https://doi.org/10.31925/farmacia.2022.5.20 **ORIGINAL ARTICLE**

CURCUMIN CO-LOADED WITH A LIPID MEDIATOR IN THE SAME NANOSTRUCTURED LIPID DELIVERY SYSTEM

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Manuscript received: June 2022

Abstract

This study focuses on comparing the ability of two kinds of lipid matrices – solid lipids and vegetable oil associated or not with a lipid mediator (phenylalaninol oleamide, PO), for an efficient loading of Curcumin (CRC) into nanostructured lipid carriers (NLC). The Curcumin-loaded NLC were analysed in terms of particle size, physical stability, and encapsulation efficiency. By using the in vitro ABTS assay this research underlined the dominance of vegetable oils on the antioxidant properties of developed NLC formulations prepared with argan oil (AO) and linseed oil (LO). Another direction was to investigate the influence of the argan oil and linseed oil for a desired *in vitro* sustained release of CRC from the designed NLC formulations. Both antioxidant activity results and controlled release study highlighted the potential role of such formulation in normalizing the *in vitro* fate of poorly soluble Curcumin.

Rezumat

Acest studiu are ca obiectiv compararea capacității a două tipuri de matrice lipidice – lipide solide și ulei vegetal asociat sau nu cu un mediator lipidic (fenilalaninol oleamidă, PO), pentru o încărcare eficientă a curcuminei (CRC) în carrieri lipidici nanostructurați (NLC). NLC-urile încărcate cu curcumină au fost analizate în ceea ce privește dimensiunea particulelor, stabilitatea fizică și eficiența încapsulării. Prin utilizarea testului ABTS in vitro, această cercetare a subliniat dominanța uleiurilor vegetale asupra proprietăților antioxidante ale formulărilor NLC dezvoltate, preparate cu ulei de argan (AO) și ulei de in (LO). O altă direcție a fost investigarea influenței uleiului de argan și uleiului de in pentru o dorită eliberare susținută *in vitro* de CRC din formulările NLC proiectate. Atât rezultatele activității antioxidante, cât și studiul cu eliberare controlată au evidențiat rolul potențial al unei astfel de formulări în normalizarea profilului *in vitro* a curcuminei slab solubile.

Keywords: nanostructured lipid carriers, curcumin; phenylalaninol oleamide, linseed oil, argan oil

Introduction

Natural compounds have been used in traditional medicine since ancient times and have represented a source of components for the development of new drugs [1]. For example, turmeric (*Curcuma longa Linn*) is a member of the *Zingiberaceae* family and is cultivated in tropical and subtropical regions around the world, coming from India, South East Asia and Indonesia [2]. Turmeric is one of the most popular medicinal herbs, with a wide range of pharmacological activities such as antioxidant [3], antimicrobial, anti-malarial [4], anti-inflammatory [5], anti-angiogenic, anti-tumoural [6] and anti-aging [7] properties. The pharmacological activity of turmeric has been attributed mainly to curcuminoids, consisting of curcumin and two related derivatives (*e.g.* demethoxy curcumin and bisdemethoxy curcumin) [1]. Curcumin (CRC) is a bright orange-yellow phenolic compound characterized as safe active by the Food and Drug Administration which is well known for its numerous beneficial effects, such as anti-tumoural [8, 9], anti-inflammatory [10], anti-viral [11], anti-fungal [12], antioxidant [9, 13] and anti-obesity [14] properties. Previous studies highlighted that nanoencapsulation protected curcumin from photodegradation and can improve its antioxidant capacity, so nanoemulsions based on curcumin showed a high radical scavenging activity as

compared with native curcumin when tested with free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'- azinobis-(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{\bullet+}$) [15].

Nanostructured lipid carriers (NLCs) are new type of lipid nanoparticles which offer many advantages in certain circumstances when compared with other colloidal carriers, especially with the first generation of Solid Lipid Nanoparticles (SLNs), developed by Muller at beginning of 1991 [16]. These lipid nanocarriers in which crystallized lipid particles (with average mean diameters ≤ 500 nm) are dispersed in an aqueous phase containing emulsifier(s), are efficient delivery systems for improving oral bioavailability of lipophilic drugs (the main applications), but in the last years their appropriate use for delivering of hydrophilic drugs has been also investigated [17, 18]. NLCs are composed by a lipid matrix containing a blend of solid and liquid lipids (responsible for a lower crystallinity and higher incidence of defects inside the lipid core), surrounded by a surfactant mixture shell [19, 20]. Owing to the many imperfections of lipid core, NLCs manifest multiple advantages, the most significant being the improved drug loading capacity (LC%) and release properties along with stable drug incorporation during storage [21, 22]. Over the last few years, new compositions of lipids have been developed, and the bioavailability enhancement of many bioactive drugs has been well argued and demonstrated.

The limited applicability of the natural active from turmeric – Curcumin, is mainly related to the poor bioavailability [23]. Ingesting curcumin by itself does not lead to the associated health benefits, which appears to be primarily due to the poor absorption, rapid metabolism, and rapid elimination [24]. In the last years, several studies describe the encapsulation of curcumin in lipid nanocarriers, the main purpose being the increase of its bioavailability. For instance, Curcumin-loaded NLC prepared by microemulsion-ultrasonication technique, showed a good release of Curcumin from NLC systems [25]. Appropriate physical-chemical stability with optimal values of mean diameter particle size and of polydispersity index (PdI) have been also underlined by Rashidzadeh *et. al*. for NLCs based on curcumin [26]. Another study highlighted that the curcumin-loaded NLCs are more effective compared to curcumin-loaded SLNs [27]. Furthermore, nanoencapsulation is shown to increase curcumin's effect, for example anti-obesity effect, showing that compared to mice fed with free curcumin, those fed with curcumin-loaded nanoemulsions had lower lipogenesis and adipogenesis [28].

Selection of an appropriate lipidic blend is crucial for successful production of NLC with suitable physical and chemical characteristics. It was reported that the lipid blend has a significant impact on chemical stability of sensitive bioactive materials [29]. In addition, the liquid lipids from the NLC core can improve the solubility of lipophilic active [30, 31]. In this study curcumin (CRC) was encapsulated into lipid nanocarrier delivery system, prepared by including in the conventional solid lipids blend (composed by glycerol monostearate (GMS) and cetyl palmitate (CP)) of a lipid mediator (phenylalaninol oleamide) and a vegetable oil (*e.g.* linseed oil or argan oil). Many vegetable oils, including linseed and argan oils are considered bioactive lipids owing to their rich content in ω-6 fatty acids, responsible for anti-inflammatory effect, hypotriglyceridemic and hypocholesterolemia properties, antioxidant, and antidiabetic actions [32]. Argan oil (AO) is a product harvested from the fruits of the argan tree (Argania spinosa). Argan oil is known to be a rich source of unsaturated fatty acids (especially essential linoleic acid, C18:2, w-6) and tocopherols; it also contains benefic phenolic antioxidants such as vanillic, caffeic and ferulic acids along with the sterols such as schottenol and spinasterol [33, 34]. In addition, linseed oil (LO) has a high calorific value and contains varying amounts of fatty acids necessary for the correct development of human cells. Phenylalaninol oleamide (PO) is a structural analogue of phenylalaninol with oleic acid that manifests several therapeutic properties such as antioxidant action and anti-obesity effect [35]. Therefore, this study focuses on comparing the ability of two kinds of lipid matrices – solid lipids and vegetable oil associated or not with PO, for an efficient loading of Curcumin into NLC formulations. Beside the physico-chemical characterization, the research discusses the dominance of vegetable oils over antioxidant properties of loaded and free-NLC formulations prepared with the two kinds of vegetable oils. Another direction was to investigate the influence of the argan oil and linseed oil for a successful *in vitro* release of highly lipophilic CRC through appropriate designing lipidic nanocarriers formulations.

Materials and Methods

Materials

The solid lipid nanocarriers have been prepared using the following solid lipids: Glycerol monostearate (GMS) from BASF (Germany) and cetyl palmitate (CP) from Acros Organics (USA). The linseed oil (LO) and argan oil (AO) were purchased from Solaris Plant.

The aqueous phase was prepared using Tween 20 (Polyoxyethylenesorbitan monolaurate) purchased

from Merck, Poloxamer 188 (Synperonic PE/F68) – a block copolymer of polyethylene and polypropylene glycol- and L-α-Phosphatidylcholine purchased from Sigma Aldrich Chemie GmbH. The herbal active curcumin (CRC, 95%) was supplied by S.C. Helcor SRL, Baia Mare, Romania. The lipid mediator phenylalaninol oleamide (PO) was provided in more than 98% purity by National Institute for Chemical-Pharmaceutical Research and Development I.C.C.F. Bucharest. The reagent used for antioxidant activity tests was 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) bought from Sigma Aldrich Chemie GmbH (Munich, Germany).

Synthesis and characterization of NLCs

Preparation of nanostructured lipid carriers

The Curcumin-nanostructured lipid carriers based on lipid mediator PO and linseed or argan oil were synthetized using melt emulsification method associated with high shear homogenization and high-pressure homogenization techniques, as was previously described [36]. The lipid phase was subjected to heating and stirring at 78°C, to assure a complete melting, and the CRC was added in the lipid phase with the formation of a homogeneous molten solution. In the aqueous phase with an optimal concentration of surfactant mixture of 2.5%, heated and stirred at 78°C, the lipid phase was added; the formed emulsion was maintained under stirring at 78°C for 30 min. The obtained pre-emulsion was subjected to an external mechanical force by high shear homogenization at 12000 rpm for 1 min and then by high pressure homogenization (HPH) at 500 bar for 196 s. The hot nanoemulsions were cooled at room temperature to allow the crystallization of lipids with obtaining of lipid nanocarriers loaded with CRC. The excess of water from nanoparticles dispersions was removed by lyophilization using an Alpha 1-2 LD Freeze Drying System, thus obtaining the NLCs formulations in solid form. The compositions of new synthetized NLCs based on curcumin and PO are shown in Table I.

Table I

Composition of lipid nanocarriers based on argan/linseed oil, PO and CRC

NLC formulations	Surfactant mixture (g)	GMS (g)	CP (g)	Argan oil (g)	Linseed oil (g)	Curcumin (g)	PO (g)
NLC-LO-CRC	2.5	3	3	-	3	0.1	-
NLC-AO-CRC	2.5	3	3	3	-	0.1	-
NLC-LO-PO-CRC	2.5	3	3	-		0.1	1
NLC-AO-PO-CRC	2.5	3	3	3	-	0.1	1
NLC-AO	2.5	3	3	3	-	-	-
NLC-LO	2.5	3	3	-	3	-	-

Particle size analysis

For the obtained nanoparticles dispersions, the mean diameter size (Zave) and the polydispersity index (PdI) were measured at a scattering angle of 90° and a temperature of 25°C, by dynamic light scattering (DLS), using a Zetasizer ZS 90 (Malvern Instruments Inc., UK), equipped with a solid-state laser (670 nm) at a scattering angle of 90° and a temperature of 25.0 ± 0.1°C. For the DLS analysis, the dispersions were initially diluted with distilled water to a suitable intensity scattering. The particle intensity distribution was used for evaluating the size data, the Zave and PdI being given as an average of three measurements.

Zeta potential analysis

The electrophoretic mobility of the nanoparticles was measured using a Zetasizer Nano ZS 90 equipped with a solid-state laser (670 nm). The Helmholtz-Smoluchowsky equation [37] was used for determining the Zeta potential (ξ) as following:

$$\xi = E \times (4\pi \times \eta/\varepsilon),$$

where, ξ = zeta potential; E = electrophoretic mobility; η = viscosity of the dispersion medium; ε = dielectric constant.

In order to set the conductivity to 50 μS/cm, the lipid nanoparticles dispersions were diluted with a sodium chloride solution 0.9% (w/v). For each average value of ξ three individual measurements were used.

Determination of entrapment efficiency and loading capacity of Curcumin into NLCs

For determination of curcumin entrapment efficiency (EE) into NLCs the UV-VIS spectroscopy was used. Thus, an amount of 0.05 g lyophilized NLC-LO/AO-CRC with or without phenylalaninol oleamide was dispersed into 1 mL ethanol, gentle mixed and then centrifuged at 15,000 rpm for 15 minutes. The unloaded active substance (contained in the supernatant thus obtained) was analysed using a Jasco V-670 spectrophotometric system, at λ = 426.5 nm; The amount of free curcumin was calculated using the calibration curves in the concentration range of 5-50 μg/mL with a correlation coefficient R^2 = 0.9996. For the UV analysis, an aliquot of 50 μL of supernatant was dispersed into 10 mL ethanol (ethanol was used as the blank sample). The following equation was used for calculating the entrapped efficiency of CRC:

$$\% EE = \frac{W_a - W_s}{W_a} \text{ x } 100,$$

To evaluate the percentage of loading capacity (%LC), the following equation has been employed:

$$\% LC = \frac{W_a - W_s}{W_a - W_s + W_L} \text{ x } 100,$$

where, Wa = weight of total CRC used for the synthesis of NLC-LO/AO- CRC; Ws = analysed weight of CRC in supernatant; WL = weight of lipid added in NLC.

ABTS assay. In vitro determination of antioxidant activity

The *in vitro* antioxidant activity of NLC-AO/LO-CRC with or without PO and of NLC-free, CRC, PO was determined by ABTS assay, using a UV–VIS Spectrophotometer Type V670 [38]. By this assay is determined the intensity of radiation transmitted through the substrate of the applied sample at 734 nm and using Trolox (an analogue of vitamin E soluble in water) as standard. The cation radicals $ABTS^{\bullet+}$ resulted by reaction of ABTS solution with potassium persulfate solution (dark conditions for 16 h, at 25°C). A volume of 3 mL $ABTS^{\bullet+}$ solution was added into 1 mL NLCs solution and then ethanol was added until the volume was 5 mL [39]; the reaction was maintained for 4 min. and then the absorbance was determined, using ethanol as reference. The blank sample was prepared with 3 mL of normalized $ABTS^{\bullet+}$ solution and 2 mL of ethanol.

The inhibition (%) of $ABTS^{\bullet+}$ was calculated with the equation described below [40], the average of absorbance being measured in triplicate for each sample:

$$\% \, Inhibition \, ABTS^{+\cdot} = \frac{A_o - A_s}{A_o} \text{ x } 100,$$

where, A_o = absorbance of the blank (unscavenged radical cation solution); A_s = absorbance after the addition of the antioxidant sample (NLCs or Trolox).

Controlled in vitro release of CRC from NLCs

To evaluate the release of curcumin from loaded lipid nanocarriers in dispersion (NLC-AO-PO-CRC and NLC-LO-PO-CRC) vertical Franz diffusion cells (25 mm diameter) were used [41, 42]. The Franz system is composed of a donor chamber and a receptor chamber between which a cellulose nitrate membrane filter (0.1 µm diameter) was placed. The release medium represented by 6 mL of ethanol: phosphate buffer solution is found into the receptor chamber. An amount of 150 µL of aqueous dispersion NLC-LO/AO-PO-CRC was placed onto a cellulose nitrate membrane (previously hydrated in distillated water for 1 h), in the donor chamber. The receptor phase consisted of ethanol and phosphate buffer solution at a ratio of 50:50 (v/v) at 37°C and sink conditions were employed in release experiments. The NLC formulations were maintained onto the membrane for 24 hours, at 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours, 500 µL of each sample being collected from the receptor chamber and diluted with ethanol. The release medium was maintained at a constant volume of 6 mL during the study. The collected samples were analysed using a UV–VIS V670 Spectrophotometer. All the measurements were performed in triplicate at 426.5 nm. The kinetics of the release of Curcumin from NLCs was described using different mathematical models [43].

Results and Discussion

Particle size and zeta potential determination

Zave and PdI of the synthesized NLC systems were determined by Dynamic Light Scattering technique (DLS) [44]. NLC-LO/AO-PO-CRC formulations presented small particle size, with Zave values less than 200 nm. For example, Zave values of NLC-LO-PO-CRC and NLC-AO-PO-CRC are 129.7 nm and 127.6 nm, respectively, while those of NLC-LO-CRC/NLC-AO-CRC and NLC-LO/NLC-AO are of 149.1 nm/130.3 nm and 130.9 nm/157.6 nm (Figure 1). A reorganization of the lipid core, obtaining smaller diameters was observed when encapsulating curcumin in NLC. The result of Zave decrease can be attributed to a loss of viscosity (which helps in HPH processing, with smaller particles), by combining PO with the other lipids selected in the preparation.

In addition to the previous size characteristics, the NLCs based on linseed or argan oil revealed appropriate PdI values ranging from 0.189 to 0.232, which suggests the existence of a relatively monodisperse population of lipid particles. According to literature data, the nanoparticles with PdI values lower than 0.25 are considered homogeneous, avoiding the phenomena of aggregation [45]. The results obtained by DLS showed that NLCs loaded with curcumin present a unimodal profile of size distribution. An exemplification for NLC-AO-PO-CRC, with mean particle size of 127.6/PdI = 0.206 and for NLC-LO-PO-CRC, 129.7/PdI = 0.191, is represented in Figure 2.

Regarding the influence of the two oils on the NLC size, no visible changes of Zave were observed (127.6 nm for NLC-AO-PO-CRC *versus* 129.7 nm for NLC-LO-PO-CRC).

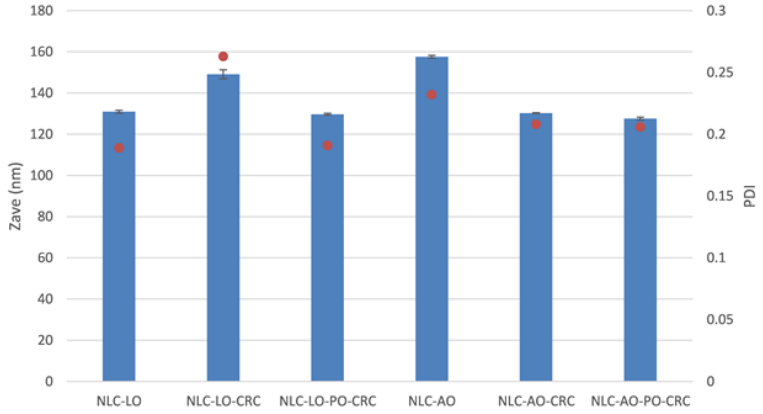

Figure 1.
Mean particle size (nm) and PdI of NLCs dispersion

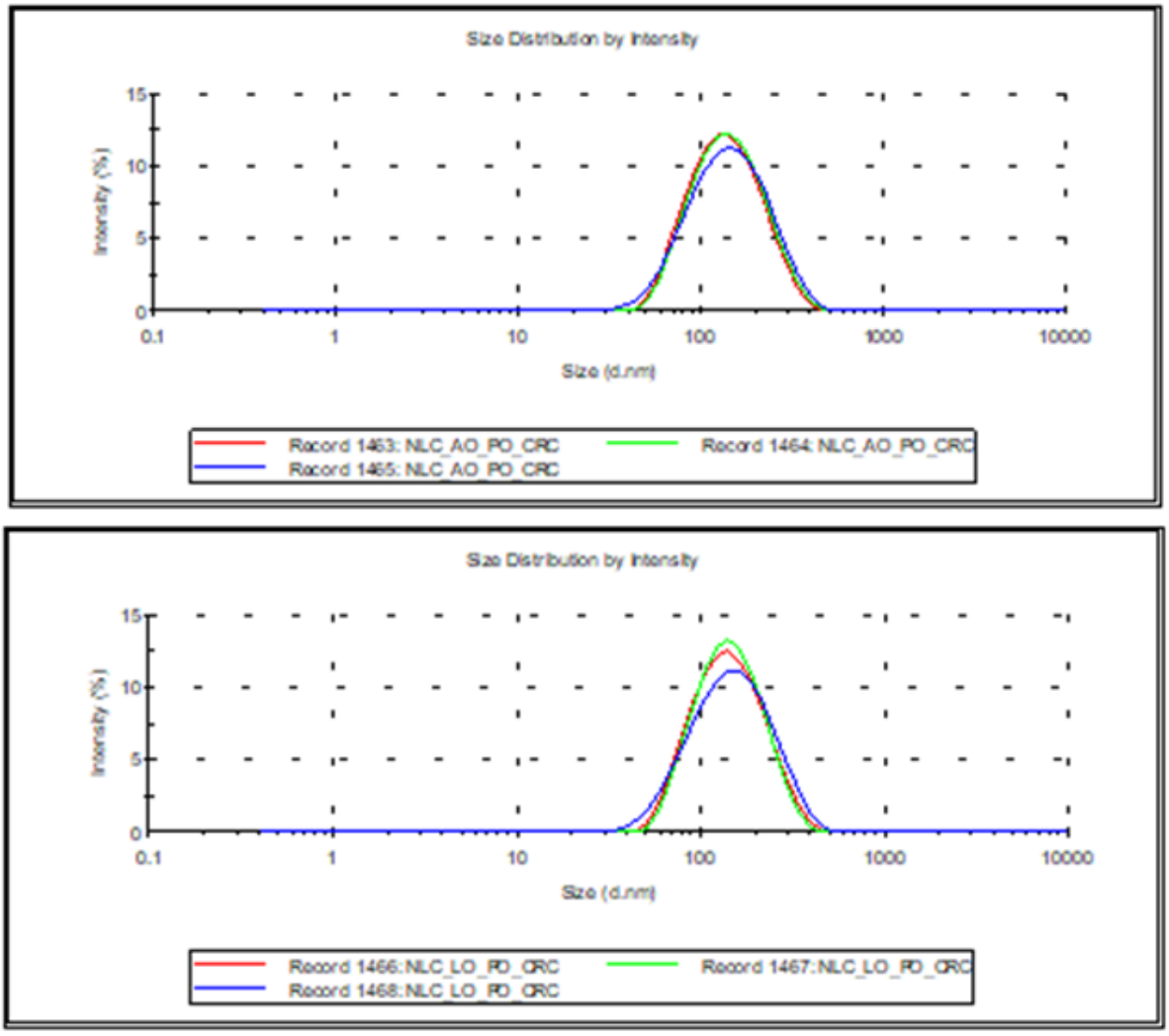

Figure 2.
Size distribution of nanoparticles population

In order to evaluate the physical stability of lipid nanostructured carriers the zeta potential (ζ) was determined. The zeta potential is an electrostatic potential which reflects the electric charge on the nanoparticle surface, which can influence particle stability and further cell absorption phenomena. It is known that a range of -30 mV and -60 mV for zeta potential values is one of the important factors to maintain the stability of the lipid nanoparticles delivery systems [46]. To avoid the aggregation phenomena and for a good physical stability,

electrostatic and steric repulsions among the lipid nanoparticles are needed [47, 48].

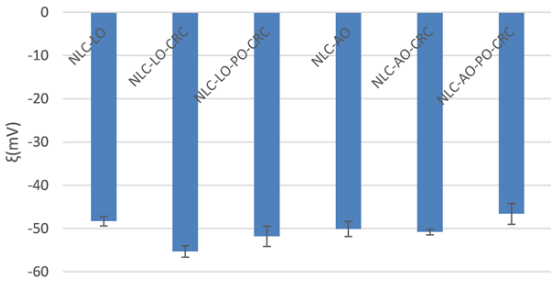

Figure 3.
Evaluation of NLCs physical stability by zeta potential

The experimental results showed that all Curcumin-loaded NLCs are stable over time, with a zeta potential average between -46.6 mV (for NLC-AO-PO-CRC) and -55.3 mV (for NLC-LO-CRC), the entrapment of CRC in the lipid core explaining the small difference between the zeta potential values of NLC-AO and Curcumin-loaded NLCs (Figure 3).

For all the developed NLCs, the zeta potential values were lower than < -40 mV, so the obtained NLC systems are expected to manifest a long-term physical stability. The negative charges of NLCs are due to the effect of surfactants mixture. The zeta potential distributions of the developed NLCs, free and Curcumin-loaded NLCs, are shown in Figure 4.

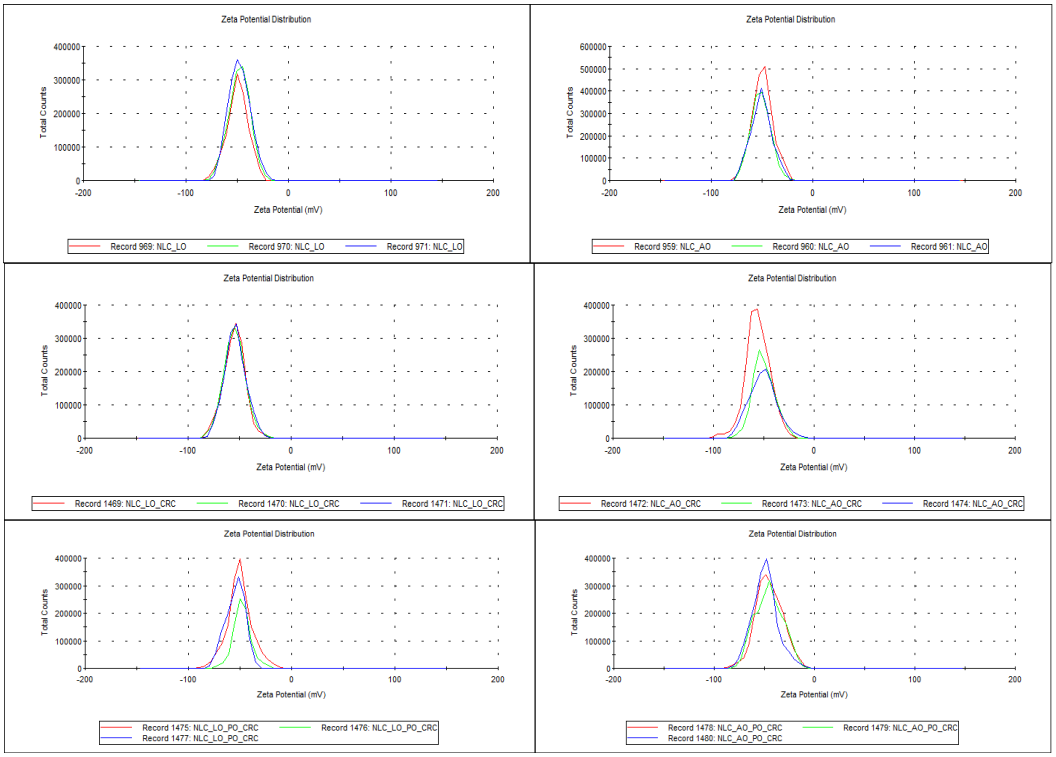

Figure 4.
Zeta potential distribution for free and CRC-loaded NLCs

Entrapment efficiency and loading capacity of CRC

The entrapment efficiency and loading capacity of CRC within different prepared lipid nanocarriers based on linseed/argan oil are shown in Figure 5 and Figure 6. The EE% values for CRC entrapped in the NLCs were higher than 96%, these results being explained by the efficiently capture of CRC in the lipidic network formed by PO in association with vegetable oils, *e.g.* from 96.2% to 99.66%. In case of Curcumin-loaded NLCs co-loaded with PO the EE% results can be explained by the high compatibility of CRC and PO with the lipids mixture consisting of AO/LO, GMS and CP.

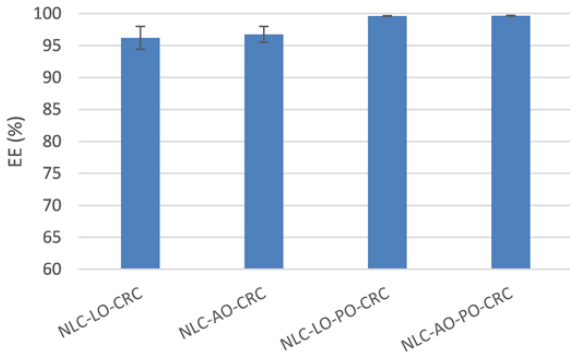

Figure 5.
Entrapment efficiency of CRC into NLCs based on linseed/argan oil

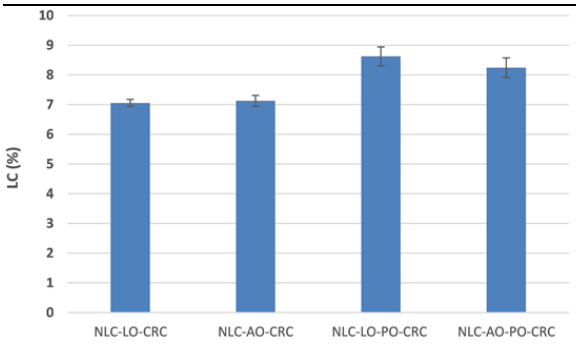

Figure 6.
Loading capacity of CRC into NLCs based on linseed/argan oil

The loading capacity of curcumin into lipid phase is well correlated to the entrapment efficiency values, from 7.05% (NLC-LO-CRC) to 8.62% (NLC-LO-PO-CRC) (Figure 6).

The in vitro determination of antioxidant activity

Lipid peroxidation produced by means of harmful reactive oxygen species (ROS) in humans, is regarded to be a major cause of ageing, carcinogenesis, and cardiovascular disease. Excess ROS leads to a continuous decrease in the biological functions of various organs, respectively to an advanced deterioration of cells and tissues, with the appearance of genetic mutations, inflammation, etc. [49]. Antioxidants are considered to prevent or delay the onset of these processes. A source of antioxidants is both vegetable oils encountered in the developed NLC-CRC.

In our research, in order to determine the free radical scavenging activity of curcumin, PO, linseed oil, argan oil, free NLC, NLC-CRC and NLC-PO-CRC, a spectrophotometric ABTS assay was used. This method is helpful to quantify the free radical trapping capacity of antioxidants [50]. The activity against in situ obtained $ABTS^{+*}$ cation radicals showed the antioxidant potential of the Curcumin in association with argan/linseed oil and with PO. The antioxidant effect of free NLC may be related to the high level of α-linolenic acid in the linseed oil and of linoleic acid in the argan oil. The presence of the native curcumin with linseed/argan oil in the same NLCs induces an improvement of antioxidant action, *e.g.* 61.62% inhibition of $ABTS^{+*}$ in case of NLC-LO-CRC and 64.2% in case of NLC-AO-CRC.

The presence of both CRC and PO, has led to a higher ability of developed NLC to inhibits $ABTS^{+*}$ cation radicals, with inhibition percentage of 69.37% for NLC-AO-PO-CRC and 75.73% for NLC-LO-PO-CRC (Figure 7).

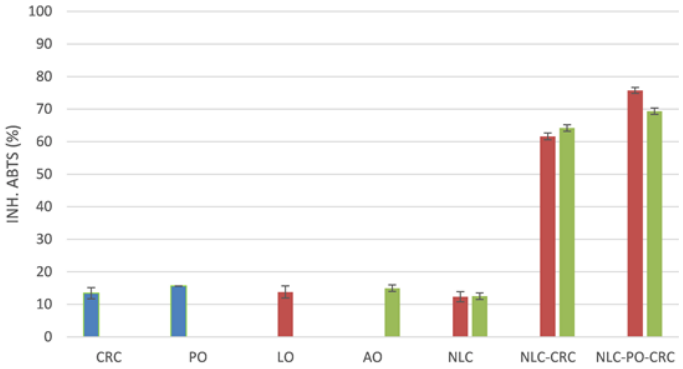

Figure 7.
Antioxidant activity of developed-NLC *versus* native curcumin and vegetable oils (ABTS assay)

These results highlight the net superior role of Curcumin-loaded NLCs to scavenge $ABTS^{+*}$ as compared to the native CRC, phenomenon which might be attributed to the size effect induced by nanoencapsulation and to different types of functional groups from curcumin and vegetable oils that quench the radical state of ABTS. It was observed that the NLCs based on LO, CRC and PO revealed the highest antioxidant capacity.

Figure 8 highlights the comparative assessment of antioxidant activity of native curcumin, PO, LO, AO, free NLC and loaded NLCs using TEAC (Trolox Equivalents Antioxidant Capacity) method. The obtained results by TEAC method sustain those obtained by ABTS assay. Although *in vitro* studies have shown a protective effect of CRC against oxidative stress by reducing the formation of free radicals [51], CRC nanoencapsulation has produced a visible amplification of antioxidant action. As was shown in other studies [52], the nano effect of particles influences the physical and biological properties compared to the native form. A comparative look of the data represented in figure 9 demonstrates the synergistic effect manifested by CRC - linseed oil/argan oil, both captured in the same lipid delivery system. The percentage of ABTS inhibition is 6-fold higher in the case of NLC-CRC and NLC-PO-CRC, compared to NLC-free and native-CRC and LO/AO (Figure 8).

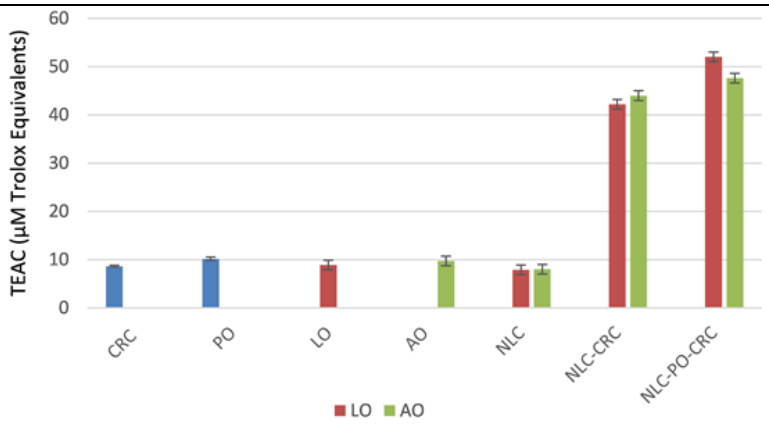

Figure 8.
Comparative assessment of antioxidant activity of native curcumin, lipids, free- and CRC loaded-NLCs, by TEAC method

In vitro release studies of CRC from NLC

Drug release is the procedure by which a drug leaves a pharmaceutical formulation (to supply one or many pharmacological actions) and is subjected to absorption, distribution, metabolism and excretion. Drug release is described in various methods including immediate, modified, delayed, extended, controlled and pulsatile release [53]. Different kinetics models describe drug dissolution from immediate and modified release dosage forms such as zero-order, first-order, Higuchi and Hixson–Crowell. The release kinetics is significantly influenced by the nature of drug, particle size, solubility, crystallinity, and their amount of pharmaceutical dosage form [54]. Solubility of drug in the lipid phase, particle size, lipid core arrangement, partition coefficient are most important factors that influence the release of an active compound from a distribution system [55].

According to the release profiles (Figure 9), in the group of Curcumin-loaded NLCs it was observed a slow release of curcumin; after 24 h of the *in vitro* experiments 17.1% of curcumin was released in case of NLC-AO-PO-CRC and 32% in case of NLC-LO-PO-CRC. This tendency can be attributed to the complex structure of the lipids blend which influenced the migration of CRC through NLC. Interestingly, NLC based on curcumin, PO and AO showed a faster release of curcumin compared to NLC based on curcumin, PO and LO, a phenomenon that may be closely correlated with the composition of vegetable oil; *e.g.* linoleic acid contained in linseed oil is about 14% [35] *versus* 34% in argan oil [33].

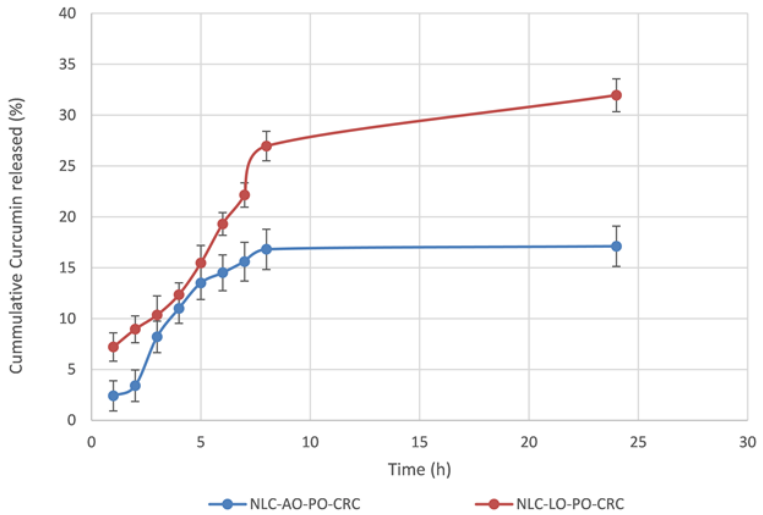

Figure 9.
Influence of the lipid nanocarriers type on the release of curcumin

The release of a drug from nanoparticles was often considered of three types [56]: firstly, a burst release caused by the dissolution or adsorption of the drug on the nanoparticles surface; secondly, a diffusion of drug, caused by the concentration gradient between the nanoparticle and the release

medium and, thirdly, a sustained released of drug incorporated in nanoparticles, caused by the corrosion and degradation of materials that forms delivery system.

Overall, the curcumin release from NLC-LO/AO-PO-CRC exhibited a controlled-release profile, which revealed a two-stage process: the initial release (during 8 h of experiments) might be related to release of a small amount of curcumin adsorbed to the surface layer of NLC and to the passive diffusion of curcumin. After 8 h, 16.8% of curcumin was released in case of NLC-AO-PO-CRC and 26.9% of curcumin in case of NLC-LO-PO-CRC. Moreover, the prolonged release profile after 8 h should be associated to the curcumin incorporated into the NLC inner core that is slowly released with the degradation of NLC.

A possible explanation for the slow release of curcumin from the NLC-AO-PO-CRC delivery system as compared with those of NLC-LO-PO-CRC, might be an increased affinity between curcumin, PO and AO due to π-stacking of phenyl moieties which causes a delayed release of the drug from the lipid network.

The *in vitro* release study was described using several kinetic mathematic models including: Zero-order, First-order, Higuchi [57], Korsmeyer-Peppas [58] and Hixson Crowell models [59]. The kinetic parameters obtained, the rate constant (k), the correlation coefficient (R^2) and the release coefficient (n) were determined for each mathematical model and are shown in Table II.

Table II

Kinetic data obtained at controlled release of curcumin 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-AO-PO-CRC	0.9423	2.181	0.9500	0.0242	0.9698	8.6244	0.9511	0.0029	0.9513	2.0502	0.4877
NLC-LO-PO-CRC	0.9298	2.669	0.9123	0.0320	0.8570	9.9885	0.9095	0.0039	0.8918	1.2032	0.8311

According to the correlation coefficients, it was observed that the release of curcumin from synthesized NLC is performed after a zero-order kinetics in case of NLC-LO-PO-CRC (R^2 = 0.9298) and after a Higuchi model in case of NLC-AO-PO-CRC (R^2 = 0.9698). After 8h, in case of NLC-AO-PO-CRC the rate constant (k = 8.62) is higher than that of NLC-LO-PO-CRC (k = 2.18), which strengthens the previous assumption regarding faster release of curcumin from the LO-based NLC compared to AO-based NLC. In the first hour curcumin is released about 2.4% (NLC-AO-PO-CRC) compared to about 7.2% (NLC-LO-PO-CRC). This trend is not maintained over a period of 24 h, the release percentage for NLC-LO-PO-CRC being 31.95% and only 17.1% in case of NLC-AO-PO-CRC. Zero order release is characteristic to a release of drug at a constant rate for any initial concentration of active, being the goal of all controlled-release drug-delivery mechanisms. It leads, in principle, to the best control of plasma concentration and offers several advantages, including improved patient compliance and reduction in the frequency of drug administration [60, 61]. Higuchi model has become one of the widely used and the most well-known controlled-release model, which is based on several assumptions: the initial concentration of the drug in the formulation is higher than the drug solubility; the drug spreads only in one dimension; the substance particle are smaller than the size of a carrier; swelling of the system and its dissolution is insignificant; the drug diffusivity does not change [62].

The results highlight that NLC based on linseed oil played an important role in controlled release of CRC, this phenomenon could be efficient to avoiding the potential irritant effect of CRC on the gastrointestinal tract as tackled in other conventional pharmaceutical forms [63] and could be useful to get an effective plasmatic concentration of CRC – which can be maintained for a long period of time by the sustained release during 24 h.

Conclusions

The experimental data described in the present study indicate that curcumin can be successfully encapsulated into lipid nanostructures prepared by association of linseed oil or argan oil with a lipid mediator – phenylalaninol oleamida. The DLS measurements highlighted that all NLCs showed desired size characteristics with values of Zave smaller than 150 nm and good physical stability over time, *e.g.* zeta potential ranging from -46.6 mV (NLC-AO-PO-CRC) to -55.3 mV (NLC-LO-CRC).

According to the *in vitro* experimental results, the antioxidant activity of the curcumin loaded NLC revealed their capacity to protect against oxidative damage. The capacity to inhibit the $ABTS^{+*}$ cation radicals was higher for the curcumin loaded NLC when compared to native curcumin. The developed NLC systems have ensured a slow release of CRC after 24 h, being feasible to control the curcumin release for further potential pharmaceutical therapy. The *in vitro* release study showed that 17.1%, respectively, 32% of curcumin was released after

24 h, NLC-LO-PO-CRC indicating a faster release of curcumin compared to NLC-AO-PO-CRC; the slowest release percentage was obtained for the NLC containing argan oil.

In conclusion, the nanoencapsulation of curcumin in association with phenyl alaninol oleamide and the argan oil/linseed oil, may improve their therapeutic effects and and can ensure a slow release of CRC. By capturing all the active principles selected in this study in the same distribution system can provide new premises on the future development of helpful drug systems with different biological effects.

Acknowledgement

This research was financially supported by "Carol Davila" University of Medicine and Pharmacy Bucharest, Romania, through Contract no. CNFIS-FDI-2022-0253, funded by the Romanian Ministry of Education.

Conflict of interest

The authors declare no conflict of interest.

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