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Entrapment efficiency and drug loading of curcumin nanostructured lipid carrier (NLC) formula

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ABSTRACT

Curcumin is a chemical compound that has low solubility and bioavailability and, for these reasons, limited biological effectiveness. For improvement, the solubility needs to be increased by nanotechnology and nanoparticles, among others. Nanostructured Lipid Carrier (NLC) is a new drug delivery system that offers several advantages, including a significant increase in drug solubility and entrapment efficiency. This study aimed to formulate curcumin into curcumin-loaded NLC preparation and determine its characteristics, absorption efficiency, and drug loading. The formulation used evaporation and solvent diffusion methods with three different concentrations, namely 5%, 10%, and 15%. Spectrophotometry and HPLC were employed to test the absorption efficiency and drug loading capacity. The results showed that the curcumin-loaded NLC preparation containing 10% curcumin had stable characteristics and produced particles sized 17.4 nm with a polydispersity index of 0.574 and zeta potential of -63.43 mV. Based on the spectrophotometry results, the entrapment efficiency was 93.212%, and the drug loading capacity was 0.708%. Meanwhile, the HPLC showed that the entrapment efficiency was 93.007%, and the drug loading capacity was 0.795%.

Keywords: drug loading, absorption efficiency, curcumin, NLC

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INTRODUCTION

Curcumin is a chemical compound responsible for the yellow color in turmeric and has a broad therapeutic effect (Soni *et al.*, 2011). It has several unfavorable properties such as low solubility and low bioavailability (Wang *et al.*, 2009; Yang *et al.*, 2007), and this combination negatively affects its biological effectiveness (Shaikh *et al.*, 2009). Therefore, developing formulas to increase the bioavailability of this natural compound becomes necessary (Ajazudin, 2010).

Nanotechnology and nanoparticles are two approaches known to enhance the poor biopharmaceutical properties of curcumin by increasing its aqueous solubility (Torchilin, 2009; Ruenraroengsak *et al.*, 2010; Sultana *et al.*, 2013). Nanostructured Lipid Carrier (NLC) is a drug delivery system with several benefits, which include increased solubility of less dissolvable drugs, physical stability, and efficiency entrapment, as well as reduced skin irritation and ease of manufacture and improvement (Sanad *et al.*, 2010; Kaur *et al.*, 2015; Jain and Jain, 2010).

Based on the description above, research on the entrapment efficiency and drug loading of the curcumin-loaded NLC formula becomes necessary. This study is expected to obtain a stable preparation, with improved %entrapment efficiency and drug loading.

MATERIALS AND METHODS

Materials

The tools used in this study were batch heater, hotplate, magnetic stirrer, homogenizer (IKA Ultra-Turrax), 130-Watt ultrasonic processor 20 kHz (Cole-Parmer), particle size analyzer (Delsa Nano Beckman Coulter), UV-1700 UV/Vis spectrophotometer (Shimadzu), Fourier-Transform Infrared (FTIR) Spectrophotometer (Shimadzu), JEOL JEM 1400 transmission electron microscope for the High Performance Liquid Chromatography (Shimadzu LC-20AT(HPLC)), and glassware. The materials included curcumin (Merck), cholesterol (Merck), oleic acid (Sigma-Aldrich), Tween 80 (Merck), distilled water, potassium bromide (Merck), ethanol (Merck), and acetone (Merck).

Curcumin-loaded NLC formulation

Curcumin-loaded NLC was created by evaporation and solvent diffusion in aqueous systems. The stages were as follows: 60 mg of a mixture of cholesterol and 15% oleic acid (AO) was dissolved in a mixture of acetone (3 mL) and ethanol (3 mL), then heated at 60^0C. Curcumin was added to the lipid phase (drug/lipid 10%), and the heating temperature was maintained at 60^0C. Meanwhile, the aqueous phase was prepared by mixing distilled water (60 mL) with tween 80 (concentration of 1%) and heating this mixture at a temperature of 60^0C. Immediately, the aqueous mixture was added to the lipid mixture, and then homogenized using the IKA Ultra-Turrax homogenizer at 800 rpm for 2 to 4 minutes. Then, the pre-emulsion mixture was screened using ultrasound for 20 minutes at an amplitude of 20%. Finally, the NLC disperse was cooled to room temperature (25^0C) and stored at 4^0C (Emami *et al.*, 2012).

Characterization of curcumin-loaded NLC

Curcumin-loaded NLC was characterized by particle size, zeta potential, and polydispersity index using Delsa™ Nano C (Beckman Coulter, Inc.). The structure and morphology of the final product were observed under the JEOL JEM 1400 transmission electron microscope at an accelerating voltage of 100 kV.

Identification of curcumin-loaded NLC

The curcumin compounds in curcumin-loaded NLC was identified by Fourier-Transform Infrared (FT-IR) Spectroscopy. Afterward, the FTIR spectrum of the liquid samples was analyzed by dripping the sample onto KBr powder, followed by drying and homogeneous grinding. The IR spectrum was checked at the wavenumbers of 400 to 4000 cm^{-1}.

Absorption efficiency analysis

The efficiency of curcumin-loaded NLC was determined by centrifugation at 12,000 rpm for 60 minutes. The supernatant was decanted and then measured using a UV-Vis spectrophotometer at a wavelength of 423 nm and by High-Performance Liquid Chromatography (HPLC). As for the drug loading, it was analyzed from the curcumin content in the NLC using a spectrophotometer at a wavelength of 423 nm and by High-Performance Liquid Chromatography (HPLC), with acetonitrile and 2% acetic acid as the mobile phase. The absorption efficiency (% EE) and drug loading (% DL) were calculated using the following equations:

$$\%EE = \left(\frac{\frac{A-B}{A}}{}\right) \times 100\% \quad \text{.....(1)}$$

$$\%DL = \left(\frac{\frac{C}{A+D}}{}\right) \times 100\% \quad \text{.....(2)}$$

A = The amount of curcumin added
B = The amount of curcumin in the supernatant
C = The amount of curcumin added (DL)
D = The number of excipients added

RESULTS AND DISCUSSION

The curcumin-loaded NLC was formulated by evaporation and solvent diffusion with several variations in curcumin's concentration (active substance), i.e., 5% or 3 mg curcumin (Formula 1), 10% or 6 mg (Formula 2), and 15 % or 9 mg (Formula 3).

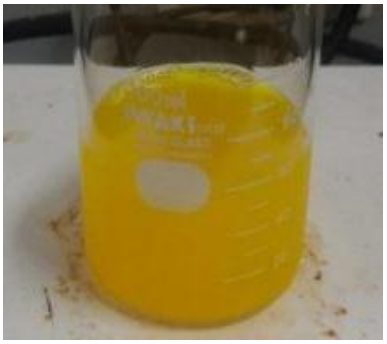

Figure1. The curcumin-loaded NLC formulated with 10% curcumin

Cholesterol was used as a solid lipid because it can increase the drug-carrying system to specifically target cancer cells (Emami *et al.*, 2012), while oleic acid was chosen as a liquid lipid because it can produce NLC with a higher entrapment efficiency than any drugs that do not contain oleic acid. Tween 80 was selected as an emulsifier because, if enriched with this surfactant, the resultant NLC has a high entrapment efficiency (Attama and Müller-Goymann 2008). The combination of homogenization and ultrasonication techniques has several advantages, including nanoparticle preparations with smaller particle size and the use of simple and effective equipment for a scale of a laboratory (Asawale *et al.*, 2014). Formula 2 (10%) produced curcumin-loaded NLC with clear yellow color, no sediment, no foaming, and favorable homogeneity (Figure 1). Meanwhile, Formula 1 and 2, each using 5% and 15% curcumin, yielded yellow liquid containing sediment. The presence of this deposit in the final product shows that the small particles are not well dispersed; in other terms, the curcumin-loaded NLC prepared using Formula 1 (5%) and Formula 3 (15%) cannot be used for further testing.

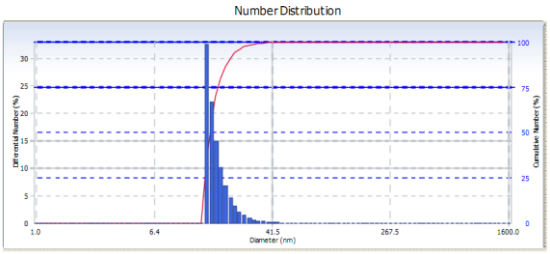

Figure 2. The PSA analysis results of the produced curcumin-loaded NLC

Particle size is an essential characteristic of nanoparticles because it determines the stability of the nanoparticle system. Smaller particle size increases the surface area and, consequently, leads to high solubility, making the particles easier to be absorbed in the body (Awad *et al.*, 2008). The diameter size of the produced curcumin-loaded NLC was 17.4 nm. It proves that the oleic acid content in NLC initiates nano lipid formation with smaller particle sizes. Similar results are reported in other studies (Hu *et al.* 2008; Agrawal *et al.*, 2010; Tiwari and Pathak, 2011).

The polydispersity index represents the distribution of nanoparticle in a dosage form. In this study, the polydispersity index started from 0.01 to 0.5-0.7 for monodispersed particles. Based on the results of the particle size analyzer (Figure 2), the polydispersity index of the curcumin-loaded NLC was 0.574. Monodisperse nanoparticles can improve the stability of the nanoparticle system because they show the size, shape, and weight of homogeneous particles. A higher polydispersity index means that more particles are aggregated, or in other words, the preparation is increasingly unstable.

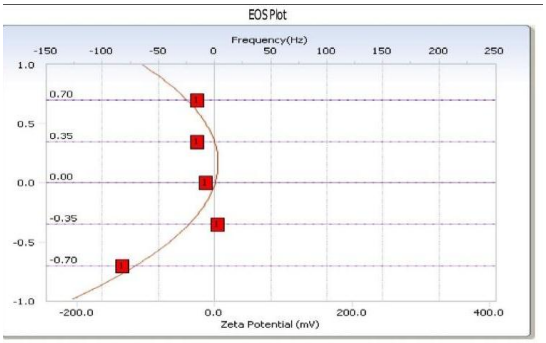

Figure 3. The zeta potential analysis of the curcumin-loaded NLC

The results showed that the zeta potential of the prepared curcumin-loaded NLC was -63.43 (mV). Zeta potential represents the aggregation of the preparation, i.e., a high zeta potential, both negative and positive, indicates a colloidal system that tends to be stable and can prevent particles from aggregating. In general, particles with a zeta potential higher (more positive) than +30 mV or lower (more negative) than -30 mV are considered stable (Rosli *et al.*, 2015).

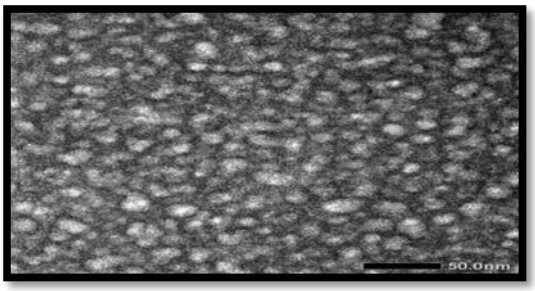

Figure 4. The morphological analysis results of curcumin-loaded NLC, as observed under a transmission electron microscope

The morphology of the NLC particle was examined under a transmission electron microscope. Based on the observation results, the curcumin-loaded NLC particles were round with a smooth surface and no appearance of particle aggregation (Figure 4). This finding is consistent with Almoussalam (2015), which suggests that nanoparticles with lipids as carriers have a round structure with a smooth surface, regardless of the type of the lipid used. The size of the curcumin nanoparticles obtained was 68.18 nm, which is similar to the size of curcuminoid nanoparticles prepared in Mujib (2011), that is, 70.21 nm. These results prove that the oleic acid content in NLC forms nano lipids with smaller particle sizes.

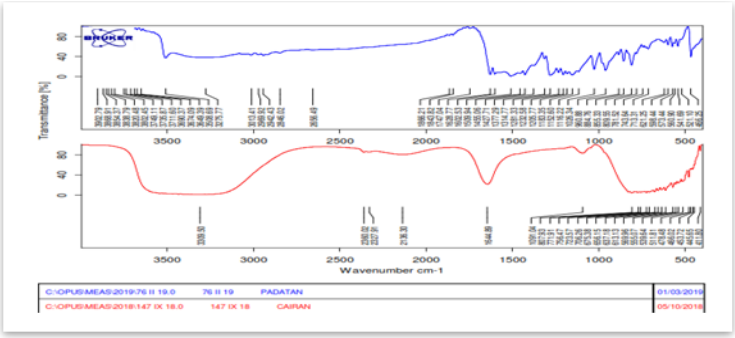

Figure 5. The identification results of compounds in curcumin-loaded NLC by FTIR (the blue curve is the NLC spectrum, and the red one is curcumin)

The infrared spectra of pure curcumin and curcumin-loaded NLC were compared (Figure 5). Based on the observation results, the standard infrared spectrum for curcumin was as follows: 1628 cm^{-1} (for C = C aromatic), 1427 cm^{-1} (for CC aromatic), 1602 cm^{-1} (for conjugated diene), 1509 cm^{-1} (for C = C ketones), 3200-3508 cm^{-1} (for OH bonded to the aromatic group), 809 cm^{-1} and 855 cm^{-1} (for substituted aromatic ring). Meanwhile, the infrared spectrum of the NLC sample did not have the main peaks of curcumin (1628, 1427, 1602, 1509, 3200-3508, 809-855 cm^{-1}), but those of oleic acid were apparent, as marked by the wavenumbers of 1644 cm^{-1} (for C = C alkene) and 3309 cm^{-1} (OH-carboxylic). The different spectra between the standard curcumin and curcumin-loaded NLC samples prove that curcumin has been encapsulated by NLC.

Table I. The entrapment efficiency and drug loading of curcumin-loaded NLC, as measured using a UV-VIS Spectrophotometer

% Entrapment Efficiency	% Drug Loading
93.212 %	0.708 %

Entrapment efficiency determines how much percentage of the active substance is absorbed in the NLC compared to the total curcumin added. The absorbance values showed that the concentration of curcumin was absorbed, while the absorption efficiency of the curcumin-loaded NLC was identified using a linear equation of the standard curcumin curve. The absorption efficiency was 93.212% (Table I). Such a high absorption efficiency is likely to increase drug release (Sonaje *et al.*, 2007). The drug loading was measured by dividing the amount of curcumin absorbed in the nanoparticle system by the total amount of solution. This calculation yielded a drug loading of 0.708%. A nanoparticulate system ideally has a high loading capacity that can reduce the quantity of material used for drug delivery.

Table II. The absorption efficiency and drug loading of curcumin-loaded NLC-, as measured by High-Performance Liquid Chromatography (HPLC)

% Entrapment Efficiency	% Drug Loading
93.007%	0.795%

A drug delivery system must have a high loading capacity. Entrapment efficiency is generally expressed in percent of drug absorption. In this study, the entrapment efficiency was determined by centrifuging curcumin-loaded NLC samples and measuring the supernatant by HPLC. The results showed that the entrapment efficiency of curcumin-loaded NLC was 93.07%. This figure is close to 100%, indicating that curcumin was entirely encapsulated by NLC. Severino *et al.* (2015) formulate NLC with 1% surfactant and produce 90% entrapment efficiency. The amount of curcumin absorbed in the nanoparticle system was divided by the total amount of solution to quantify loading capacity. This calculation result showed that the drug loading of the produced curcumin-loaded NLC was 0.795%. An ideal nanoparticle system has a high loading capacity, and in this condition, the quantity of materials used for drug delivery can be reduced.

CONCLUSION

Curcumin-loaded NLC that is prepared with 10% curcumin has stable characteristics, a particle size of 17.4 nm, a polydispersity index of 0.574, and zeta potential of -63.43 mV. Morphologically, it is round in shape with a smooth surface. Based on the spectrophotometry results, this preparation has an entrapment efficiency of 93.212% and a drug loading capacity of 0.708%. Meanwhile, the HPLC shows that the entrapment efficiency is 93.007 %, and the drug loading capacity is 0.795%.

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