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Preparation, characterization and bioavailability by oral administration of O/W curcumin nanoemulsions stabilized with lysophosphatidylcholine

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Curcumin is the main and most abundant bioactive component in *Curcuma longa* L. with documented properties in the prevention and treatment of chronic degenerative and infectious diseases. However, curcumin has low solubility in aqueous media, hence low bioavailability when administered orally. The use of nanoemulsions as carriers can provide a partial solution to bioavailability restrictions. In our study, O/W nanoemulsions of curcumin were prepared using lysophosphatidylcholine, a phospholipid with proven emulsification capacity; nevertheless, such qualities have not been previously reported in the preparation of nanoemulsions. Lysophosphatidylcholine was obtained by enzymatic removal of one fatty acid residue from phosphatidylcholine. The objective of our work was to formulate stable curcumin nanoemulsions and evaluate their bioavailability in BALB/c mice plasma after oral administration. Formulated nanoemulsions had a droplet size mean of 154.32 ± 3.10 nm, a polydispersity index of 0.34 ± 0.07 and zeta potential of –10.43 ± 1.10 mV; stability was monitored for 12 weeks. Lastly, *in vivo* pharmacokinetic parameters, using BALB/c mice, were obtained; namely, C_{max} of 610 ± 65.0 μg mL^{-1} and T_{max} of 2 h. Pharmacokinetic data revealed a higher bioavailability of emulsified as opposed to free curcumin. Research regarding other potential emulsifiers that may provide better health benefits and carry nano-encapsulated bioactive compounds more effectively, is necessary. This study provides important data on the preparation and design of nanoencapsulated Curcumin using lysophosphatidylcholine as an emulsifier.

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Introduction

Curcumin (diferuloylmethane) is a yellow pigment found in turmeric and curry. It is used extensively in food and chemical industries as a coloring, flavoring and preservative agent.[1,2] Additionally, curcumin exhibits antioxidant,[3] anti-inflammatory,[4,5] antiviral, antibacterial, antifungal,[6] and anticancer activity.[7–10] Thus, curcumin has been proven to exert potential benefits against various malignant diseases, such as diabetes, allergies, arthritis, Alzheimer's disease, and other chronic and degenerative illnesses. These effects are mediated through the regulation of various transcription and growth factors, inflammatory cytokines, protein kinases, and other enzymes.[11,12] Various studies in cell culture, animal models, and humans have validated the preventive or therapeutic roles of curcumin.[13,14] However, when assessing the absorption and metabolism of curcumin after oral administration, several reports determined that its absorption in the gut was poor, resulting in extremely low bioavailability.[15–18] When curcumin was administered orally at a 2 g kg^{-1} dose to rats, a maximum serum concentration of 0.23 μg mL^{-1} was observed at 0.83 h; whereas in humans the same dose resulted in either undetectable or extremely low (0.006–0.005 ng mL^{-1} at 1 h) serum levels.[19–21] To increase the bioavailability of curcumin, several delivery systems have been of great interest, including nanoemulsions (NEs).[22–26]

NEs contain both a dispersed and a continuous phase, stabilized by a surfactant called an emulsifier.[26–28] NEs usually range between 50 and 500 nm (ref. 30 and 31) in size; their physicochemical properties are interesting for practical applications such as bioactive compound delivery systems[32,33] to prevent degradation.[29,31] NEs improve, due to their small droplet sizes, compound transport through cell membranes, and consequently their bioavailability and concentration in

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plasma.[30] To understand the precise function of some surfactants, two concepts must be considered. The first concept is the packing parameter, as the ratio of volume to surface area. The packing parameter varies with the number of hydrophobic groups, chain unsaturation, chain branching and chain penetration by other compatible hydrophobic groups. It is essential to understand how surfactants can pack; this information is useful since it allows the prediction of aggregate shape and size. The second concept is the hydrophilic–lipophilic balance (HLB), which refers to the balance between the hydrophobic and hydrophilic groups in a molecule. The HLB range for NEs O/W is between 8–28.[34] The use of phospholipids as surfactants has proven to provide superior physicochemical characteristics and storage stability.[35]

Lysophosphatidylcholine (LPC) is obtained by hydrolysis of one fatty acyl residue from naturally abundant sources of phosphatidylcholine (PC). The LPC molecule has two distinctly different regions: a long hydrocarbon chain of non-polar, lipophilic character and a phosphoric acid-choline moiety, polar in nature. The most common enzymes used for this purpose are phospholipase A1 (PLA1),[36–38] phospholipase A2 (PLA2)[39,40] or lipases.[41–44] LPC can be found in low concentrations in the cell membrane, where it plays an essential biological role as a membrane-derived bioactive lipid mediator. LPC can affect fundamental cellular functions, which include proliferation, differentiation, survival, migration, adhesion, invasion, and morphogenesis. These functions influence many biological processes that include neurogenesis, angiogenesis, wound healing, immunity, and carcinogenesis.[45] Hence, LPC is a good emulsifying and solubilizing agent, and a useful intermediate for the preparation of NE for food, cosmetics, agrochemicals and pharmaceutical applications.[46–48] One of the most important factors influencing the formation of nanoemulsions is believed to be the packing of the surfactant molecules at the oil–water boundary, which can be characterized by their molecular geometry. A surfactant's molecular geometry is classified by a packing parameter, which is the cross-sectional area of the tail group relative to that of the head group: $p = aT/aH$. Differences in surfactant packing at oil–water boundaries influence interfacial characteristics such as surface energy and dynamics, which are likely to play an important role in the spontaneous formation of ultrafine oil droplets using spontaneous emulsification. The packaging parameter for LPC is 0.4; this molecule tends to form micelles in the form of an "inverted cone", with smaller droplet sizes in aqueous solutions, compared to PC, whose packaging parameter is 0.6, and the droplet size is greater in aqueous solutions.[49–52,81]

LPC can also result as a by-product of de-esterification in the modification of PC to obtain structured phospholipids. LPC represents a reduction in the total yield of the interesterification reaction.[34,38,43,53,54] We used LPC as a novel surfactant to prepare NEs as carriers of bioactive compounds such as curcumin. To continue previous studies with this compound performed by our research group, PC was used as an emulsifier with good bioavailability results.[73]

The objectives of this study were to: (1) prepare and characterize LPC by partial hydrolysis of PC catalyzed by phospholipase A1; (2) produce and characterize NEs containing curcumin stabilized with LPC, and (3) study the absorption of curcumin NEs after oral administration to BALB/c mice.

Materials and methods

Materials

Soybean lecithin (95% phosphatidylcholine, PC) was purchased from Shenyang Tianfeng Bioengineering Technology Co. (Shenyang, Liaoning, China). Phospholipase A1 (PLA1) (Lecitase Ultra®) was kindly provided by Novozymes (Salem, VA). Curcumin was purchased from LKT laboratories (St. Paul, MN). Medium chain oil was obtained from Now Foods (Bloomindale, IL). For all the experiments, Milli-Q water was employed. All solvents were HPLC grade from Tecsiquim (Mexico City).

Hydrolysis reaction

The procedure to obtain LPC was based on our previous report. Hydrolysis reactions between PC and PLA1 were carried out in 50 mL glass vials containing methanol and 15% of enzyme with respect of the total PC weight (PC contained in glass vials was 1 g). The reaction mixtures were incubated under nitrogen atmosphere in an orbital shaker set at 65 °C and 200 rpm for 48 h. During the reaction, samples of 30 μL were withdrawn at 0.5, 1, 2, 4, 8, 12, 24 and 48 h, and analyzed by HPLC; conversions of PC into LPC were calculated.[55,79,80]

Identification of phospholipids by liquid chromatography (HPLC)

PC and LPC were analyzed according to a previous report with some modifications.[56] A Waters HPLC system equipped with a UV–visible detector (Waters model 2487), a binary pump (Waters model 1525) and an auto-injector (Waters model 717plus) was employed. A normal phase (250 × 4.6 mm) silica column (Econosil Silica, Alltech Associates, Inc.) was used. The mobile phase consisted of hexane : isopropanol : water (17 : 66 : 17 v/v/v) at an isocratic flow rate of 1 mL min^{-1} and the detector was set at 205 nm. Samples of 10 μL were injected and a calibration curve was prepared with a $R^2 = 0.992$.

Purification of lysophosphatidylcholine

LPC was separated from free fatty acids (FFA), phosphatidylglycerol and unreacted PC according to a published procedure.[57] A glass column (46 cm height, 5.7 cm diameter) was packed with silica gel (500–600 mesh). The mobile phase consisted of consecutive applications of 100 mL of chloroform; chloroform : methanol (65 : 35 v/v); and methanol. Fractions of 100 mL were collected and analyzed by HPLC as described above. The solvent was eliminated from the fractions by rotary evaporation flushed with nitrogen and stored frozen until analyzed.

The recovery of LPC after modification was calculated as follows:

$$\text{LPC recovery}\,(\%) = [\text{recovered LPC}\,(\text{g})/\text{used PC}\,(\text{g}) \text{ on the hydrolysis reaction}] \times 100.$$

Characterization of the fatty acid composition of LPC by gas chromatography (CG)

The fatty acid (FA) composition was determined by gas chromatography following a previously published procedure.[35,56] Briefly, 100 μL of recovered LPC were mixed with 1 mL of 0.5 N sodium methoxide in methanol at 25 °C for 5 min; then, 100 μL of distilled water were added. The methyl esters were extracted with 2 mL of hexane and 1 μL was injected into an HP Model 6890 gas chromatograph fitted with a flame ionization detector (FID) and a HP-INNOWAX (60 m × 0.25 mm × 0.25 mm) capillary column. The column was set to an initial temperature of 50 °C for 1 min, followed by heating to 200 °C at 15 °C min^{-1}. The injector temperature was set at 200 °C and the FID was set at 230 °C. The total running time was 24 min.

Development of curcumin NE formulation

The O/W NEs were formulated using curcumin dispersed in medium chain oil as the dispersed phase at a rate of 5 : 95. Curcumin was placed in test tubes to which the oily phase was added, put on an orbital shaker for 5 min, and then the mixture was positioned in an ultrasonic bath for 1 min. A curcumin load of 50 mg g^{-1} was employed. The continuous phase consisted of either pure water, or 25% glycerol, and LPC was used as emulsifier; LPC was added to water or water-25% glycerol and placed in an ultrasonic bath for two minutes. The continuous phase was added to the oily phase and a combination of high energy methods was used to reduce the droplet size: first, coarse emulsions were prepared by high speed stirring using an UltraTurrax T25 (IKA Works, Inc., Wilmington, NC) at 20 000 rpm for 3 min, according to previous reports;[58,59] then, the emulsion was subjected to ultrasonic emulsification using a S-450D Branson Sonicator (Emerson Electric Co., St. Louis, MO) set at a 30% amplitude. Samples were withdrawn for analysis as described in the following section. The sonication process was carried out under the emulsification conditions described in the experimental design below.

The effect of three variables was explored using a 2^3 factorial design. With this design the mean value, three main effects, three interaction effects of two factors and an interaction effect of three factors were studied. The variables studied were: the concentration of the emulsifier, glycerol addition and the concentration of the bioactive compound. The formulations are depicted in Table 1. Treatments were characterized and the storage stability was determined at 5 °C for 12 weeks. Then, we selected one treatment for a pharmacokinetic study in BALB/c mice, according to the results for the storage stability.

Table 1 Treatments of the factorial design for the preparation of NEs with curcumin using LPC

Treatment	LPC as emulsifier (% total of NE)	Glycerol[a] (% total continuous phase)	Curcumin content (mg g^{-1} NE)
1	5	0	0
2	5	0	5
3	5	25	0
4	5	25	5
5	10	0	0
6	10	0	5
7	10	25	0
8	10	25	5

[a] Glycerol was used as a co-emulsifier in the curcumin nanoemulsions.

Characterization and storage stability of curcumin nanoemulsions

The characterization of nanoemulsions was made by monitoring the droplet size, globule size distribution (reported as polydispersity index, or PDI) and the globule electrical charge (ζ potential) according to our previous report.[60] These properties were determined using a Zetasizer Nano S90 dynamic light scattering device (Malvern Instruments Inc., Worcestershire, UK). The Zetasizer uses the patented Non-Invasive Back-Scatter (NIBS) technology which illuminates a larger number of particles and uses efficient fiber detection.[74]

Curcumin nanoemulsions were diluted 1 : 200 in deionized microfiltered water. For ζ potential measurements, the formulations were diluted 1 : 100 in deionized microfiltered water and placed in the electrophoretic cell, and the average surface charge was determined. All measurements were made in triplicate at 25 °C.

Pharmacokinetic study

To evaluate the bioavailability of the curcumin into NEs stabilized with LPC, *in vivo* pharmacokinetic studies were performed using male BALB/c mice of 8 weeks of age (22–26 g), purchased from Envigo (Mexico City). The mice were randomly divided into two groups (n = 24) and held in vivarium racks for laboratory animals. The mice were standardized under laboratory conditions, *i.e.* at 25 ± 5 °C, with relative humidity of 50% cycles of 12 h/12 h of light/darkness and *ad libitum* access to water and feed (Envigo Teklad Global 18% protein rodent diet 2018S). All animal model experiments were performed in accordance with Mexican norms (NOM-062-ZOO-1999) and institutional bioethics committee guidelines.

After the adaptation period, NEs were administered orally, according to previous reports[60,61] corresponding to 50 mg of active compound per kg of body weight. Group 1 was fed with 50 mg of NEs per kg b.w. without the active compound as a control group; group 2 was fed with 50 mg of curcumin nanoemulsion per kg b.w. We employed this type of control group because our laboratory reported that the modified PC and pure PC are effective nano-emulsifying agents.[73] Therefore, this study focused on the efficacy of nanoemulsions to achieve the absolute bioavailability of curcumin. For this reason, a

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negative control group was chosen, since NEs have been demonstrated to be more effective than suspensions and emulsions as bioactive carriers. For each group, the mice were sacrificed at each of the following sampling points: 0, 0.5, 1, 2, 4, 8, 12, and 24 h.

Blood samples (0.5 mL) were obtained from heart puncture, and processed according to previous reports.[62,63] Samples were placed in heparinized micro centrifuge tubes containing 20 μL of 1000 IU heparin per mL of blood and immediately centrifuged at 4000 rpm for 10 min at 4 °C, to obtain the plasma. To stabilize curcumin, plasma was added with 2.8% v/v acetic acid solution (25 μL/250 μL plasma) and vortexed for 20 s.

To extract curcumin from blood plasma, 1.2 mL of ethyl acetate were added to a 500 μL sample aliquot, mixed in a vortex for 10 min, and then centrifuged at 10 000 rpm for 10 min. The upper organic layer containing curcumin was transferred to another tube and dried under a stream of nitrogen to remove the ethyl acetate. The residue was reconstituted in 125 μL of methanol followed by vortex mixing for 1 min and filtered through a membrane (0.45 μm pore size), and 10 μL of the filtrate were injected into the HPLC system for analysis, according to a method previously reported.[62] A Waters HPLC system consisting of a UV–visible detector (Waters model 2487), a quaternary pump (Waters model 600) and an autoinjector (Waters model 717plus) was used. A reverse phase silica column (250 × 4.6 mm, C18 Alltech) was employed. The mobile phase consisted of 100% methanol in an isocratic flow of 1 mL min^{-1}, and the detector was set at 430 nm. Calculations were made using an external standard.

The pharmacokinetic analysis was performed for formulations using a non-compartmental design. The area under the drug concentration *versus* time curve from zero to 12 hours ($AUC_{0-12\ h}$) was calculated using the trapezoidal rule. The maximum plasma concentration of curcumin (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma analyses. Plasma half-life values ($t_{1/2}$) were also estimated.[46]

Statistical analysis

The results are depicted as the mean values of curcumin nanoemulsions duplicates, and as the mean of the pharmacokinetic study by triplicates. The data were subjected to ANOVA using Minitab® v. 17 statistical software. Differences between means were compared using Tukey's test with a significance level of $p < 0.05$.

Results and discussion

Preparation, characterization and purification of LPC

The main reason to pursue the enzymatic reaction was to produce and use the LPC as an innovative emulsifier. In most investigations, LPC is considered as an unwanted by-product of the enzymatic reaction. LPC decreases the yield of the reaction in the production of structured phospholipids. The enzymatic reaction proceeds as follows: the PLA1 is specific to ester bonds at position *sn*-1 of the PC. In this case, the hydrolysis of phospholipids occurred in one step of the reaction, thereby separating free fatty acids (FFA) in the position *sn*-1 of the molecule PC and hence the LPC is obtained.[36,58]

To estimate the hydrolysis reaction yield, the conversion of PC to LPC was quantified, and the results show a 29% conversion that was attained with 15% enzyme–substrate ratio for a reaction time of 72 h at 65 °C (Fig. 1). The hydrolysis reaction can be affected by a number of factors such as substrate and enzyme concentrations, and the direction of the reaction depends on the equilibrium constant and the reactant concentrations. The obtained yield is attributed to the following reasons: during the reaction, production of several intermediates occurred, caused by the hydrolysis of PC and further re-esterification of the lysophospholipid; therefore, a net reduction in LPC yield occurred.[36,44,53,57,63]

Previous reports dealing with enzymatic modification of phospholipids proposed that increasing conversion to LPC decreased the yield of other phospholipids as PC. Then, LPC appears as an unwanted product and therefore has not been previously considered for delivery applications. For example, phospholipids were modified using free n-3 fatty acids, and the results were products esterified with n-3 fatty acids by acidolysis using immobilized phospholipase A1, of which 34% corresponded to PC and 13.7% to LPC.[35]

In order to estimate the FA content of the LPC, analyses were performed by gas chromatography and the results are shown in Table 2. The sources of PC and fatty acid composition determine the reaction product, and therefore will also determine the properties, structures and the behavior of the LPC in NEs during preparation, stability and pharmacokinetic studies.

As described above, LPC has two important features of the surfactants used in O/W nanoemulsions: the packing parameter of LPC is 0.4, and it has an HLB value of 16, which was calculated from the data obtained by gas chromatography. These features render LPC suitable for the preparation of O/W NEs.[54]

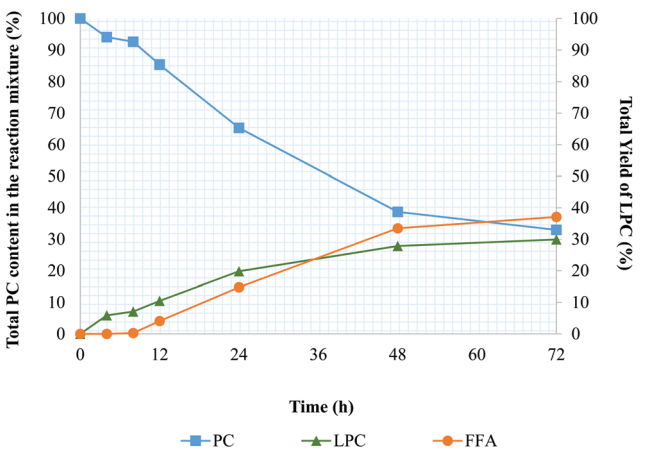

Fig. 1 Composition of the reaction mixture as a function of time. The reaction mixture in glass reactors contained 1 g of PC and 15% enzyme–substrate ratio; 72 h at 65 °C. The yield of the reaction was calculated according to total reaction products.

Table 2 Fatty acid composition (mol%) in phosphatidylcholine (PC) and lysophosphatidylcholine (LPC)

Fatty acid	LPC	PC
C8:0	15.34 ± 0.32	—
C10:0	07.95 ± 0.05	—
C14:0	02.92 ± 0.00	—
C16:0	03.82 ± 0.21	36.81 ± 0.45
C18:0	00.24 ± 0.01	03.25 ± 0.22
C18:1(9)	00.55 ± 0.30	18.58 ± 0.76
C18:1(7)	11.26 ± 0.01	31.17 ± 0.43
C18:2(6)	56.23 ± 0.00	05.08 ± 0.09
C18:3(3)	04.58 ± 0.24	02.15 ± 0.08

Reaction conditions to obtain LPC: The reaction mixture consisted of PC and phospholipase A1; a loading of 15% with respect to the weight of PC was incubated for 72 h and maintained at 65 °C and 200 rpm. Values reported are the mean of triplicate determinations.

Characterization and storage stability of curcumin nanoemulsions

All treatments produced emulsions in the nanoscale range (<500 nm); addition of the active compound had a significant effect on the droplet size as shown in Table 3. According to at least one report, this is attributed to the encapsulation of crystalline particles of curcumin, which increased the droplet size.[64] In another report, O/W emulsions were prepared using medium chain triacylglycerols (MCT) as the oil phase and Tween 20 as the emulsifier, with mean droplet sizes ranging from 79.5 nm to 618.6 nm. Droplet size increased with increased amounts of curcumin.[54]

To the best of our knowledge, there are no reports in the literature that indicate the use of LPC as a surfactant in curcumin NEs. Concentrations of 5 to 10% LPC were tested and there were no significant differences between the concentrations of LPC and droplet size. For this reason, it is necessary to further investigate the efficacy of LPC as a surfactant in diverse delivery systems.

The nanoemulsions had polydispersity indexes below 0.2, which suggests that the NEs were monodispersed. These results indicated that in all cases a good distribution of the hydrophobic phase in NEs stabilized with LPC. Polydispersity values near 1.0 are indicative of a polydispersed system.[65] Addition of the active compound had a significant effect on the PDI. This could be caused by a higher amount of LPC in the medium, causing viscosity increases that lead to a slower mobilization around the droplets, which probably prevented suitable coating, and thus generated higher PDI values.[66]

Finally, regarding zeta potential, it has been proposed that values lower than −30 mV may suggest good stability caused by the strong repulsive forces between the droplets.[67] In nanoemulsions prepared with 10% LPC and in those containing curcumin, zeta potential values increased in comparison with those containing 5% as emulsifier. The data are depicted in Table 3. This can be explained by the addition of macromolecules that provide charges such as van der Waals forces and electrostatic and steric repulsion, which are the main stabilizing forces for NEs which interact with the layer of ions and the associated counter-ions, thus modifying the potential around the droplets. When droplets come so close together that the adsorbed emulsifier layer begins to overlap, some short-range interactions become significant, such as steric interactions, thermal fluctuation and hydration forces.[21]

Comparing our results with data from other works, curcumin NEs, stabilized with Tween 80, MCT as the oil phase, and 40 mg of curcumin, showed a droplet size of 141.6 nm, a zeta potential of −6.9 mV and a PDI of 0.273.[21] In another report, curcumin NEs were stabilized with phosphatidylcholine-MCT, which produced a mean droplet size of 156 nm, a zeta potential of −12.2 mV and a PDI of 0.48.[49]

Table 3 Initial characteristics of curcumin nanoemulsions stabilized by LPC

Treatment	Droplet size (nm)	PDI	Zeta potential (mV)
1	114.80 ± 9.83^{c}	0.26 ± 0.02^{d}	-11.54 ± 0.87^{bc}
2	154.32 ± 3.10^{a}	0.34 ± 0.07^{cd}	-10.43 ± 1.10^{e}
3	98.66 ± 1.53^{c}	0.29 ± 0.04^{cd}	-10.77 ± 1.10^{b}
4	124.7 ± 3.10^{b}	0.38 ± 0.04^{cd}	-18.59 ± 0.09^{a}
5	92.02 ± 8.91^{c}	0.25 ± 0.18^{a}	-15.87 ± 0.94^{d}
6	176.8 ± 8.40^{ab}	0.47 ± 0.10^{ab}	-5.47 ± 0.68^{cd}
7	92.078 ± 3.62^{c}	0.30 ± 0.24^{b}	-13.50 ± 0.35^{b}
8	235.3 ± 3.52^{a}	0.54 ± 0.15^{a}	-4.20 ± 0.5^{bc}

PDI = polydispersity index; nanoemulsions were prepared as described in Table 1. Values are reported as mean ± SD. $n = 6$. Means with different letters represent significant differences ($p < 0.05$) by one-way ANOVA and Tukey's mean comparison test.

Stability of curcumin nanoemulsions

The characteristics of the treatments in the initial and final weeks of the evaluation are shown in Table 4. All treatments showed a significant difference during storage. The stability of a wide range of preparations has been assessed whereby the

Table 4 Characteristics of curcumin nanoemulsions stabilized by LPC in storage for 12 weeks at 5 °C

Treatment	Week	Droplet size (nm)	PDI	Zeta potential (mV)
1	0	114.80 ± 9.83^{a}	0.26 ± 0.02^{a}	-11.54 ± 0.87^{a}
	12	198 ± 7.6^{b}	0.21 ± 0.01^{a}	-6.2 ± 1.8^{b}
2	0	154.32 ± 3.10^{a}	0.34 ± 0.07^{a}	-10.43 ± 1.10^{a}
	12	241 ± 2.4^{b}	0.43 ± 0.04^{b}	-18.7 ± 3.47^{b}
3	0	98.66 ± 1.53^{a}	0.29 ± 0.04^{a}	-10.77 ± 1.10^{a}
	12	233.1 ± 33.9^{b}	0.4 ± 0.05^{b}	-4.32 ± 2.9^{b}
4	0	124.7 ± 3.10^{a}	0.38 ± 0.04^{a}	-18.59 ± 0.09^{a}
	12	242.42 ± 18.29^{b}	0.66 ± 0.05^{b}	-2.87 ± 1.3^{b}
5	0	92.02 ± 8.91^{a}	0.25 ± 0.18^{a}	-15.87 ± 0.94^{a}
	12	345.9 ± 6.54^{b}	0.2 ± 0.005^{a}	-6.2 ± 0.3^{b}
6	0	176.8 ± 8.40^{a}	0.47 ± 0.10^{a}	-5.47 ± 0.68^{a}
	12	387.8 ± 10.1^{b}	0.79 ± 0.07^{b}	-5.6 ± 0.5^{a}
7	0	92.078 ± 3.62^{a}	0.30 ± 0.24^{a}	-13.50 ± 0.35^{a}
	12	486 ± 32.7^{b}	0.52 ± 0.09^{b}	-5.6 ± 0.88^{b}
8	0	235.3 ± 3.52^{a}	0.54 ± 0.15^{a}	-4.20 ± 0.5^{a}
	12	663 ± 37.7^{b}	0.8 ± 0.009^{b}	-2.42 ± 0.6^{b}

PDI = polydispersity index. Values are reported as mean ± SD. $n = 6$. Means with different letters represent significant differences ($p < 0.05$) by one-way ANOVA and Tukey's mean comparison test.

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storage period varied amply. For example, limonene encapsulated in nanoparticles stabilized with Tween 80 were stable for 3.5 months;[68] curcumin nanoemulsions were prepared using egg lecithin as the emulsifier and were stable for 3 months.[69] Finally, the significance of different emulsifiers for the characteristics and stability of the nanoemulsions were evaluated. In the single report found in the literature concerning LPC NEs, nanoemulsions prepared with LPC were more stable compared to other emulsifiers.[70]

The droplet size of curcumin nanoemulsions during storage is depicted in Fig. 2. It can be seen that all treatments had significant changes with respect to droplet size. We thus selected one treatment for a further pharmacokinetic study using BALB/c mice. The droplet size of curcumin nanoemulsions was selected; curcumin NEs formulated with 5% of emulsifier, 5% medium chain oil, 0% glycerol in the aqueous phase and 5 mg mL^{-1} of active compound produced nanoemulsions of 154.32 nm, and a PDI value of 0.34. The storage period increased by 56.65%. However, the particle size was still within the nanometer scale, and it was considered stable for the 12 weeks of the study. These data are shown in Table 5.

The choice of treatment 2 was based on this stability study, because stability in the formulation of nanoemulsions is important if one desires to apply the procedure to other animal models.

Pharmacokinetics of curcumin nanoemulsions

In Fig. 3 concentrations in BALB/c mice plasma after an oral administration of curcumin nanoemulsions (5% of emulsifier, 5% medium chain oil, 25% glycerol in the aqueous phase and 5 mg of the active compound per mL) are depicted. The results were: a C_{max} of 610 μg mL^{-1} of blood plasma and a T_{max} of 2 hours. The observed pharmacokinetic behavior is associated with a fast adsorption of the active compound. For these systems, the bioavailability of curcumin concomitantly increased with the content of phospholipids. This may be caused by the increased number of mixed micelles available to incorporate curcumin. Bioavailability may increase with increasing phospholipid content, because more mixed micelles are formed to solubilize the released curcumin and they are best incorporated into cell membranes.[71]

A summary of pharmacokinetic parameters is displayed in Table 6. The LPC content in the nanoemulsions had a favorable effect on the bioavailability of curcumin. One theory is that being a smaller molecule than PC, with a single fatty acid,

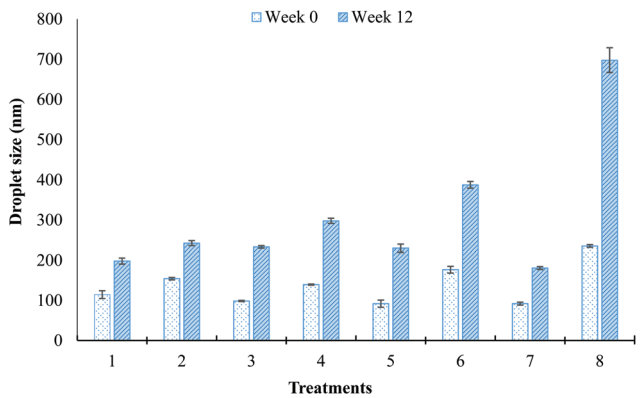

Fig. 2 Stability of NEs prepared according to conditions depicted in Table 1. Values are reported as mean ± SD, n = 4. Storage was made for 12 weeks at 5 °C.

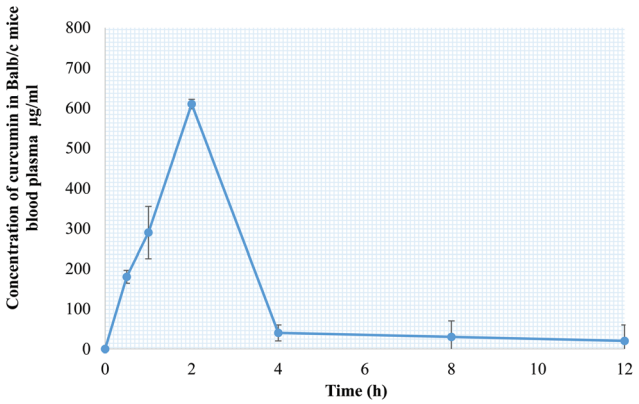

Fig. 3 Pharmacokinetic data showing maximal plasma concentration of the bioactive (C_{max}), 610 ± 65.0 μg mL^{-1} and the time to reach maximum plasma concentration (T_{max}), 2 h.

Table 5 Evolution during storage at 5 °C of curcumin nanoemulsions was selected to continue the BALB/c mice study

Weeks	Droplet size (nm)	PDI	Zeta potential (mV)
0	154.32 ± 3.10^{a}	0.34 ± 0.07^{a}	−10.43 ± 1.103^{a}
2	207 ± 8.34^{a}	0.34 ± 0.04^{a}	−15.36 ± 5.67^{ab}
4	229 ± 4.93^{a}	0.45 ± 0.07^{b}	−13.15 ± 0.8^{ab}
8	243 ± 6.81^{b}	0.47 ± 0.05^{b}	−13.54 ± 2.73^{ab}
12	241 ± 2.4^{b}	0.43 ± 0.04^{b}	−18.7 ± 3.47^{b}

Nanoemulsion composition was: 5% of LPC; 5% medium chain oil; 0% glycerol in the aqueous phase, and 5 mg mL^{-1} curcumin. Values are reported as mean ± SD, n = 4. Means in rows with different letters are significantly different (p < 0.05) by one-way ANOVA and Tukey's mean comparison test.

Table 6 Summary of the plasma pharmacokinetic parameters of curcumin in male BALB/c mice

Parameters	Units	NE curcumin
Dose	mg kg^{-1}	50
C_{max}	μg mL^{-1}	610 ± 65.0
T_{max}	h	2.0
AUC_t	μg h mL^{-1}	2387.5
K_{el}	h^{-1}	0.5021
$T_{1/2}$	h	1.38

The mice were administered with 50 mg of curcumin per kg of body weight. ($AUC_{0-12\ h}$) was calculated using the trapezoidal rule. The maximum plasma concentration of drug (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were obtained for BALB/c mice plasma.

the droplet sizes may be smaller. The second likely reason for this improvement is that the LPC could adhere to the cell membranes more effectively favored by their structural characteristics, thus releasing faster the active compound in the bloodstream.[72,73]

There are several reports of emulsified systems that deal with the pharmacokinetics of curcumin. For example, SNEDDS were prepared and showed that the adsorption of these systems is better (approximately 93.8%) than a curcumin suspension with a T_{max} of 3.86 h compared with the suspension (24%).[72] Comparing these results with other reports made with modified nanoemulsions containing medium chain fatty acids, T_{max} and C_{max} values were greater in this study, but the reason why this happens is still under consideration.[52,59] The length of the phospholipid chain could be responsible for this event.

Other reports portray the emulsifying properties of phospholipids;[70] for example, a phospholipid containing a single fatty acid in its structure, as the LPC, facilitated the absorption of particles through the intestinal villi attributed to the nanometric size of the LPC-stabilized curcumin NEs, thus allowing for increased bioavailability of the compound in plasma. The LPC nanoemulsions provided higher bioavailability of the bioactive compared with the curcumin suspension; however, the C_{max} of our preparations was higher than that from nanoemulsions made with PC modified with MCT, which produced 316.81 ng mL^{-1}.[52] The obtained data are consistent with previous reports, and thus propose the LPC as a suitable stabilizer for formulations of curcumin NEs with particle sizes smaller than 500 nm.[1,59]

In general, phospholipids act as excellent wall materials for entrapping bioactive molecules and aid in the emulsification process. Emulsification is an essential step for the digestion and absorption of lipids. These combined effects of phospholipid emulsions may enhance the bioavailability of lipophilic molecules such as entrapped curcumin.[75–78] The objective of this work, to assess the bioavailability of curcumin in nanoemulsions stabilized with LPC, was fulfilled. As previously mentioned, curcumin suspensions did not produce favorable results in the plasma concentration of curcumin, but absorption was improved by the use of phospholipids. These results did not compare formulations that have already been reported by our group.[73] Instead, the possibility of using LPC as an emulsifier was analyzed and confirmed our hypothesis that the LPC was an effective nanoemulsifier. We propose that LPC should be used to prepare delivery systems for problematic bioactive compounds in aqueous solutions or for aqueous solutions that have low absorption rates.

Conclusions

The use of LPC to prepare nanoemulsions resulted in particle sizes of less than 500 nm. Droplet size, polydispersity index and zeta potential values were maintained for at least 12 weeks and improved the bioavailability of curcumin nanoemulsions after oral administration. The data suggest that LPC is an effective surfactant for encapsulating curcumin in nanoemulsions.

Abbreviations

PC	Phosphatidylcholine
LPC	Lysophosphatidylcholine
NEs	Nanoemulsions
MCT	Medium chain triacylglycerides
T_{max}	Maximum time
C_{max}	Maximum concentration

Ethical statement

All experiments were approved by the Institutional Ethics Committee (CEI-ITVER), with authorization CEI-ITVER/012/2013, conducted in compliance with the Mexican standard NOM-062-ZOO-199. Additionally, the Occupational Health and Safety in the Care and Use of Research Animals (1997), issued by the National Academy of Sciences of the US was followed. All experiments were performed using institutional guidelines and supervised by a DVM.

Conflicts of interest

The authors confirm that this article content has no conflict of interest.

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