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Is it possible to improve antioxidant activity of curcumin with the structure of lipid- based nanocarriers?

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ABSTRACT: Although curcumin is a commonly used antioxidant in cosmetic and pharmaceutical products, the dermal efficacy of curcumin is limited due to its very low solubility and poor permeability. Considering dermal application, lipid-based nanocarriers (LbNs) are highlighted as promising delivery systems for enhancing the efficacy of active substances. The aim of this study is to evaluate the effect of the components of LbNs on the antioxidant activity of curcumin. For this purpose, the mixture of Precirol® ATO5 and Tristearin was selected as solid lipid; while vitamin E, pomegranate seed oil (PSO) and Labrafac® Lipophile WL 1349 were used as liquid lipids. Solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and nanoemulsion (NE) formulations were designed by using different combination of these lipids and Gelucire®50/13 as an amphiphilic surfactant. The particle size, PDI, zeta potential analysis, encapsulation efficiency and in-vitro release studies were performed as particle characteristics. The contribution of LbNs with different structures to the antioxidant capacity of curcumin was evaluated by in-vitro ABTS scavenging experiment. While SLN has an average particle size of 106.7 nm with negative zeta potential, the size of NLC and NE formulations were below 100nm. The highest curcumin release upon 24h was obtained by NLC formulation prepared with PSO. A significant contribution to the antioxidant activity of curcumin was obtained when vitamin E and PSO were used as liquid lipid in NLC and NE formulations. In conclusion, it is possible to improve the antioxidant effect of curcumin by the modification of the structure of LbNs.

KEYWORDS: Lipid-based nanocarriers; Solid lipid nanoparticles; Nanostructured lipid carriers; Nanoemulsions; Curcumin; Antioxidant effect.

1. INTRODUCTION

Curcumin is a natural, bioactive polyphenolic compound in diketone structure extracted from turmeric (*Curcuma longa*) [1-2]. In general, it has a wide range of biological activities, including antioxidant, anti-atherosclerosis, anti-tumor, anti-inflammatory, lipid regulation, antimicrobial and anti-Alzheimer's disease, etc [3-6]. Its antioxidant activity is a remarkable feature as it makes curcumin very valuable in many fields such as food, medicine and cosmetics.

It is known that damage to cell membranes and biomolecules such as lipids, proteins, and deoxyribonucleic acid (DNA) is caused by free radicals. Free radicals are highly reactive because the unpaired electrons cause other compounds to impart high reactivity. They can occur as a part of the normal physiological cell metabolism, but is also produced during biochemical redox reactions in response to environmental factors such as UV light and plays a role in the formation of various metabolic disorders in the organism, including many types of cancer [7]. While free radicals are any molecules that possess unpaired electrons and are therefore highly unstable and reactive; Reactive Oxygen Species (ROS) are the subset of oxygen-containing free radicals and they are the major cause of oxidative stress. It has been emphasized in many studies that oxidative stress is the leading factor underlying cardiovascular, neurological, and respiratory diseases, cancers, kidney defects, rheumatoid arthritis and even skin aging [8]. Antioxidant molecules are also known as free-radical scavengers and the antioxidant activity can be defined as the effect of molecules that have the ability to react with free radicals that damage body structures by limiting oxidative chain reactions [9]. Antioxidant compounds neutralize free radicals, prevent the body from being affected by them and enable it to renew itself. On the other hand, the unique antioxidant activity of curcumin is due to

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the fact that it contains a phenolic structure and curcumin fulfills its antioxidant role by scavenging free radicals involved in peroxidation reactions [2,10]. In addition, curcumin, one of the world's best-selling natural food additives, is classified in the "generally recognized as safe" (GRAS) category by the American Food and Drug Administration (FDA) [11-13].

Nanotechnology, which includes the study and application of extremely small objects, is very popular in pharmaceuticals as well as being adapted to different field of science. Because nanoscale and nano-drug delivery systems are highly important for the pharmaceutical sciences as they provide a solution for the API, which is insufficient in treatment alone. From this point of view, especially lipid-based nanocarriers (LbNs) will provide the opportunity for the effective incorporation of natural compounds into pharmaceutical and cosmetic products based on their lipid structures [14]. On the other hand, the profits of LbNs such as their low toxicity due to their biodegradable and biocompatible nature, their non-allergic or non-irritant skin response performance, improved physicochemical stability, and the ability to modify their drug release profile make them advantageous carriers over other nanosystems [15]. Nanoemulsions are transparent/translucent, heterogeneous systems in which two immiscible liquids are stabilized with an interfacial layer of emulsifiers and co-emulsifiers. They are both thermodynamically and kinetically stable systems [16]. With nanotechnological advances, unlike conventional emulsions, the droplet size in nanoemulsions has become incredibly small. They generally exhibit uniform and small droplet size ranging from 20 to 200 nm and high solubility capacity for lipophilic drugs [17-19]. On the other hand lipid-based nanoparticulate systems such as nanostructured lipid carriers (NLC) are obtained when some of the liquid lipid used in nanoemulsions is replaced with solid lipids at room and body temperature, and solid lipid nanoparticles (SLN) are obtained when all of the liquid lipid is replaced with solid lipid.

Since the structures of the aforementioned lipid-based nanocarriers vary according to the ratio and type of lipids used in their production, the effect of the physical states of nanocarriers on the behavior of the encapsulated active substance was investigated. Hence the aim of this study is to investigate the contribution of LbNs with different structures to the antioxidant capacity of curcumin. In addition, the synergistic effects of liquid lipids on antioxidant capacity of curcumin, which are known to have antioxidant effects were also examined.

2. RESULTS AND DISCUSSION

Herein, curcumin loaded lipid-based nanocarriers with different structures according to the ratio and type of lipids were succesfully produced. Three different liquid lipids were used for preparation of NLC and NE formulations. PSO is an vegetable oil which has an important antioxidant activity because of its phytochemical composition enriched with high punicic acid content and the presence of phenolic compounds, especially ortho-diphenols [20-22]. Vitamin E is a non-enzymatic lipid-soluble antioxidant which protects skin from oxidative stress and photoaging as well as stabilize the compounds in an oxidative enviroment [23]. The other liquid lipid used in this study is Labrafac™ Lipophile WL 1349. Labrafac is a medium-chain triglycerides which consists of caprylic and capric acids and it does not have a known antioxidant property. In this study, lipid-based nanocarriers were selected as the drug delivery system in order to increase the expected therapeutic effect of the curcumin which is known as highly soluble in oil or lipids and practically insoluble in water at room temperature, acidic and neutral pH. On the other hand, LbNs with different structures were preferred due to the high lipid solubility of curcumin and considering the advantages of lipids in dermal application. Another major factor of entrapping curcumin in LbNs is to increase the chemical and physical stability of curcumin. It has been emphasized in many studies that curcumin is degraded at physiological pH and it has been determined that the water dispersibility and chemical stability of curcumin are improved by oil-in-water emulsions [24, 25].

Gelucire® 50/13 was choosen as surfactant to form LbNs since it is a biocompatible amphiphilic lipid excipient [26, 27]. Gelucire®s are multifunctional lipid excipients which are generally accepted in GRAS (generally recognized as safe) category by FDA [28]. They are usually identified by two numbers, the first representing the lipid melting point and the second indicating the HLB value. Therefore, Gelucire®s have properties such as emulsification, increasing drug solubility and granule formation [29]. Gelucire® 50/13 is generally used to increase the solubility and bioavailability of drugs and to prepare the self emulsifying drug delivery systems [30-33]. Due to its high HLB value it can act as a stabilizer for lipid nanosuspensions, thus eliminating the requirement for additional surfactants [34].

The average particle size and polydispersity index of all formulations were measured by dynamic light scattering (DLS) which is the most useful method for physicochemical characterization of nanoparticles in a dispersion. This method measures the fluctuation of the intensity of scattered light caused by particle motion [35-37]. While the average particle size of the SLN prepared with Gelucire® 50/13 was 106.7 nm, the PDI value

was measured as 0.362. When different liquid lipids were used, means the SLN structure was changed to the NLC structure, a significant reduction in particle size was observed. NLCs prepared using different oils have particle sizes between 71.73 nm and 90.22 nm and their PDI values were between 0.230 and 0.422. On the other hand droplet sizes of NEs were between 74.15 nm and 96.68 nm, while the PDI values of NEs were ranged from 0.240 to 0.351. The particle size of NLC and NE formulations were found smaller compared to SLN. The mixing of solid and liquid lipids could cause viscosity differences that affect the size of nanoparticles. In addition, it was previously reported that the liquid lipid has a less ordered structure than the solid lipid. This provides a higher flexibility in rearranging the oil molecules, therefore reducing the surface tension to form smaller particles [38-41]. In our study, the smallest particle size was obtained in the presence of Labrafac among the prepared NLC formulations and when Vitamin E was used among the NEs. On the other hand, the PDI of all formulations were found to be below 0,5 and it was indicated that all LbNs have narrow size distribution [42-44].

Zeta potential is the estimation of the surface charge gained by the particles in the dispersed state and is an important parameter for the colloidal physical stability of the particle dispersions [45]. LbNs with high zeta potential values are considered stable and less prone to aggregate formation or increase in particle size [46]. For all formulations negative zeta potential values were measured in the range of (-24.7 – -16.4) mV as reported in Table 1. Zeta potential values measured in our study are similar to the results obtained in the literature [47, 48].

Table 1. Particle size, PDI and zeta potential results of lipid based nanocarriers

	Particle size (nm ± SD)	PDI ± SD	Zeta Potential (mV ± SD)
SLN	106.7 ± 1.766	0.362 ± 0.009	-20.4 ± 1.04
Vitamin E NLC	90.22 ± 0.572	0.422 ± 0.018	-19.7 ± 3.98
PSO NLC	80.31 ± 0.618	0.381 ± 0.010	-18.2 ± 1.87
Labrafac NLC	71.73 ± 0.302	0.230 ± 0.007	-17.1 ± 1.46
Vitamin E NE	74.15 ± 0.805	0.351 ± 0.004	-24.7 ± 2.93
PSO NE	91.79 ± 1.174	0.240 ± 0.003	-23.9 ± 1.85
Labrafac NE	96.68 ± 0.756	0.264 ± 0.006	-16.4 ± 0.77

The encapculation efficiency (EE%) of LbNs were determined by indirect method. EE% is strongly depend on the solubility of the drug in the carrier, the size of the carrier as well as the chemical interactions between the drug and the carrier. Therefore, EE% is one of the most important parameters in developing nanoparticle drug delivery systems which is the indicator of loading efficiency of the drug due to the size of the reservoir in which the drug molecule is loaded is extremely small. In this study, The EE% values of all formulations were found higher than 99% and it was concluded that almost all of the curcumin was entrapped into the nanocarriers. The high drug loading is assumed to be the oil soluble nature of curcumin [49]. From another point of view, high encapsulation of curcumin, which has low water solubility and low bioavailability, in the lipid structured nanoparticle platform is a convenient and advantageous way to achieve its targeted dermal delivery. Therefore, it is predicted that increased efficiency can be achieved when the high encapsulation efficiency values of curcumin and the surface areas of the nanoparticles as well as their ability to enter the cell are taken into account.

The release profiles of curcumin loaded LbNs were shown in Figure 1. The curcumin release was significantly higher when NLC structure were formed than SLNs and NEs as well. It was concluded that the imperfections in the lipid matrix of NLC structure in comparison with the perfect lipid crystal matrix of SLN contributes the higher release rate of curcumin. At the end of the 24th hour, the maximum release of curcumin was found as 53% from PSO NLC coded formulation while 48% and 46% were determined for Vit E NLC and Labrafac NLC respectively. On the other hand, the lowest release amounts were obtained by NE formulations. It was revealed that, the type of liquid lipid that used for preparation of nanoemulsions has no effect on the release profile of curcumin. Mathematical models, including zero-order, first-order, Higuchi and Hixson-Crowell were studied to examine the release behavior of curcumin from formulations of SLN, PSO-containing NLC, and PSO-containing NE. The suitability of a kinetic model can be predicted by the high r-squared (r^2) value while the high Anova F value supports the r^2. Therefore, the release constant (k), r-squared (r^2) and Anova F values were calculated to estimate the suitability of these models, as shown in Table 2. The release of

curcumin from the SLN formulation which has a rigid lipid core can be explained by the first-order kinetics since the highest r^2 and F values were calculated. Similarly, the obtained release data of NLC and NE were fitted into first order kinetic even if the liquid lipid increase in the nanocarrier structure. Hence it is clear that the increase in liquid lipid in the nanocarrier structure has no effect on the drug release mechanism.

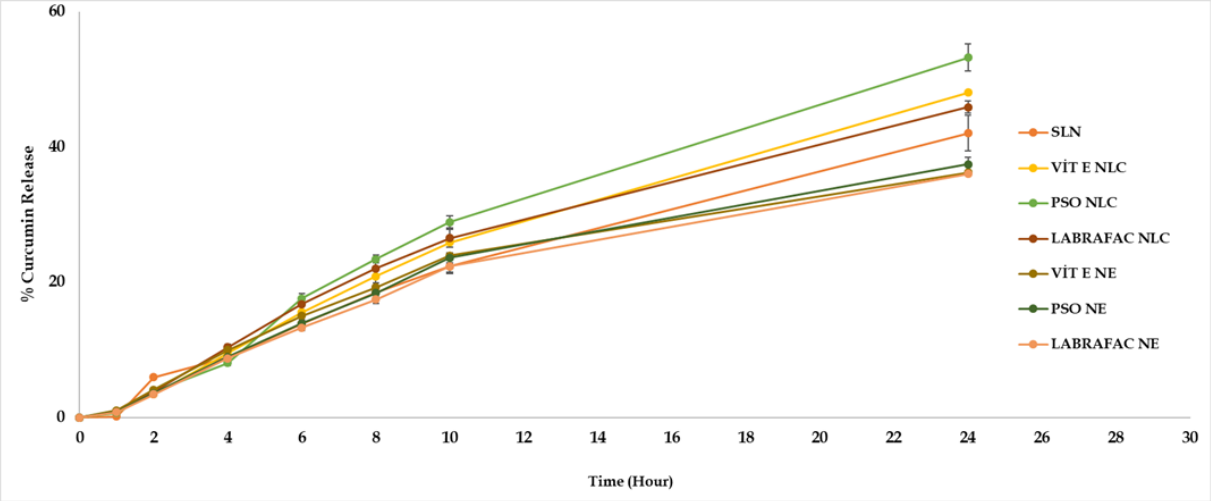

Figure 1. *In vitro* curcumin release profiles of LbNs (n=3)

Table 2. The results of the kinetic evaluation of release profiles

Kinetic models		Formulations		
		SLN	PSO NLC	PSO NE
Zero order	k_r^0	5.005	2.305	1.533
	r^2	0.330	0.280	0.350
	RMS	5800.396	5680.850	5732.380
	F	3.442	2.716	3.764
	SE	2.698	2.670	2.682
First order	k_r^1	0.023	0.033	0.019
	r^2	0.993	0.995	0.969
	RMS	0.000	0.000	0.001
	F	837.196	1244.346	189.772
	SE	0.001	0.001	0.001
Higuchi	k	7.076	9.398	0.149
	r^2	0,945	0.952	0.958
	RMS	22.952	34.916	0.331
	F	119.972	139.096	159.117
	SE	0.646	0.797	0.012
Hixson-Crowell	k	1.152	1.540	0.857
	r^2	0.928	0.944	0.957
	RMS	11.703	14.673	3.790
	F	90.402	119.004	154.462
	SE	0.121	0.136	0.069

According to the particle size and *in vitro* release results, NLC and NE formulations preparing using PSO were choosen and morphological properties were compared with SLN. For morphological evaluation, SLN, PSO NLC and PSO NE were visualized by TEM analysis and the micrographs were shown in Figure 2. Screening of formulations revealed that the formulations have spherical particles and droplets, uniformly dispersed without aggregation. The size of nanoparticles observed with TEM was also found to be compatible with DLS measurement.

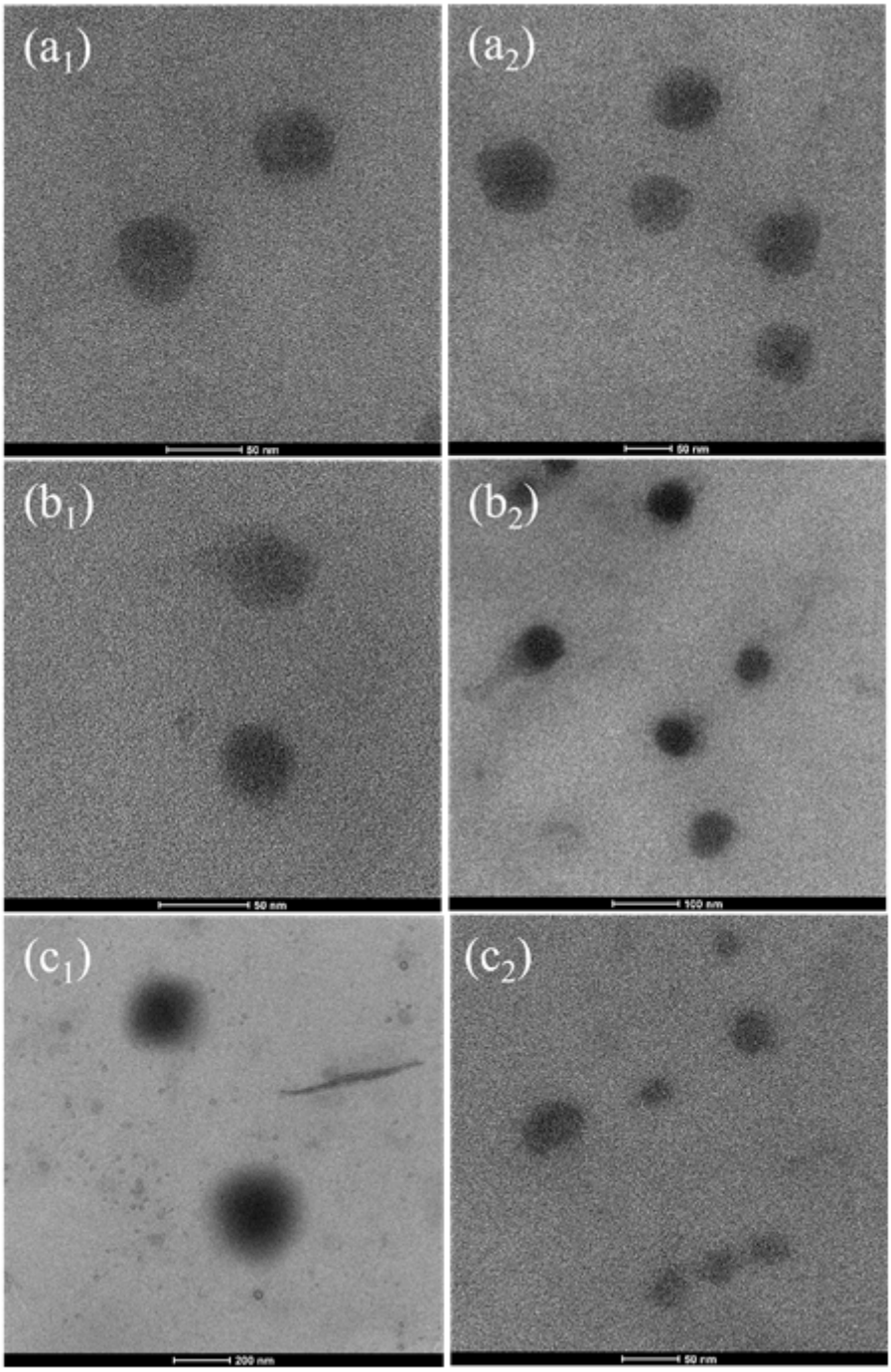

Figure 2. TEM images of optimized SLN (a_1, a_2), PSO-NLC (b_1, b_2), and PSO-NE (c_1, c_2) formulations.

The free radical scavenging (antioxidant) capacity of SLN, NLC and NE formulations were determined by Trolox equivalent antioxidant capacity assay (TEAC). TEAC is a common method in order to evaluate the ability of a compound to scavenge ABTS (2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radicals since it is a rapid and an easy method which correlates with the biological activity of antioxidants (Arts et al.,2004). This method is based on formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide which oxidizes the ABTS to produce $ABTS^{\cdot+}$. This radical cation can be determined at 405 nm by spectrophotometer [50]. In our study, the *in vitro* antioxidant capacity of LbNs were investigated and the results were expressed as the TEAC value. The $ABTS^{\cdot+}$ scavenging activity of SLN, NLC and NE formulations equivalent to two different curcumin concentrations (0.2 and 0.4 µg/mL) was given in Figure 3. The TEAC value of curcumin loaded SLN formulation was found to be 0.47mM and 0.72mM for the curcumin

concentrations of 0.2 and 0.4 µg/mL, respectively. It was proved that the antioxidant activity of curcumin is concentration dependent. When the NLC formulations were obtained by adding liquid lipids to the SLN structure; an increase in the antioxidant activity was obtained with Vitamin E and PSO, which are known to have antioxidant effects, but no significant difference was observed in the presence of Labrafac. Similar results were also obtained for NE formulations of curcumin. The effect of the structural difference of LbNs and the existence of antioxidant active liquid lipids (PSO and Vitamin E) on the free radical scavenging capacity of curcumin was confirmed by the results obtained.

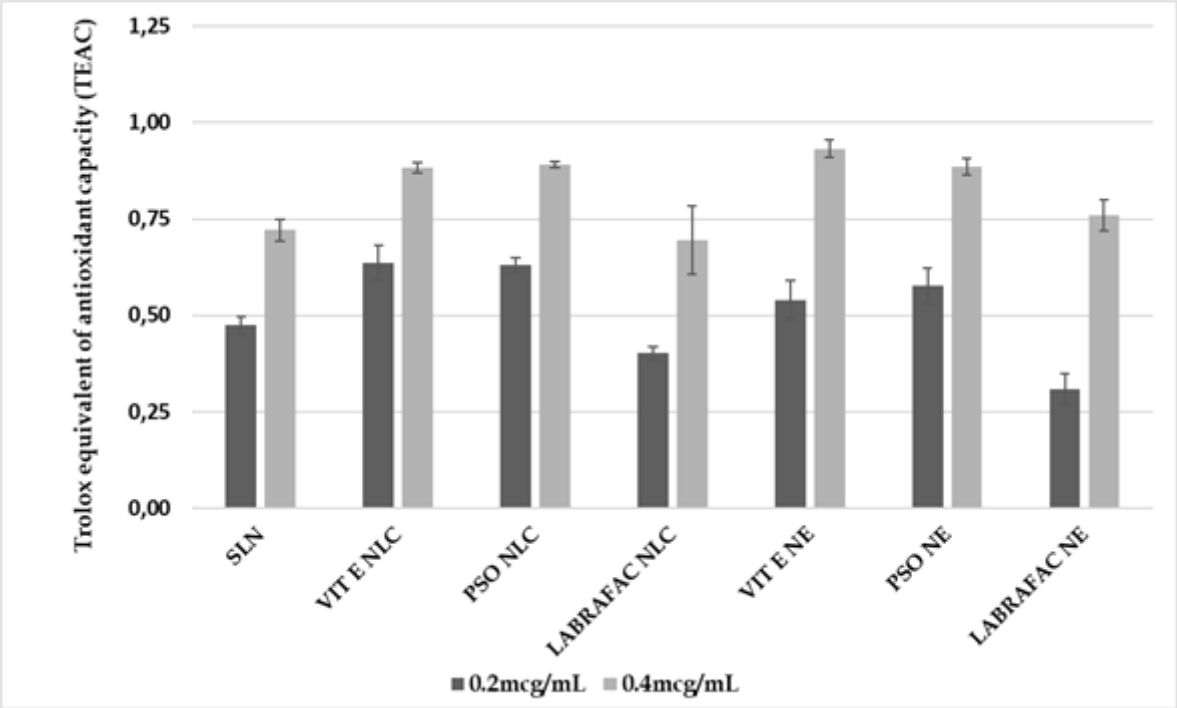

Figure 3. Trolox equivalent of antioxidant capacity of LbNs (n=3)

3. CONCLUSION

In this study, different lipid-based nanocarriers containing curcumin, which is an important antioxidant active ingredient, were prepared and the contribution of the excipients of the nanocarriers to the antioxidant activity was investigated. A significant contribution to the antioxidant activity of curcumin was obtained when vitamin E and PSO were used as liquid lipid in NLC and NE formulations in comparison with SLN formulation or in presence of Labrafac. In conclusion, it is possible to improve the antioxidant effect of curcumin by the modification of the structure of LbNs.

4. MATERIALS AND METHODS

4.1. Materials

Curcumin was purchased from Alfa Aesar (USA). Tristearin was bought from Sigma Aldrich (USA). Precirol® ATO 5, Labrafac® Lipophile WL 1349 (Labrafac) and Gelucire® 50/13 were kindly provided by Gattefosse (France). Vitamin E and pomegranate seed oil (PSO) were purchased from Galenik (Turkey) and Zadevital (Turkey), respectively.

4.2. HPLC Assay Method

Curcumin was analyzed by high performance liquid chromatography (HPLC) with a NovaPak C18 (4 µm, 150 x 3.9 mm) (Waters, Ireland) column using the Agilent 1260 series (Agilent, USA) equipped with a photodiode array detector. The mobile phase was prepared by mixing 0.2% (v/v) glacial acetic acid buffer (pH 3), methanol and acetonitrile at a ratio of 30:35:35, respectively. Column temperature was set at 30 °C and flow rate was determined as 0.8 mL/min. The detection wavelength (λ_{max}) was choosen as 422 nm and under this

conditions the retention time was 2.5 min. Curcumin stock solution was prepared by dissolving it in methanol and a linear response was obtained in the range of 0.1 - 4 µg/mL (r^2= 0,998).

4.3. Preparation of Curcumin – loaded SLN, NLC and NE Formulations

Binary mixtures of Precirol ATO 5® and Tristearin (1:1) were used as solid lipids in the preparation of SLN formulations. For the NLC formulation, in addition to the 1:1 mixture of Precirol ATO 5® and Tristearin as solid lipid, vitamin E, PSO or Labrafac were used as liquid lipid. On the other hand, nanoemulsion formulations were prepared using Vitamin E, PSO or Labrafac alone. Gelucire® 50/13 was used as surfactant for all formulations.

Blank and curcumin-loaded formulations were prepared by the high shear homogenization method followed by ultrasonication [51, 52]. First, 150 mg of solid lipid mixture for SLN, solid lipid-liquid lipid mixture for NLC and liquid lipid for nanoemulsion were weighed as lipid phase of the LbN formulations. 5 mg of curcumin was added into lipid phase and was mixed homogeneously. Also, 0.5 mL of absolute ethanol was added after the lipid mixture of curcumin was heated to increase the lipid solubility of curcumin. 15 mL of 2% Gelucire® 50/13 aqueous solution was prepared for the aqueous surfactant phase. The composition of LbN formulations were given in Table 3. After the lipid and drug were heated above the melting point of the lipid (80°C), the aqueous surfactant solution at the same temperature added onto the melted lipid. Emulsification was performed for 5 minutes at 20,000 rpm using a high shear homogenizer (Ultra Turrax T18 IKA, Germany), followed by sonication (Bandelin Sonoplus HD2070, Germany) for 5 minutes. The hot oil-in-water nanoemulsion was cooled at room temperature and finally kept at 4 °C overnight.

Table 3. The code and compositions of lipid based nananocarriers

	SLN	Vitamin E NLC	PSO NLC	Labrafac NLC	Vitamin E NE	PSO NE	Labrafac NE
Precirol (g)	0.15	0.075	0.075	0.075	-	-	-
Tristearin (g)	0.15	0.075	0.075	0.075	-	-	-
Vitamin E (g)	-	0.15	-	-	0.3	-	-
PSO (g)	-	-	0.15	-	-	0.3	-
Labrafac (g)	-	-	-	0.15	-	-	0.3
Gelucire® 50/13 (%w/v)	%2	%2	%2	%2	%2	%2	%2
Ethanol (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Aqueous phase (mL)	15	15	15	15	15	15	15

4.4. Physicochemical Characterization of the SLNs, NLCs and NEs.

4.4.1. Particle size and zeta potential analysis

The particle size and polydispersity index (PDI) of the curcumin-loaded SLNs, NLCs and NEs were determined by a dynamic light scattering technique (Malvern Instruments, UK). Before measurement, the samples were pipetted 100 µL and diluted to 1 mL with ultra purified water. Measurements were made in 5 replicates and the results were given as the mean ± standard deviation.

4.4.2. Drug content and encapsulation efficiency

The encapsulated amount of curcumin into LbNs were calculated by indirect method by measuring the unloaded curcumin in the supernatant after centrifugation. 0.5 mL of LbNs dispersion was placed into Amicon Ultra-0.5 Centrifugal Filter Unit (Merck, Germany) with the molecular weight cut off 30kDa and it was centrifugated at 10000 rpm for 20 min. The obtained supernatant was analysed by HPLC to detect the amount of curcumin.

4.4.3. In vitro drug release study and release kinetics of Curcumin

In vitro drug release studies were performed by the dialysis bag method using a thermostated shaker. 1.5 mL of each formulation was placed in a pre-hydrated cellulose acetate dialysis membrane (MWCO 12–14 kDa) and immersed in 25 mL dissolution medium at 37 °C. A mixture of phosphate buffered saline pH 5.5 and ethanol (70:30) was used as dissolution medium in order to provide the sink condition since it is a common approach to add organic solvents to the dissolution medium in order to provide the sink condition for the active substances with low aqueous solubility [53, 54]. The medium was stirred at 100 rpm for 24 hours.

Release studies for each formulation were performed in triplicate and the release medium was completely changed after each sampling. The amount of drug released from the formulations at different time points (1. 2. 4. 6. 8. 10. and 24. h) was quantified using an HPLC at 422 nm.

Then, various kinetic equations were applied to clarify the release mechanism and the effect of the structure of nanocarriers on the *in vitro* release data of the SLN, NLC and NE. The first-order, zero-order, Hixon–Crowell and Higuchi mathematical models were compared using linear regression and ANOVA by IBM SPSS Statistics for Windows, Version 26.0.

4.4.4. Morphological analysis

The morphological features of LbNs were characterized by transmission electron microscopy (TEM) at an acceleration voltage of 200 kV (TEM-FEI 120kV HCTEM, Japon).

4.4.5. In vitro antioxidant activity

The antioxidant activity of LbNs was evaluated by the TEAC (Trolox equivalent antioxidant capacity) assay using commercially available antioxidant assay kit (Sigma Aldrich Chemie GmbH, Germany). This method is a fast, simple and inexpensive. Since it is commercially available, it is widely used to evaluate the antioxidant activity of different compounds. The kit provides all the necessary reagents for effective measurement of the total antioxidant capacity of the samples. The test was carried out following the instruction. At the end of the test, the absorbance of the samples was measures at 405 nm using a plate reader (CLARIOstar Plus, BMG LABTECH, Germany). All analyzes were carried out using two different concentrations of curcumin as 0.2 and 0.4 µg/mL.

Author contributions: Concept – G.A.; U.B.; Design – G.A.; U.B.; Supervision – N.O.; Resources – N.O.; U.B; Materials – G.A.; U.B.; Data Collection and/or Processing – G.A.; U.B.; O.Y.; Analysis and/or Interpretation – G.A.; U.B.; O.Y.; Literature Search – G.A.; U.B.; O.Y.; Writing – G.A.; U.B.; O.Y.; Critical Reviews – N.O.; G.A.; U.B.; O.Y.;

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