

Research Article

Effective Lipid Nanocarriers Based on Linseed Oil for Delivery of Natural Polyphenolic Active

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The main purpose of the present research was to test the ability of nanostructured lipid carriers (NLCs) to efficiently host a hydrophilic polyphenol active with health-promoting activities (caffeic acid (CA)). The caffeic acid-loaded lipid nanocarriers (CA-NLCs) were obtained by high-pressure homogenization technique using a surfactant mixture of Tween 20 and L- α -phosphatidylcholine in association with a lipid mixture of linseed oil, hexadecyl palmitate, and glycerol monostearate. In the first stage, the proportion between surfactant mixture and lipid phase has been varied to obtain appropriate stable nanocarriers. The optimized NLCs have been further loaded with different amounts of caffeic acid and were analyzed in terms of physical stability, size characteristics, and encapsulation efficiency. The antioxidant activity of CA-loaded NLCs and their release behavior have been tested by specific *in vitro* methods, e.g., ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay and release experiments, by Franz cell diffusion. The ABTS assay highlighted a high antioxidant potential of the caffeic acid in association with linseed oil. The capacity to capture ABTS cationic radicals was superior for the NLC entrapping an initial amount of 1.5% CA, the level of antioxidant capacity being 91.3%. The *in vitro* release experiments showed a different release behavior, depending on the initial amount of caffeic acid used. NLC loaded with a higher concentration of CA manifests a gradual slow release, e.g., 45% CA after 24 h of *in vitro* experiments, while the NLC loaded with smaller concentration of CA assured a higher release in time, around 65%.

1. Introduction

During the past decade, lipid nanoparticles have been investigated as platforms for efficient drug encapsulation and sustained release at the specific site of many localized diseases. The solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), respectively, are derived from oil/water emulsions, with the remark that the solid lipids or a mixture of solid and liquid lipids, respectively, replace the liquid lipids [1]. Lipid nanoparticles have an average diameter ranging between 50 and 1000 nm, a spherical morphology containing a solid lipid core surrounded by a surfactant shell. One of the most important features of the lipid matrix is its ability to

maintain in solid form at both ambient and body temperatures [2]. NLCs are known as improved systems of SLNs, showing a better stability and higher encapsulation efficiency for numerous active compounds. Concomitantly, the NLCs prevent the active substances' expulsion from the lipid matrix during storage, thus increasing their stability and efficacy during administration [3].

NLCs are considered modified SLNs in which the lipidic phase contains both solid and liquid lipids at ambient temperature, which generate imperfections within the matrix, decreasing its crystallinity and facilitating the accommodation of drug molecules [4, 5]. In this sense, improved drug encapsulation, minimized drug leakage during storage, and

controlled drug release kinetics are demonstrated for these delivery systems [1]. Additional advantages of NLCs include high biocompatibility and simple production at large scale [6]. These nanocarriers have been widely developed in the last years aiming at the improvement of the therapeutic index of drugs by means of modifying their pharmacokinetics and biodistribution. Several examples include the encapsulation into SLN and NLC systems of synthetic and/or natural active principles, i.e., anti-UV, anti-inflammatory, antitumor, and antioxidant actives, such as ethylhexyl salicylate, octocrylene, bemotrizinol [7, 8], naringenin [9], tocopherols [10], azelaic acid [11], sodium pemetrexed [6], ivy leaf extract [1], willow bark extract [12], and natural carotenoid extracts from *Tagetes patula* and *Calendula officinalis* L. [13]. In the present work, nanostructured lipid carriers (NLCs) based on bioactive linseed oil have been developed in order to test their ability to encapsulate a hydrophilic polyphenol active with potential antioxidant, anti-inflammatory, and antineoplastic activities—caffeic acid (CA)—and to assure an efficient release of this natural active.

Caffeic acid (3,4-dihydroxycinnamic acid, Figure 1) is a hydroxycinnamic acid derivative with potential antioxidant, anti-inflammatory, and antihyperglycemic action and antineoplastic activities [14]. It is a naturally occurring phenolic compound which is produced in many fruits, vegetables, and herbs, including pears, basil, thyme, verbena, tarragon, oregano, wood betony, burning bush, turmeric, dandelion, yarrow, horsetail, rosemary, hawthorn, and coffee [15]. The amount of caffeic acid is strongly dependent on the plant species [16]. Their variety of potential pharmacological effects in *in vitro* studies and in animal models and the inhibitory effect of caffeic acid on cancer cell proliferation by an oxidative mechanism in the human cell line have recently been established [17]. Caffeic acid is an efficient antioxidant *in vitro* and also *in vivo*, e.g., upon administration, it prevents oxidative stress, thereby preventing DNA damage induced by free radicals [18]. Caffeic acid also shows immunomodulatory and anti-inflammatory activity; it reduces the aflatoxin production by more than 90% [19]. Another study showed that oxidative stress that would otherwise trigger or enhance *Aspergillus flavus* aflatoxin production can be stopped by caffeic acid [20]. Despite the large therapeutic efficiency of caffeic acid, oral administration of high doses of caffeic acid in rats has caused stomach papilloma [21].

In this context, the main objective of the present work is to encapsulate different amounts of caffeic acid and to determine the influence of CA concentrations on the antioxidant properties and CA release under controlled conditions. Firstly, the influence of surfactant concentration was investigated on the most important characteristics of NLCs such as mean diameter particle size and zeta potential. The CA was encapsulated into NLCs with the adequate characteristics. For the CA-loaded NLCs, mean diameter particle size, zeta potential, and entrapment efficiency were evaluated. The efficiency of NLCs as pharmaceutical formulations has been characterized by *in vitro* analysis of the antioxidant activity and CA *in vitro* release from NLCs.

The research presented in this study is aimed at providing new insights about the association of two categories of natu-

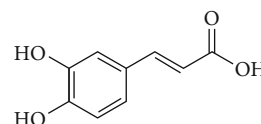


FIGURE 1: Caffeic acid (3,4-dihydroxycinnamic acid).

ral active principles—hydrophilic and lipophilic—in a lipid delivery system. Although there are several studies in which caffeic acid has been loaded into solid lipid nanoparticles (SLNs) [22, 23], to our knowledge, its capture in lipid nanocarriers has not been achieved so far. As such, the novelty achieved by performing this work refers to the design of CA-loaded nanocarriers containing linseed oil to obtain a synergic effect between linseed oil and caffeic acid and the enhancement of antioxidant activity of both natural actives (caffeic acid and linseed oil).

2. Materials and Methods

2.1. Materials. Polyoxyethylene (20) sorbitan monolaurate (Tween 20) and caffeic acid (CA) were purchased from Merck, while Synperonic PE/F68 (poloxamer 188) and L- α -phosphatidylcholine (~99%, from egg yolk, lyophilized powder) were purchased from Sigma Aldrich Chemie GmbH (Munich, Germany). The solid lipids, n-hexadecyl palmitate (CP) and glycerol monostearate (GMS), were obtained from Acros Organics (New Jersey, USA) and Cognis GmbH (Monheim, Germany), respectively. 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Sigma-Aldrich (Munich, Germany). The linseed oil (LO) was purchased from Hofigal S.A., Bucharest, Romania. The LO composition, evaluated by gas chromatography (Thermo-GC, DSQ P 5000 mass spectrometer, Macrolog 2000 column with $\Phi = 0.25$ mm and $l = 30$ m; helium carrier gas, injection temperature of 25°C, flow rate of 1 mL/min), is 50.59% α -linolenic acid (C18:3, ω -3), 24.33% oleic acid (C18:1, ω -9), 14.34% linoleic acid (C18:2, ω -6), 5.19% palmitic acid, 4.34% stearic acid, 0.8% *cis*-vaccenic acid, and 0.11% γ -linolenic acid (C18:3, ω -6).

2.2. Synthesis and Characterization of NLCs

2.2.1. Preparation of Nanostructured Lipid Carriers. Lipid nanocarriers were prepared using melt emulsification method coupled with high-shear homogenization and high-pressure homogenization [24]. For preparation of NLCs, both the lipid phase (a mixture of CP, GMS, and LO) and the aqueous phase (different concentrations of surfactant mixture, namely, 2%, 2.5%, 3%, and 3.5%) were heated under stirring conditions, at 78°C. The active substance was added into the aqueous phase, in which then was also slowly added the lipid phase under stirring, at 78°C. The obtained pre-emulsion was exposed to an external mechanical energy by high-shear homogenization with a lab rotor-stator homogenizer by applying 15000 rpm for 1 min and then by high-pressure homogenization by applying 500 bar for 196 s. Furthermore, the formed NLC dispersions were subjected to lyophilization in order to remove the water in excess.

TABLE 1: Composition of nanostructured lipid carriers (NLCs).

NLC formulation*	Surfactant (% w/w)	Caffeic acid (% w/w)
NLC-LO1	2.0	—
NLC-LO2	2.5	—
NLC-LO3	3.0	—
NLC-LO4	3.5	—
NLC-LO-CA1	2.5	0.5
NLC-LO-CA2	2.5	1.0
NLC-LO-CA3	2.5	1.5

*All NLCs were prepared with 10% (w/w) lipid mixtures, in a ratio of CP/GMS/LO = 1 : 1 : 1 and a ratio of Tween 20 : poloxamer : L- α -phosphatidylcholine = 70 : 15 : 15.

Table 1 shows different compositions of the synthesized NLCs.

2.2.2. Particle Size Analysis. The Dynamic Light Scattering (DLS) technique was used for measuring the mean diameter size (Z_{ave}) and the polydispersity index (PDI), at a scattering angle of 90° and 25°C, with a Zetasizer Nano ZS, Malvern Instruments, Malvern, United Kingdom. The aqueous NLC dispersions have been adequately diluted in deionized water to fulfill the sensitivity range of the instrument in terms of an accurate achievement of the light scattering intensity (dilution of NLC dispersion: deionized water = 0.02 : 1). The intensity distribution was used to evaluate the particle size data, with Z_{ave} and PDI being expressed as an average of three DLS measurements. DLS measurements (Z_{ave} and PDI determination) were performed on the day of preparation of free and caffeic acid-loaded NLC.

2.2.3. Zeta Potential Analysis. The Helmholtz-Smoluchowski equation [25, 26] was used to calculate the zeta potential (ZP) after determining the electrophoretic mobility of the NLC dispersion. Prior to analysis, each NLC dispersion was diluted with ultrapurified water (in a ratio of NLC aqueous dispersion : ultrapurified water = 1 : 0.01, v/v) and was adjusted by adding a minimum volume of sodium chloride 0.9% solution (e.g., to 20 mL of NLC aqueous dispersion was added 65 μ L NaCl solution, 0.9%) in order to reach a conductivity of 50 μ S/cm (a value required in the electrophoretic light scattering technique). The zeta potential measurements were performed on Zetasizer Nano ZS; zeta potential values were determined on the day of NLC preparation. The ZP was expressed as an average of three ZP measurements.

2.2.4. Entrapment Efficiency of Caffeic Acid into NLC Systems. The entrapment efficiency of caffeic acid into NLCs was determined by UV-VIS spectroscopy, an amount of 0.05 g of lyophilized NLC-LO-CA being dispersed into 1.0 mL ethanol, mixed, and centrifuged at 15000 rpm, for 15 min. Then, the unloaded CA from the obtained supernatant was analyzed by a spectrophotometric system Jasco V-670, at 325 nm. Before analysis, 50 μ L of supernatant was dispersed into 10 mL ethanol. The blank sample consisted of ethanol. The following equation was used for calculating the percent of EE:

$$\%EE = \frac{W_a - W_s}{W_a} \times 100, \quad (1)$$

where W_a is the weight of total CA used for the preparation of NLC-LO-CA and W_s is the analyzed weight of CA in the supernatant.

Equation (2) was used to calculate the percentage of loading capacity (%LC):

$$\%LC = \frac{W_a - W_s}{W_a - W_s + W_L} \times 100, \quad (2)$$

where W_a is the weight of CA added in the NLC, W_s is the analyzed weight of CA in the supernatant, and W_L is the weight of lipid added in the NLC.

2.2.5. In Vitro Antioxidant Activity Measurements. The *in vitro* determination of antioxidant activity was conducted using a UV-VIS Spectrophotometer Type V670 (Jasco, Japan), according to ABTS assay [27], at 734 nm. The blank sample consisted of normalized ABTS solution and ethanol. Each analyzed sample was prepared with ABTS solution and ethanol.

Firstly, an aqueous 7 mM solution of ABTS was prepared. The cationic radical $ABTS^+$ was generated after 16 hours of reaction between 7 mM ABTS solution and 2.45 mM potassium persulfate solution, in the dark conditions, at 25°C. The $ABTS^+$ solution was normalized with ethanol to an absorbance of 0.700 (± 0.02) at 734 nm. Each sample was prepared by adding 3.0 mL of normalized $ABTS^+$ solution to 1 mL NLC solution (prepared with 0.05 g lyophilized nanocarriers into 10 mL ethanol) and 2.0 mL ethanol into a 5 mL flask.

The absorbance of samples was determined after 4 min of reaction of initial mixture, with ethanol being used as reference. Each absorbance of sample was determined in duplicate, the average of absorbance values being used for calculating the percentage inhibition of the sample on $ABTS^+$ according to

$$\%Inhibition\ ABTS^+ = \frac{A_0 - A_s}{A_0} \times 100, \quad (3)$$

where A_0 is the absorbance of the blank and A_s is the absorbance after the addition of the antioxidant.

The antioxidant capacity of NLC systems was also highlighted by TEAC (Trolox Equivalent Antioxidant Capacity) method, with the Trolox solution concentrations ranging between 2 and 60 μ M ($R^2 = 0.999$).

2.2.6. In Vitro Release of Caffeic Acid from NLC. The release of caffeic acid from loaded lipid nanocarriers in dispersion was evaluated using Franz diffusion [28]. The Franz system has two chambers—a donor chamber and a receptor chamber. Between the chambers, a Tuffryn membrane filter was placed.

The receptor chamber contains a release medium of ethanol with a volume of 6 mL. In the donor chamber, an amount of 150 μ L of each formulation NLC-LO-CA was

placed onto the Tuffryn membrane which was initially hydrated in ethanol for 1 h. The receptor phase consisted of ethanol and phosphate buffer, at a ratio of 50 : 50 (v/v), maintained at 37°C. At 1, 2, 3, 4, 5, 6, 7, 8, and 24 hours, aliquots of 500 μ L of sample were collected from the receptor chamber and discharged. The sample was then diluted with ethanol. During the study, the volume of release medium was maintained constant. Finally, the collected samples were measured using the UV-VIS Spectrophotometer Jasco V670. All the measurements were performed in duplicate at 325 nm.

3. Results and Discussion

3.1. Optimization of NLC and Physical Stability. Lipid nano-carriers were firstly characterized in terms of mean diameter, polydispersity index, and zeta potential (ξ) by DLS technique. To identify the optimized surfactant composition, the NLCs with different concentrations (2%, 2.5%, 3%, and 3.5%) of surfactants have been synthesized (Table 1). The variation of Z_{ave} and PdI of NLCs is shown in Figure 2.

By tracking the behavior of the four types of NLCs, it was shown that all the synthesized formulations have an appropriate distribution of particle size, e.g., between 110.9 and 131.4 nm. These results could be correlated with the efficiency of the surfactant mixture but also with the presence of the linseed oil which contributes to the viscosity decrease of the lipid mixture, which reduces the surface tension to form smaller and smoother surface particles [29].

Different values of Z_{ave} and PdI are influenced by the different molecular weights of surfactants [30]. The best experimental data were obtained for NLCs with a surfactant concentration of 2.5%: $Z_{ave} = 110.9 \pm 2.3$ nm and $PdI = 0.156 \pm 0.004$. According to literature data, it is known that the NLCs with a PdI lower than 0.25 show a minimum tendency to aggregation [31].

It was observed that the extreme concentrations selected for the surfactant mixture, e.g., 2% and 3.5%, lead to higher values of Z_{ave} . This result can be attributed on the basis of two considerations: (1) a high amount of phosphatidylcholine and poloxamer favors the formation of liposomes, which would lead to higher mean values of Z_{ave} ; (2) too small amounts of surfactants do not ensure full coverage of the lipid core.

NLCs prepared with the highest amount of surfactant mixture exhibit higher values of Z_{ave} ($Z_{ave} = 131.4 \pm 1.1$ nm) and PdI ($PdI = 0.222 \pm 0.005$), while by using lower surfactant concentrations (NLC-LO2 and NLC-LO3), the average diameter has been drastically decreased (Figure 2). According to the literature data, compared with a single surfactant, the surfactant mixture could decrease the interfacial tension [32].

3.2. Physical Stability of NLC Dispersions. The zeta potential (ZP) (ξ) is one of the main characteristics that defines the behavior of nanoparticles in aqueous solution, which highlights the physical stability of NLCs. The zeta potential is the electrostatic potential at the shear plane and reflects the electric charge on the particle surface, being a measure of surface charge. It can influence particle stability as well as cellular uptake and intracellular trafficking [33]. The ZP data of

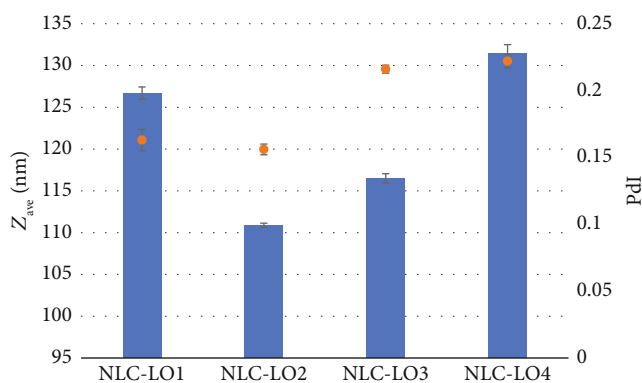


FIGURE 2: Mean diameter (columns) and PdI (circles) of NLCs prepared with variable content of surfactants.

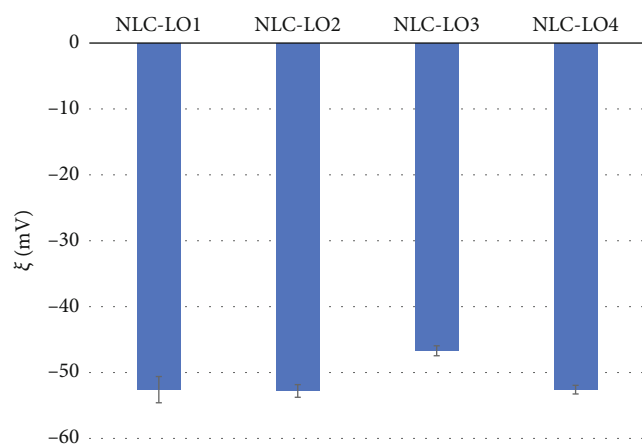


FIGURE 3: Evaluation of NLC stability by zeta potential.

the synthesized NLCs are shown in Figure 3. All the developed NLCs are stable, with the zeta potential distribution being between -52.8 mV and -47.6 mV. The negative charges of NLCs are due to surfactant mixture, with the best ZP result being obtained with 2.5% surfactant mixture, $\xi = -52.8$ mV ± 0.56 (Figure 3), which reveals a high stability of NLC and a low potential of aggregation of solid lipid nano-carriers suspended in aqueous media.

3.3. Size Characteristics and Physical Stability of CA-NLCs. The NLC-LO2, having a reduced mean diameter and appropriate physical stability, was used for encapsulating the natural antioxidant. The DLS results showed that CA-NLCs present a unimodal profile of size distribution, with a Z_{ave} value of 145.5 ± 0.8 nm for NLC-LO-CA1, 119.7 ± 0.9 nm for NLC-LO-CA2, and 195.1 ± 5.3 nm for NLC-LO-CA3 (Figure 4(a)). One example of size distribution is included in Figure 4(b).

After CA encapsulation in the NLC, a significant increase of the mean diameter compared to that of the free NLC is observed ($Z_{ave,NLC-LO} = 110.9 \pm 2.3$ nm). This increase mainly denotes trapping of caffeic acid in the coating created by the surfactant mixture, most probably by hydrogen bonds between the OH groups of caffeic acid, the OH groups of Tween 20, and the polar groups of phosphatidylcholines. If

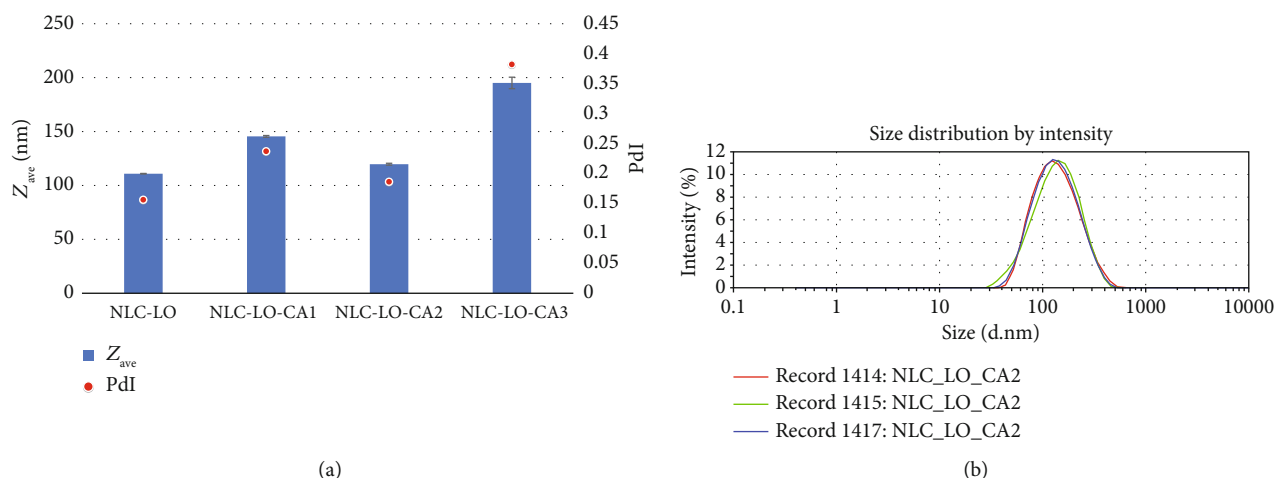


FIGURE 4: (a) Evaluation of loaded NLC stability by mean diameter and PDI. (b) Exemplification of particle size distribution for NLC-LO-CA2.

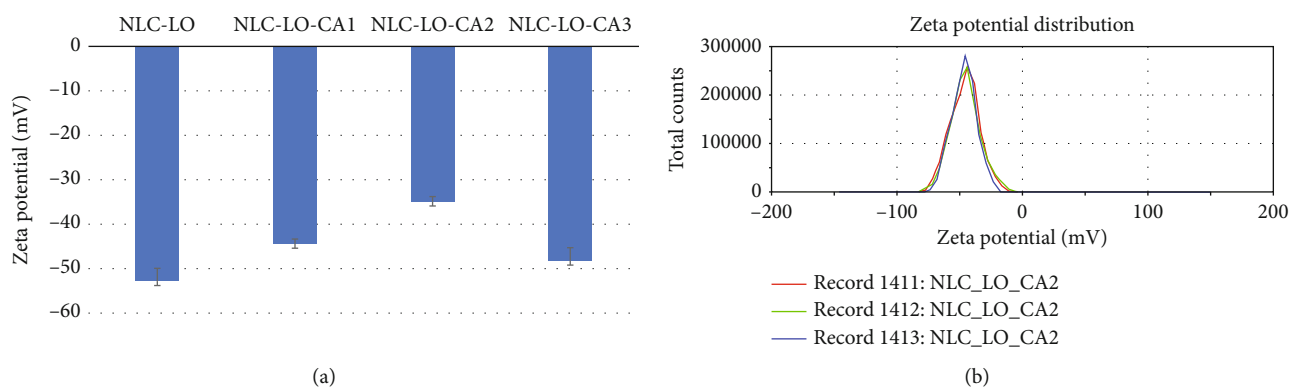


FIGURE 5: (a) Zeta potential values of empty and CA-loaded NLCs. (b) Exemplification of zeta potential distribution for NLC-LO-CA2.

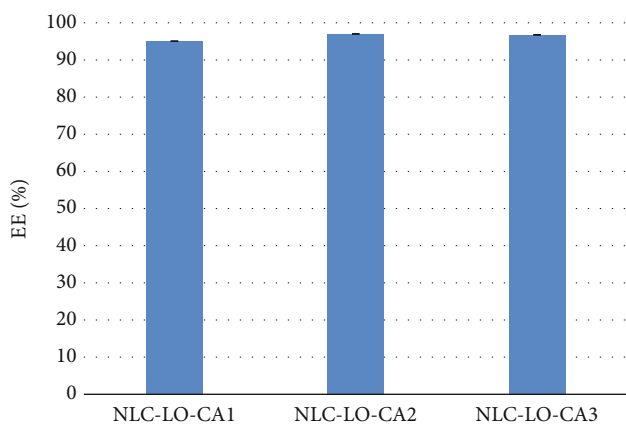


FIGURE 6: Entrapment efficiency of CA into lipid nanocarriers based on linseed oil.

there was a capture of caffeic acid in the voids/imperfections in the lipid core, it would have resulted in an insignificant change in the average diameter. This result was not observed, but on the contrary, a Z_{ave} increase was detected which confirms a preferential distribution of caffeic acid in the surfactant layer. These results are predictable having in view the

greater affinity and solubility of caffeic acid in surfactant coating than in solid lipids.

A drastic increase of Z_{ave} was observed for NLCs loaded with a high amount of CA, e.g., 195.1 ± 5.3 nm for NLC loaded with 1.5% CA versus 119.7 ± 0.9 nm for NLC loaded with 1% CA. This could be attributed to the hydrophilic character of CA; the surfactant coating can capture a limited concentration of CA, with the remainder being trapped outside the nanocarrier system, thus leading to the appearance of larger mean diameters of lipid nanocarriers.

The results obtained after determination of electrokinetic potential showed that the NLC formulations loaded with CA are negatively charged (Figure 5). As compared to the free NLC, there is a significant perturbation of electric surface after retention of caffeic acid. The addition of caffeic acid resulted in an increase in zeta potential, e.g., from -52.8 mV in free NLC to -48.2 mV and -34.9 mV in NLC-LO-CA1 and NLC-LO-CA2 (loaded with 0.5% and 1% CA, respectively). The presence of anionic active ingredient (CA) has led to a notable modification in zeta potential values because of surface charge rearrangements. A potential explanation may be related to masking of negative charges from phosphate groups (from phosphatidylcholine), due to the caffeic acid trapped by weak bonds in the surfactant coating or

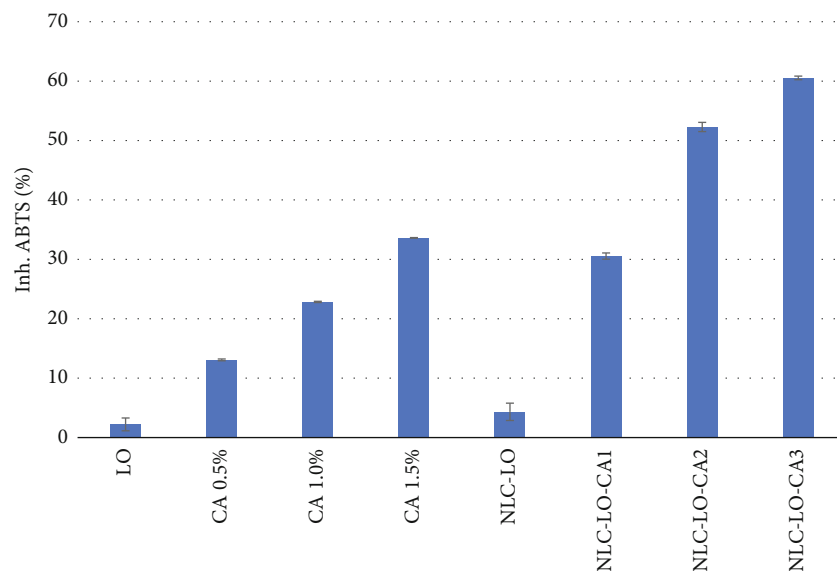


FIGURE 7: Comparative assessment of antioxidant activity of native CA, NLC-LO, and NLC-LO-CA.

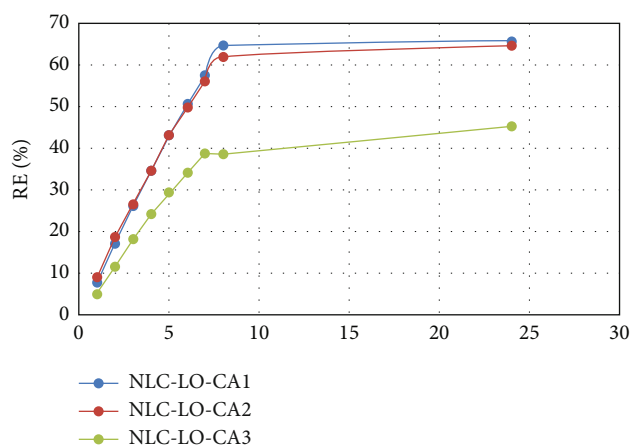


FIGURE 8: Influence of the lipid nanocarrier type on the release of caffeic acid.

attached to the surfactant layer. These aspects justify a certain compensation of surface charges or a covering of negative charges from phosphatidylcholine.

The colloidal systems are considered stable when the zeta potential values of particles are integrated in the -30 mV and -60 mV potential ranges [34]. All the zeta potential values determined for NLC loaded with CA were below the critical value. Moreover, according to the values lower than < 40 mV (reported for NLC-LO-CA1 and NLC-LO-CA3), the obtained NLC systems are expected to manifest a long-term physical stability, which involves the existence of desired electrostatic and steric repulsions between the lipid nanocarriers suspended in aqueous dispersion. This last aspect avoids the aggregation of lipid particles dispersed in water over time.

3.4. Entrapment Efficiency of Caffeic Acid into NLC Systems. The entrapment efficiency (EE%) of LO-based CA-NLCs is shown in Figure 6. There was no relevant difference between

EE values, when variable content of CA was included in the NLC formulations. All synthesized NLC-CA showed high EE values, exceeding 95% CA. These results revealed that CA was efficiently entrapped into the NLC, mainly captured in the layer formed by the mixture of surfactants and cosurfactant, showing a high compatibility with the surfactant mixture.

3.5. The In Vitro Determination of Antioxidant Activity. Oxidative processes are represented by a lost balance between the oxidative and antioxidative systems of the cells and tissues, with the production of oxidative free radicals and reactive oxygen species (ROS). Excessive ROS generated could attack the cellular proteins, lipids, and nucleic acids leading to cellular disorders including loss of energy metabolism, altered cell signaling and cell cycle control, genetic mutations, altered cellular transport mechanisms and overall decreased biological activity, immune activation, and inflammation [35].

The ABTS method highlights the antioxidant activity of complex biological systems [36] and was used in our study to determine the scavenging activity of developed NLC-LO-CA 1/2/3 on $ABTS^{+}$ radical. The experimental data highlighted the high antioxidant capacity of the CA in association with linseed oil. Figure 7 reveals that $ABTS^{+}$ inhibition depends on CA concentration. The obtained data showed a higher antioxidant capacity for the CA-NLCs compared to native CA. The smaller concentration of the native CA (0.5%) shows a 21.5 ± 0.15 antioxidant capacity, while the encapsulation of CA into NLC revealed a radical $ABTS^{+}$ inhibition activity of 47.2 ± 0.5 .

The incorporation of higher CA concentration into NLC has led to stronger antioxidant properties, an improved antioxidant activity of 91.3 ± 0.3 being determined for NLC-LO-CA3. Figure 7 shows an inhibition of 79.2 ± 0.8 for NLC loaded with 1% CA and an inhibition of 47.2 ± 0.5 for NLC loaded with 0.5% CA.

The improved results of $ABTS^{+}$ inhibition of NLC-LO-CA compared to CA can be explained by the size effect

TABLE 2: Kinetic data obtained after release of caffeic acid from NLC prepared with linseed oil.

NLC formulations	Zero order		First order		Higuchi		Hixson–Crowell		Peppas–Korsmeyer		
	R^2	k_0	R^2	k_1	R^2	k_2	R^2	k_3	R^2	k_4	n
NLC-LO-CA1	0.9966	8.1046	0.9938	0.1353	0.9928	31.528	0.9897	0.0175	0.9967	2.0321	0.4754
NLC-LO-CA2	0.9946	7.5755	0.9972	0.1247	0.9946	29.528	0.9948	0.0161	0.9972	1.8555	0.4462
NLC-LO-CA3	0.9701	5.0423	0.9812	0.0667	0.9905	19.859	0.9823	0.0083	0.9941	2.1039	0.5973

produced by trapping the CA into NLC. The free radical scavenging activity is a surface reaction since only the surface meets the free radicals [37]. According to several studies, the radical scavenging potential is dependent on various factors such as particle size, morphology, and network defects [38, 39]. An enhancement in free radical scavenging which coincided with a decreasing of nanofiber diameters has been underlined in a study of Kumar et al. This has been associated with the increase in the surface reaction sites with the size reduction [40].

Similar to other nanomaterials, NLCs exhibit unique properties attributed to their high surface to volume ratio. The average diameters of NLC lower than 200 nm offer more reactive sites for capturing the ABTS⁺⁺ cationic radicals, determining an enhancement of antioxidant activity.

In conclusion, the *in vitro* assays evidenced that NLC-LO-CA has an important role in reducing the cellular oxide species [41]. It is also known that the physical and biological properties of CA-NLCs are influenced by a reduced size of bioactive [22].

3.6. In Vitro Release of Caffeic Acid from NLC. The series of release profiles of CA from different NLCs loaded with 0.5%, 1%, and 1.5% CA showed a different behavior. Despite the similar sustained release pattern encountered for all NLC formulations in the first hours of release experiments, significant delimitation has been noted after 5 h as a function of the initial percent of CA loaded into NLC. Regarding the CA-NLCs, it was not evidenced a burst release, with a minimum CA release being found after the first hour of experiment (with about 8–10% of total CA released); a sustained release with a maximum degree of about 65% CA was followed. It is interesting to note that during the entire release period, the amount of CA released from NLC loaded with 0.5 and 1% CA was almost similar (Figure 8).

The evolution of caffeic acid release could be illustrated as follows: the initial release (during 8 h of experiments) might be related to the release of CA slightly bonded to the surface of NLC. After 8 h, the release trend has been maintained almost constant. NLC-LO-CA3 assures a gradual slow release of CA, e.g., 45.1% CA after 24 h of *in vitro* experiments, while from the NLC-LO-CA2 and NLC-LO-CA1 the amount of CA released was significantly higher, 64.7% and 65.6%, respectively. The slowest release percent obtained for NLC-LO-CA3 containing a higher amount of CA could be potentially assigned to the size characteristics of developed NLC. According to DLS measurements, an increase of CA percent into nanostructured formulation has led to higher Z_{ave} value (e.g., from 119 nm for NLC loaded with 1% to about 195 nm for NLC loaded with 1.5% CA).

This higher loading resulted in a delay in the diffusion process of CA in the receptor medium.

To describe the *in vitro* release study, different kinetic mathematic models were used, and the kinetic parameters, the rate constant (k), the release coefficient (n), and the correlation coefficient (R^2), have been determined. Table 2 shows the kinetic parameters of caffeic acid release from CA-NLCs for different mathematical models: zero order, first order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell [42, 43].

It is observed that the CA release from NLC is best reflected by the Korsmeyer–Peppas kinetics ($R^2 > 0.994$ in all cases studied). According to the release coefficient value ($n = 0.5973$, Table 2), NLC-LO-CA3 assures a release of CA following a non-Fickian diffusion model, while the release of CA% from NLC-LO-CA1 and NLC-LO-CA2 unfolds after a Fick diffusion mechanism ($n < 0.5$, Table 2) [44]. The almost constant trend of CA released remained constant over a period of 24 h, with a percentage of release of about 65% CA (from NLC-LO-CA1 and NLC-LO-CA2) and 45% in the case of NLC-LO-CA3. All experimental results demonstrate that LO from NLC influenced the controlled release of CA; this is a desired purpose in order to avoid the irritant effect of CA and to reach an effective plasmatic concentration of CA (after one hour). Indeed, the controlled release of CA, during a period of 24 h, could maintain an effective plasmatic concentration of CA.

4. Conclusions

The experimental results obtained by this study indicate that lipid nanocarriers prepared by the association of two solid lipids with a bioactive vegetable oil (linseed oil) are appropriate delivery systems for a hydrophilic active compound—caffeic acid. The DLS measurements showed that the CA loaded-NLCs have mean diameters less than 200 nm, with polydispersity index lower than 0.25. Developed lipid nanocarriers that capture caffeic acid are covered by a strong negative charge on the surface (zeta potential values more electronegatively than -35 mV). Despite the hydrophilic feature of caffeic acid, the entrapment efficiency has reached 95%.

CA-NLCs manifested a high antioxidant activity; the capacity to scavenge the ABTS⁺⁺ cationic radicals was significantly improved as compared to native caffeic acid. The *in vitro* capacity to capture ABTS cationic radicals was superior for the NLC entrapping an initial amount of 1.5% CA, with the level of antioxidant capacity being 91.3%.

The *in vitro* release and kinetic modeling of lipid nanocarriers containing caffeic acid were also pursued. CA-NLCs have shown a desired sustained release behavior of

caffeic acid during release experiments. After 24 h of *in vitro* experiments, NLC-LO-CA3 assured a gradual slow release of CA with a percent of 45% CA released, while from NLC-LO-CA2 and NLC-LO-CA1 the amount of CA released was significantly higher, about 65%.

These results showed that the NLCs based on linseed oil and loaded with caffeic acid may be promising delivery systems which could be exploited in several interest area such as antioxidant cosmetic formulations or to produce functional food with improved antioxidant properties.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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