formulations: SLN8 and NLC2 which were stored away from light and water in sealed amber colored glass vials. From the table 3, it was found that the entrapment efficiency and the particle size of freshly prepared SLN8 showed a significant decrease ($p<0.05$) and a significant increase ($p<0.05$) respectively compared to the stored SLN8 during the 3 mo. However, no significant change was recorded in the particle size and the entrapment efficiency in case of NLC2.

CONFLICT OF INTERESTS

The authors who have taken part in this study declared that they don't have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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