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RESEARCH ARTICLE

Enhanced Bioavailability of Curcumin Nanoemulsions Stabilized with Phosphatidylcholine Modified with Medium Chain Fatty Acids

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Abstract: ***Background***: Curcumin is a natural, oil-soluble polyphenolic compound with potent anticancer, anti-inflammatory, and antioxidant activities. In its free form, it is very poorly absorbed in the gut due to its very low solubility. The use of nanoemulsions as carrier is a feasible way for improving curcumin bioavailability. To this end, the choice of emulsifying agent for stabilizing the nanoemulsions is of the upmost importance for achieving a desired functionality.

Methods: Phosphatidylcholine (PC) and phosphatidycholine enriched (PCE) with medium chain fatty acids (42.5 mol %) in combination with glycerol as co-surfactant, were used for preparing oil-in water nanoemulsions coded as NEPC and NEPCE, respectively.

Results: NEPCE displayed significantly smaller mean droplet size (30 nm), equal entrapment efficiency (100%), better droplet stability and suffered lower encapsulation efficiency loss (3%) during storage time (120 days, 4ºC) than NEPC. Bioavailability, measured in terms of area under the curve of curcumin concentration versus time, and maximum curcumin plasma concentration, was in general terms significantly higher for NEPCE than for NEPC, and for curcumin coarse aqueous suspension (CCS). Also, NEPCE produced significantly higher curcumin concentrations in liver and lung than NEPC and CCS.

Conclusion: These data support the role of phosphatidylcholine enriched with medium chain fatty acids to increase the bioavailability of nanoemulsions for therapeutic applications.

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INTRODUCTION

Curcumin is a natural, oil-soluble polyphenolic compound with potent anticancer, antiinflammatory, and antioxidant activities [1]. It poses no toxicity risks in humans after consuming several grams per day for months [2]. The pharmacological safety and efficacy of curcumin makes it a compound with potential for treatment and prevention of a wide variety of chronic illnesses [3]. Furthermore, the effectiveness of curcumin against various malignant diseases including cancer has been established [4]. However, curcumin has a very low oral bioavailability due to its poor absorption in the gastrointestinal tract, rapid metabolism, and elimination [5]. Some of the possible approaches to overcome these problems are the addition of adjuvants, which can block metabolic pathways of curcumin; or the use of promising novel formulations like nanoparticles, nanoliposomes, nanomicelles, and nanoemulsions, which appear to provide longer circulation, better permeability, and resistance to metabolic processes [6, 7].

Nanoemulsions (NEs) are a class of extremely small droplet emulsions, usually in the range of 50 to 200 nm, that appear to be transparent or translucent [8]; possess high kinetic or thermodynamic stability, and both, hydrophilic or lipophilic phytochemicals can be incorporated into the same nanoemulsion. Because of their small droplet sizes, bioactive

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compounds can be transported through cell membranes more effectively, resulting in increased bioactive compound bioavailability and concentration in plasma [9, 10]. A proper selection of the emulsifying agent is of paramount importance as it greatly influences the globule size and other properties of the NEs [11].

Lecithin, a mixture of phospholipids (PLs) including phosphatidylcholine (PC), is perhaps the most widely used natural emulsifier in the food, pharmaceutical and biotechnology industries. Moreover, PC is among the safest emulsifying agents in the market [12]. However, PC has a dimensionless packing parameter (S_p), which represents the ratio between hydrocarbon volume, optimum head group and tail length, close to unity. This molecular geometry is not perfectly suited for the formation of curved surfaces. The ability of PC as an emulsifying agent is influenced by the length and degree of unsaturation of the acyl hydrocarbon chains. Unsaturation of the acyl hydrocarbon chains weakens the emulsification ability, attributed to vulnerable double bonds. PCs with shorter and saturated acyl hydrocarbon chains are considered more effective emulsifying agents for producing smaller droplet sizes [13, 14]. PLs can be modified by physical, chemical, or enzyme-catalyzed methods to improve their emulsifying properties. In particular, enzymatic modification of PLs offers the advantages of the specificity of the enzymes and safety considerations [15, 16]. Incorporation of saturated fatty acids with a chain length of 6-10 carbons into PLs has proven to enhance their emulsifying properties [17].

The aims of this study were to: (1) obtain phosphatidylcholine enriched (PCE) with medium chain fatty acids by acidolysis catalyzed by immobilized phospholipase A_1; (2) produce oil-in-water nanoemulsions containing curcumin stabilized with PC and PCE; (3) determine the curcumin encapsulation efficiency and mean droplet size and distribution of the NEs; (4) evaluate the bioavailability of the curcumin loaded NEs, and its distribution in body tissues after oral administration to experimental mice, in comparison with a coarse curcumin aqueos suspension.

MATERIALS AND METHODS

Materials

Phospholipase A_1 (Lecitase® Ultra) was provided by NOVO (Salem, VA) and Duolite A568 as immobilization support was a donation by Rohm & Haas (Philadelphia, PA). Curcumin of high purity (≥ 98% of (1E,6E)-1,7-Bis (4-hidroxi-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, was purchased from LKT Laboratories (St. Paul, MN) and soybean lecithin (95% of PC) was acquired from Avanti Polar Lipids (Alabaster, AL). A mixture of free medium chain fatty acids (MCFAs) was obtained by saponification of medium chain oil (MCO; Original Thin Oil®, Sound Nutrition, Dover, ID) following a preparation process as described previously [18]. Glycerol (99.5% USP grade, vegetable-based, KIC Chemicals, Inc., New Paltz, NY) was used as aqueous phase co-surfactant. HPLC grade solvents were purchased from Tecsiquim (Mexico City) and all other reagents were purchased from SIGMA (Mexico City). Deionized distilled water was used in all the experiments.

Enrichment of PC with MCFAs

PC was enriched with MCFAs by acidolysis with phospholipase A_1 immobilized on Duolite. Acidolysis reaction was conducted in a solvent-free system. Substrates (PC and MCFAs in a molar ratio of 1:15) were placed in an Erlenmeyer flask and mixed in a heating plate with magnetic stirring at 300 rpm and 40°C until complete dissolution. Sixty grams of substrates were placed in 500 mL Erlenmeyer flasks and mixed with a loading of 12% of phospholipase A_1 previously immobilized on Duolite, according to our previously published procedure [19]. The reaction was carried out in an orbital shaker operating at 300 rpm, at 45°C for 24 h.

Recovery and Analysis of PCE

The PCE with MCFAs was recovered from the acidolysis reaction mixture by column chromatography, according to a method previously reported [20]. The column (460 x 57 mm) was packed with 600 g of silica analytical grade (60-200 mesh, 150 Å). Free fatty acids were eluted with chloroform, while chloroform/methanol (65:35 v/v) was used to elute the PCE, and methanol to elute lisophosphatidylcoline (LPC).

PCE recovery was determined by HPLC. Samples were prepared by diluting with ethanol and injected into a Waters HPLC System fitted with a Partisil silica column 5 µm (4.6 x 250 mm). The mobile phase, according to a previously published procedure [21], consisted of acetonitrile/methanol/phosphoric acid (130:5:1.5 v/v/v) run isocratically, at a flow rate of 1.5 mL/min. PCE was detected at 205 nm with a UV-visible detector (Waters model 2487). Retention time for PCE was 16.1 min. This peak was identified using an external standard.

The fatty acid composition of PCE was determined by gas chromatography following our previously published procedure [22]. Briefly, 100 µL of recovered PCE was mixed with 1 mL of 0.5 N sodium methoxide in methanol and held at room temperature for 5 min; then, 100 µL of distilled water was added to stop the reaction. The methyl esters were extracted with 2 mL of hexane. One µL was injected into an HP Model 6890 gas chromatograph fitted with a flame ionization detector and a HP-INNOWAX (60 m x 0.25 mm x 0.25 mm) capillary column. The temperature program consisted of an initial temperature of 50 °C for 1 min, followed by heating to 200°C at 15°C/min. Then, this temperature was maintained for 24 min. Injector and FID temperatures were set at 200 and 230°C, respectively.

Curcumin NEs Preparation

The oil-in-water (O/W) NEs were prepared following a thin-film hydration method [23], with slight modifications. In a first instance, preliminary NEs were prepared as follows: PC (10% w/w NE) was dissolved in 5 mL of ethanol by stirring for 5 min and then added to MCO (5% w/w NE), with further stirring for 3 min. Then different curcumin loads (0, 2.5, 5.0, 7.5, or 10.0 mg/g NE) were added to the mixture using vigorous mixing, followed by sonication for 15 min using an Aquawave 9376 ultrasonic bath (Barnstead International, Dubuque, IA). The resulting blends were then rotary evaporated to generate dried thin films which were subsequently hydrated with deionized distilled water (DW; 85%

w/w) warmed at 45°C and stirred for 15 min. The mixture was homogenized for 3 min at 20,000 rpm using a T25 digital ULTRA-TURRAX homogenizer (IKA Works, Inc., Wilmington, NC) to produce a coarse curcumin oil-in-water emulsion.

The coarse emulsion was subjected a ultrasonic emulsification at 20% amplitude and 50% duty cycle using a Branson Digital Sonifier S-450D (Emerson Electric Co., St. Louis, MO) with a 13 mm diameter probe for 12 min to obtain the nanosized droplets. The NE displaying the smallest mean droplet size (DS) and polydispersity index (PDI), and highest curcumin entrapment efficiency (EE) and curcumin concentration (CC) was used for preparing NEs incorporating glycerol as co-surfactant. To this end, NEs were prepared as indicated before. PC (10% w/w NE) was used as emulsifier. The thin-films were hydrated in DW-glycerol solutions (85% w/w NE; ratios of 1:0; 0.875:0.125; 0.750:0.250; 0.625:0.325; and 0.5:0.5).

Optimized NEs (NE_{PC} and NE_{PCE}) were prepared using 10% w/w PC or PCE as emulsifying agent, 5% w/w MCO, and the curcumin load and glycerol concentration that yielded the preliminary NEs with the smallest DS, narrowest PDI and highest EE. All the NEs were prepared in duplicate.

Characterization and Storage Stability of Curcumin NEs

NEs were characterized for droplet size (DS), polydispersity index (PDI), zeta potential (ζ), curcumin concentration (CC), and entrapment efficiency (EE). A Zetasizer Nano-ZS90 dynamic light scattering device (Malvern Instruments Inc., Worcestershire, UK) at a 90° fixed angle at 25°C was used to determine the DS, PDI and ζ of the NEs. For DS and PDI measurements NEs were diluted 1:200 in deionized water to avoid multiple scattering effects. DS was reported as "Z-average" diameter (the scattering intensity-weighted mean diameter), which was calculated from the signal intensity versus droplet diameter data. For ζ measurements, NEs were diluted 1:100 in deionized water, placed in the electrophoretic cell, and the average surface charge was determined.

CC in the NEs was determined by HPLC. A standard curve was prepared from known concentrations of curcumin in ethanol. After centrifugation, 5 mL of ethanol were added to 5 µL of the NEs samples and 10 µL were injected into a Waters HPLC System fitted with a Waters Econosphere C18 (5 µm, 250 X 4.6mm) column. Curcumin was detected at 428 nm with a Waters UV-visible detector model 2487. The mobile phase, according to previously published method [24], consisted of acetonitrile, 2.8% acetic acid and methanol, by gradient, at a flow rate of 1 mL/min. Retention time for curcumin was 4.6 min. The amount of curcumin in the nanoemulsions was calculated from the final concentration of curcumin after its preparation and centrifugation, and EE of curcumin in the NE was estimated with the following equation:

$$\%EE = \left(\frac{MAC}{TAC}\right) * 100$$

where: *% EE* is the entrapment efficiency of curcumin in the NE, *MAC* is the measured amount of curcumin in NE, and *TAC* is the total amount of curcumin used to prepare the NE.

NEs were maintained in incubation chambers at 4°C for 16 weeks to analyze the changes in DS and EE, as a measure of NEs stability.

Pharmacokinetic Study

For *in vivo* pharmacokinetic studies, male BALB/c mice of 8 weeks of age (22-26 g) were purchased from Harlan Teklad (Mexico City). The mice were acclimated for at least 8 d before the experiments and housed in cages (6 each) under constant temperature (23 ± 0.5°C) with free access to food (Harlan Teklad Global 18% protein rodent diet 2018S) and drinking water. Animal maintenance and handling were performed according to Mexican Official Standards [25]. After the adaptation period, animals were randomly divided into three groups (n = 21); group 1 was administered with 50 mg of curcumin coarse suspension (CCS) per kg bw; group 2 was fed with 50 mg of NE_{PC} per kg bw; and group 3 was administered with 50 mg of NE_{PCE} per kg bw, by oral gavage. For each group, three mice were sacrificed at each of the following seven time points: 0.5, 1, 2, 3, 4, 8, 12, and 24 h. Blood and tissues (stomach, small intestine, liver, kidney, and lung), were collected.

Blood samples (0.5 mL), obtained from heart puncture, were placed in heparinized microcentrifuge tubes (containing 20 µL of 1000 IU heparin/mL of blood) and immediately centrifuged at 4000 rpm for 10 min at 4°C, to get 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. Tissue samples were excised and rinsed three times in ice-cold physiological saline; samples were dried on filter paper, accurately weighed, cut into chips, and homogenized with the Ultra Turrax homogenizer after adding 0.9% NaCl solution to make a uniform concentration (1 mL/g tissue). Tissue homogenates were added with 2.8% v/v acetic acid solution (100 µL/mL tissue homogenate) and vortexed for 60 s to stabilize the curcumin. The plasma and tissue homogenates were stored at -70°C until extraction and quantification of curcumin.

For curcumin extraction from blood plasma, 1.2 mL of ethyl acetate were added to a 500 µL aliquot of sample, mixed on a vortex for 10 min, and then centrifuged at 10,000 rcf for 10 min. The upper organic layer containing curcumin was transferred to another tube and dried under a stream of nitrogen to remove ethyl acetate. The residue was reconstituted in 125 µL of methanol followed by vortex mixing for 1 min. After filtering through a membrane (0.22 µm pore size), 50 µL were injected into the HPLC system for analysis, according to the method previously reported [24]. For tissue homogenates, curcumin was extracted with ethyl acetate (2 mL/500 µL tissue homogenate) by mixing on a vortex for 3 min and then on an orbital shaker (Thermo Scientific MaxQTM 4450) for 30 min. The upper organic layer was transferred to another tube and dried under a stream of nitrogen to remove ethyl acetate. The residue was reconstituted in 125 µL of methanol, mixed on a vortex for 1 min, filtered through a membrane, and 10 µL were injected into the HPLC system for analysis.

Non-compartmental pharmacokinetic analysis was performed for all of the formulations. The area under the drug concentration versus time curve from zero to 12 hours

($AUC_{0\text{-}12\ h}$) 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 directly obtained from plasma data. The relative bioavailability (% F_{rel}) of curcumin in NE (NE_{PC} or NE_{PCE}) versus crude curcumin aqueous suspension (CCS) was calculated as:

$$\% F_{rel} = [AUC_{0\text{-}12\ h}\ NE/AUC_{0\text{-}12\ h}\ CCS] \times 100$$

Statistical Analysis

The results are presented as the average of NEs duplicates, and as the average of the pharmacokinetic study triplicates. The data were subjected to ANOVA using STATISTICA V. 6.0 software (StatSoft, Inc., Tulsa, OK). Differences among the means were compared using a Tukey's test with a significance level of $p < 0.05$.

RESULTS

PCE with MCFAs

The mixture of free MCFAs obtained had the following composition: caproic acid C6:0 (0.04 ± 0.01 mol %), caprylic acid C8:0 (71.14 ± 0.08 mol %), capric acid C10:0 (28.62 ± 0.07 mol %), and lauric acid C12:0 (0.20 ± 0.01 mol %). The acidolysis reaction was conduced at the optimal conditions for maximum PC enrichment and recovery [26]. These conditions were: molar ratio of PC to free MCFAs of 1:15, enzyme loading of 12%, and 45°C. Under these conditions, an enrichment of PC with free MCFAs of 43 mol %, after 24 h of reaction and a PC recovery of 64% could be achieved.

PCE was separated from lysophosphatidylcholine (LPC) and free fatty acids by column chromatography; it required 31 volumes (125 mL) of chloroform for separation of free fatty acids, 19 volumes of chloroform/methanol (65:35 v/v) for the separation of PCE and 15 volumes of methanol to separate LPC. The solid phase separation yielded a 60% PCE that was confirmed by HPLC. The MCFAs in the PCE fraction obtained after 24 h of acidolysis reaction were quantified by gas chromatography. The PCE was composed by 42.52 mol % of MCFAs; 31.39 ± 0.81 mol % of caprylic and 11.13 ± 0.43 mol % of capric acids, while the mole percentages of palmitic, oleic, and linoleic fatty acid residues were substantially reduced. Table **1** shows the composition (mol %) of the fatty acid residues in PC and PCE.

Characterization of the Curcumin NE_{PC}

The effect of curcumin load on the initial DS and PDI of the preliminary NEs formulations is showed in Fig. (**1a**). These results indicate that as the load of curcumin increased from 0 to 10 mg/g in the NE, both DS and PDI increased, probably because larger loads of curcumin tended to increase the dispersed phase viscosity, hampering the fragmentation of large liquid drops into small droplets during the final stages of emulsificaction. The NE made with a curcumin load of 2.5 mg/g NEs exhibited the smallest DS (108 nm) and narrowest PDI (0.13) (Fig. **1a**), and the highest encapsulation efficiency (100%) (Fig. **1b**). NEs formulated with higher curcumin loads exhibited larger DS, wider PDI and lower EE.

Table 1. Fatty acid composition (mol %) in phosphatidylcholine (PC) and phosphatidylcholine enriched with medium chain fatty acids (PCE).

Fatty Acid	PC	PCE*
C8:0		31.39 ± 0.81
C10:0		11.13 ± 0.43
C16:0	14.18 ± 0.45	2.46 ± 0.37
C18:0	3.51 ± 0.21	0.52 ± 0.08
C18:1(9)	10.65 ± 0.59	6.97 ± 0.21
C18:1(7)	1.58 ± 0.12	0.40 ± 0.05
C18:2(6)	64.03 ± 0.37	43.24 ± 0.88
C18:3(3)	6.05 ± 0.43	3.89 ± 0.08
Total MCFAs		42.52 ± 0.73

*Reaction conditions for enrichment of PC: substrates mixture consisting of PC and free medium chain fatty acids (MCFAs) in molar ratio of 1:15; a loading of 12% of phospholipase A_1 immobilized on Duolite; incubated for 24 h in an enclosed reactor, maintained at 45°C and stirred at 300 rpm. Values reported are mean of duplicate determinations from different experimental trials.

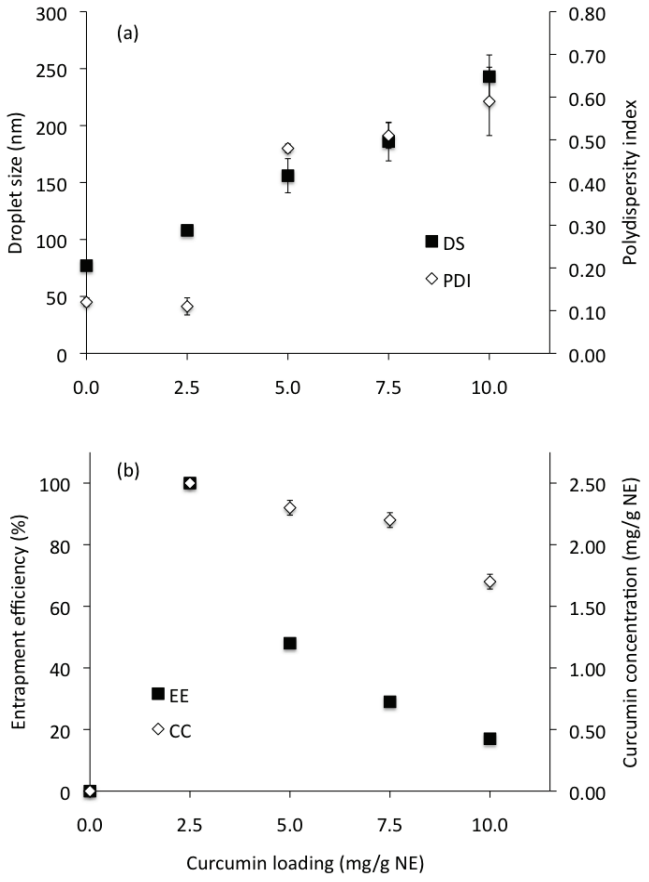

Fig. (1). Curcumin loading effect on characteristics of nanoemulsions stabilized with phosphatidylcholine (NE_{PC}). (**a**) Droplet size (DS) and polydispersity index (PDI). (**b**) Entrapment efficiency (EE) and curcumin concentration (CC).

Based on the above results, a new set of preliminary NEs were prepared containing a curcumin load of 2.5 mg/g NE with different glycerol concentrations, seeking to attain smaller DS and narrower PDI. Often the use of co-surfactants helps to optimize NEs formation. Fig. (**2**) shows that when a glycerol concentration of 21.25% w/w was used, the DS was significantly decreased to 84 nm, while the PDI was maintained at more or less the same value. This means that the addition of glycerol in the proper amount can help to enhance the quality of the formed NE, which by the way was optically translucent. Thus, from these studies it was concluded that an optimized NE_{PC} with a DS of 84 nm, a PDI of 0.15, an EE of 100% was achieved with the following formulation: 5% w/w MCO, 2.5 mg/g curcumin, 10% w/w PC and 21.25% w/w glycerol.

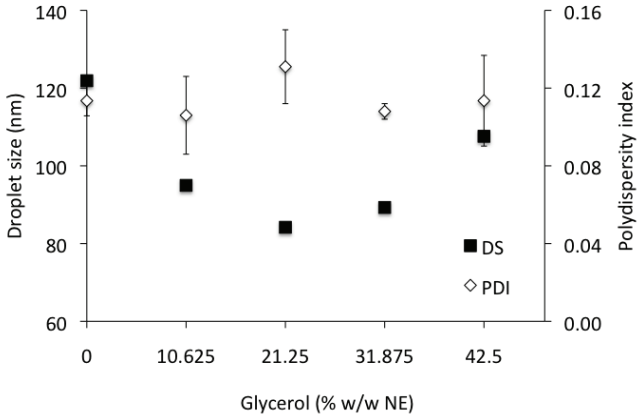

Fig. (2). Glycerol concentration effect on droplet size (DS) and polydispersity index (PDI) of curcumin nanoemulsions stabilized with phosphatidylcholine (NE_{PC}).

Curcumin NEs Stabilized by PCE

In order to try to further reduce the DS of the optimized NE_{PC} described in the previous subsection, we substituted the use of PC by PCE with MCFAs. The result of this is displayed in Table **2**. NE_{PCE} exhibited an initial DS of 29.6 nm that was significantly smaller ($p < 0.05$) than that of NE_{PC}, while the EE efficiency remained unchanged. The droplet diameter obtained in a nanoemulsion depended strongly of the molecule size emulsifier; small-molecule surfactants formed smaller droplets than large-molecule surfactants. PCE is smaller than PC, because of the substitution of longer chain fatty acids by MCFAs.

DS and EE Variation with Storage Time on NE_{PC} and NE_{PCE}

NE_{PC} and NE_{PCE} were stored at 4°C for 120 days, and their DS and EE were examined at different times (Fig. **3**). NE_{PCE} droplet size increased from 29.6 nm after freshly made to 43.2 nm, and its entrapment efficiency decreased from 100% to 97% during the 120 days storage time. On the other hand, NE_{PC} droplet size increased from 84.4 nm to 144.3 nm, and entrapment efficiency decreased 7% in the same time interval. In both NEs the sharpest droplet increase occurred during the first 30 days of storage time, followed by a steady smaller droplet increase in the subsequent storage time. If two oil-water interfaces of droplets coated with surfactant are brought close to one another, then a thin film of water between the interfaces is formed. Due to the like charges of the surfactants, on NE_{PC} or NE_{PCE} (see the zeta potential data in Table **2**), the two interfaces repel each other and the surfactant stabilized the film against rupturing, so the droplets would not coalesce. Based on the zeta potential data, it is likely than an electrostatic repulsion between the nanoemulsion droplets played a role in slowing down droplet flocculation, and contributed to their long-term stability. Although only particles with zeta potential values greater than +30 mV or smaller than −30 mV are generally considered to be stable caused by the strong repulsion forces [27], apart from charge-induced stabilization, modified phospholipids also lead to improved molecular packing at the interface, and the nanoemulsion stability. Phospholipids with short and saturated acyl hydrocarbon chains decrease the molecular packing and promote the formation of stable nanoemulsions [13].

Pharmacokinetic Study

The plasma concentration-time course profiles of curcumin in male BALB/c mice following oral administration of NE_{PC}, NE_{PCE} and coarse curcumin aqueous suspension (CCS) are shown in Fig. (**4**). Incorporation of curcumin into NEs significantly enhanced its bioavailability ($p < 0.05$), compared to that achieved with CCS, as measured by the area under the curve (*AUC*). After 12 h, the $AUC_{0\text{-}12\,h}$ was 3.5 times higher for NE_{PCE} than for CCS, and double for NE_{pc} than for CCS (Table **3**). The reason why NE_{PCE} showed a significantly higher bioavailability than NE_{PC} was probably because of its smaller globule size, that produced a much higher surface area, which increased the rate at which NE were absorbed into the body. Furthermore, the administered CCS had very low bioavailability because curcumin solubility was poor in the aqueous phase, remaining mainly insolu-

Table 2. Characteristics of curcumin nanoemulsions stabilized by phosphatidylcholine (NE_{PC}) and phosphatidylcholine enriched with medium chain fatty acids (NE_{PCE}).

NE Code	DS (nm)	PDI	ζ (mV)	EE (%)	CC (mg/g NE)
NE_{PC}	84.4 ± 2.2^{a}	0.15 ± 0.02^{a}	-8.3 ± 0.5^{a}	100 ± 0.5^{a}	2.5 ± 0.01^{a}
NE_{PCE}	29.6 ± 2.3^{b}	0.24 ± 0.08^{b}	-16.3 ± 2.2^{b}	100 ± 0.3^{a}	2.5 ± 0.01^{a}

NE = Nanoemulsion; DS = droplet size; PDI = polydispersity index; ζ = zeta potential; EE = entrapment efficiency; CC = curcumin concentration. Nanoemultions were prepared with medium chain oil (MCO) 5% w/w, phosphatidylcholine (PC) or phosphatidylcholine enriched with medium chain fatty acids (PCE) 10% w/w, glycerol 21.25% w/w, and curcumin (2.5 mg/g nanoemulsion). Values are reported as mean ± SD. n=2. Means with different lowercase letters (a or b) in the same column are significantly different ($p < 0.05$) by one-way ANOVA.

ble, and absorption from the bulk of the suspension was limited. Also NE_{PCE} produced the highest curcumin plasma concentration (C_{max}), in shorter time than CCS (Table **3**).

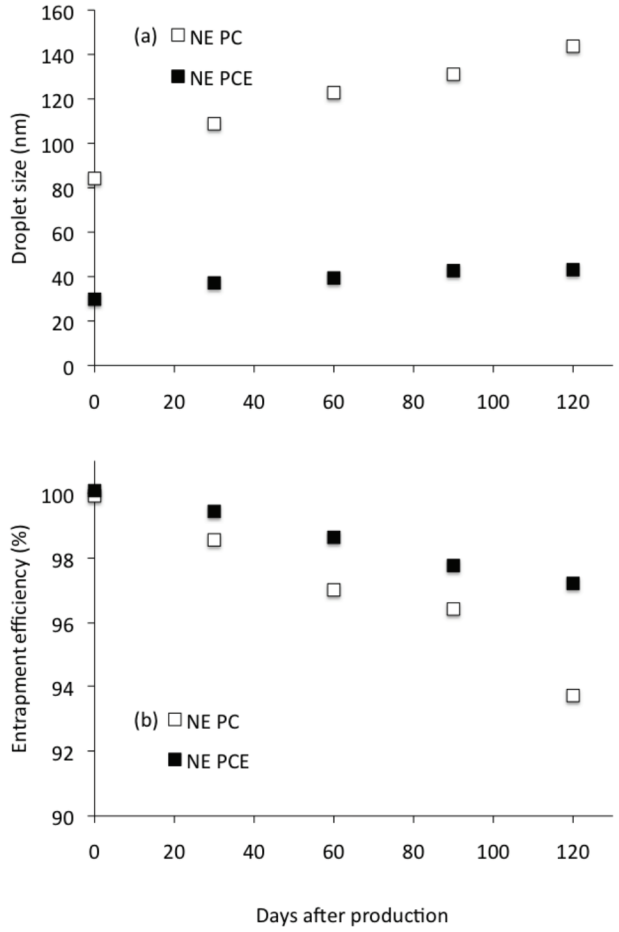

Fig. (3). Droplet size (**a**) and entrapment efficiency (**b**) variation with storage time of curcumin nanoemulsions stabilized with phosphatidylcholine (NE_{PC}) and phosphatidylcholine enriched with medium chain fatty acids (NE_{PCE}).

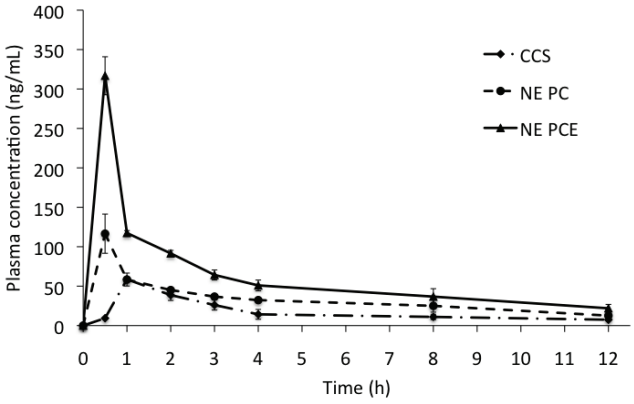

Fig. (4). Comparative *in vivo* blood plasma concentration vs time profiles of different curcumin formulations: (CCS) coarse curcumin suspension, (NE_{PC}) curcumin nanoemulsion stabilized by phosphatidylcholine, and (NE_{PCE}) curcumin nanoemulsion stabilized by phosphatidylcholine enriched with medium chain fatty acids.

The distribution of curcumin in body tissues is important because it sheds light regarding the biological activity of curcumin on specific target sites. In this study the distribution of curcumin in tissues of male BALB/c mice after oral administration of 50 mg of curcumin per kg of body weight was obtained. The results showed that the maximum concentracion of curcumin was found in the stomach (1,437 μg/g of tissue), while significantly lower concentrations were found in the intestine (234 μg/g of tissue), liver (6.00 μg/g of tissue), lung (5.52 μg/g of tissue), and kidney (1.98 μg/g of tissue). These concentrations were significantly higher for NE_{PCE} than for NE_{PC}, and for CCS in liver and lung (see Table **4**).

DISCUSSION

PLs by design bearing new physical and chemical properties can be obtained by exchanging fatty acids in the sn-1 and sn-2 positions. Some works in this direction focus on the incorporation of saturated fatty acids (including both medium chain and long chain) to improve emulsifying properties, or heat and oxidation stability of the PLs [28-30]. In this work it was possible to obtain a PCE with a greater incorporation of MCFAs and a higher PC recovery at shorter reaction time than in others studies previously reported [31-34].

In order to obtain NEs with reduced DS, PCE with MCFAs was used in its preparation. NE_{PCE} exhibited a significantly smaller DS than NEs that were prepared with PC unmodified. These results are in agreement with other study, which indicated that the minimum droplet diameter that could be obtained in a nanoemulsion depended strongly on the emulsifier type [35]. Small-molecule surfactants formed smaller droplets than proteins, which was attributed to their ability to rapidly adsorb to the droplet surfaces during homogenization. PCE is smaller than PC, because of the substitution of longer chain fatty acids by MCFAs. In another study [14] the emulsifying properties of various phosphatidylcholines, with different acyl hydrocarbon chains lengths and degrees of unsaturation, were investigated. The authors found that PCs with shorter and saturated acyl hydrocarbon chains produced oil-in-water emulsions with smaller droplet sizes.

NE_{PCE} also exhibited a suitable value of PDI and encapsulation efficiency. Values close to or lower than 0.1 are indicative of good quality colloidal suspensions, while values close to 1 are indicative of poor quality samples, either with droplet sizes out of the colloidal range or with a very high polydispersity [36]. The NE_{PCE} exhibiting smallest DS and lowest PDI, had a higher curcumin concentration than that reported by other researchers [23, 37-39]. Many authors have reported a direct relationship between NE DS and encapsulation efficiency: smaller DS, generally corresponds to higher encapsulation efficiency [40-42].

NEs with prolonged physical stability in most cases are achieved by means of electrochemical stabilization [43, 44]. The surface electrical charge of globules has an important influence in stability; causes repulsion with other globules, keeping them apart from each other and then unable to form aggregates. NE_{PCE} showed a long-term stability more likely because of its droplet surface charge. It has been reported that NEs prepared with PCs usually have a droplet surface

Table 3. Summary of plasma pharmacokinetic parameters of curcumin in male BALB/c mice obtained after oral administration*.

Parameter	Formulation		
	CCS	NE_{PC}	NE_{PCE}
$AUC_{0\text{-}12\,h}$ (ng.h/mL)	207.78 ± 20.23[a]	389.72 ± 58.82[b]	720.25 ± 86.63[c]
C_{max} (ng/mL)	58.63 ± 1.03[a]	116.50 ± 24.86[b]	316.81 ± 24.09[c]
T_{max} (h)	1.0	0.5	0.5
F_{rel} (%)	-----	188	347

*The mice were administered with 50 mg of curcumin per kg of body weight. CCS = coarse curcumin suspension; NE_{PC} = curcumin nanoemulsion stabilized by phosphatidylcholine; NE_{PCE} = curcumin nanoemulsion stabilized by phosphatidylcholine enriched with medium chain fatty acids; $AUC_{0\text{-}12\,h}$ = area under concentration curve; C_{max} = maximum concentration; T_{max} = time to reach C_{max}. Values are reported as mean ± SD (n=3). Means with different lowercase letters (a, b, or c) in the same row are significantly different ($p < 0.05$) by one-way ANOVA.

Table 4. Summary of pharmacokinetic parameters of curcumin in organs of male BALB/c mice after oral administration*.

Organ	Parameter	Formulation		
		CCS	NE_{PC}	NE_{PCE}
Liver	$AUC_{0\text{-}12\,h}$ (µg.h/g)	52.75 ± 3.44[a]	63.19 ± 1.47[a]	92.22 ± 14.21[b]
	C_{max} (µg/g)	6.65 ± 0.96[a]	6.85 ± 1.24[a]	8.57 ± 1.21[b]
	T_{max} (h)	1	1	0.5
	F_{rel} (%)	-----	120	175
Lung	$AUC_{0\text{-}12\,h}$ (µg.h/g)	48.43 ± 4.55[a]	62.41 ± 2.37[a]	80.49 ± 9.52[b]
	C_{max} (µg/g)	6.60 ± 1.65[a]	6.81 ± 1.27[a]	7.89 ± 0.59[b]
	T_{max} (h)	1	1	0.5
	F_{rel} (%)	-----	129	166
Kidney	$AUC_{0\text{-}12\,h}$ (µg.h/g)	18.63 ± 2.56[a]	21.60 ± 2.51[a]	25.11 ± 3.48[a]
	C_{max} (µg/g)	1.97 ± 0.45[a]	2.71 ± 0.60[a]	2.81 ± 0.47[a]
	T_{max} (h)	1	0.5	0.5
	F_{rel} (%)	-----	116	135

*The mice were administered with 50 mg of curcumin per kg of body weight. CCS= coarse curcumin suspension; NE_{PC} = curcumin nanoemulsion stabilized by phosphatidylcholine; NE_{PCE} = curcumin nanoemulsion stabilized by phosphatidylcholine enriched with medium chain fatty acids; $AUC_{0\text{-}12\,h}$ = area under concentration curve; C_{max} = maximum concentration; T_{max} = time to reach C_{max}. Values are reported as mean ± SD (n=3). Means with different lowercase letters (a or b) in the same row are significantly different ($p < 0.05$) by one-way ANOVA.

charge in negative range due to the presence of negatively charged PLs, especially free fatty acids and phosphatidic acid [13].

The enhanced bioavailability of curcumin on NE_{PCE}, compared with the achieved with CCS, measured by the area under the curve (*AUC*) is attributed to several factors. Curcumin (crystalline) in the aqueous suspension needs to be solubilized in the digestion environment, which may be time and energy consuming. Moreover, the pH value in the major portion of the small intestine ranges from neutral to weakly alkaline, conditions under which curcumin undergoes rapid degradation, resulting in low oral bioavailability [45]. In NEs, the adsorbed interfacial films protect curcumin from chemical or enzymatic degradation. Curcumin NEs are transported through cell membranes more effectively because of the very small DS; curcumin solubility in the oil phase droplets is increased by the surfactants, which upon contact with the digestion juices, form micellar structures that enhance the absorption of curcumin [46]. Lipid based formulations can influence the absorption of active ingredients through stimulation the lymphatic transport of active ingredients and interaction with enterocyte based transport or formation of colloidal particles with bile components, which are able to maintain a larger amount of poor water soluble drugs in solution *via* micellar solubilization [47]. Improved oral bioavailability of various hydrophobic bioactive compounds in oil-in-water emulsions has been reported in the literature [48-50]. PLs have been used as excipients in oral formulations to increase solubility of active substances, keep them solubilized in the gastrointestinal tract, promote their

absorption, and improve their bioavailability [51], and particularly PC could improve the absorption of curcumin [52, 53].

The distribution of curcumin in tissues of male BALB/c mice showed its maximum concentracion in the stomach, while lower concentrations was found in the intestine, liver, lung and kidney. However, NE_{PCE} produced higher curcumin concentrations than NEPC and CCS, in liver and lung tissues. These results are in agreement with another study that reported, after oral administration of 400 mg of curcumin to rats, only traces of the drug in the liver and kidney, while 90% of curcumin was found in the stomach and small intestine [54]. In another study, using a mouse model, a curcumin dose of 0.1 g/kg *via* i.p. showed a maximum concentration of curcumin in the intestine; while the spleen, liver, and kidney contained only moderate concentrations of curcumin, and only a trace amount were found in the brain [55].

CONCLUSION

NEs enriched with MCFAs were successfully prepared for use in oral administration of curcumin. NEs reached a small mean DS, low PDI, high CC, high EE, and displayed long term stability in terms of DS variation and curcumin EE loss. The NE_{PCE} showed significantly improved oral bioavailability of curcumin than the formulation stabilized with unmodified PC. This work established a methodology for the obtention of stable nanoemulsions with high curcumin loading that can be used for an efficient curcumin bioavailability and delivery of curcumin to specific body organs.

CONFLICT OF INTEREST

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

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PATIENT CONSENT

Declared none.

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