Research Article

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Assessing encapsulation of curcumin in cocoliposome: *In vitro* study

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Abstract: Curcumin has been known and used in the medical and industrial world. One way to improve its stability, bioavailability and its medical applications is using encapsulation method. In this research, we studied cocoliposome (coconut liposome) as the encapsulation material. The encapsulation efficiency (EE), loading capacity (LC), release rate (RR), as well as the free radical scavenging activity, measured by inhibition ratio (IR), of curcumin in encapsulation product were studied on varying cholesterol compositions and in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4) conditions. We found that curcumin encapsulation in cocoliposome (CCL) formulation was influenced by cholesterol composition and pH conditions. The EE, LC and free radical scavenging activity diminished under both the SIF and SGF conditions when the cholesterol concentration enhanced. However, the RR increased as the cholesterol intensified. The condition to acquire the most favorable encapsulation parameter values was at 10% cholesterol composition. Furthermore, the IR results at 10% cholesterol concentration of CCL was 67.70 and 82.13% in SGF and SIF milieu, respectively. The CCL formulation thrived better under SIF conditions for free radical scavenging activities.

Keywords: coconut, *Curcuma longa*, drug delivery, liposome, phospholipid

1 Introduction

Curcumin has been known and used in the medical and industrial world. Curcumin or diferuloat methane is a natural polyphenol phytochemical compound obtained from *Curcuma longa* (turmeric) extract [1]. Curcumin has been widely used as a drug due to its bioactivity as an antioxidant, anti-inflammatory, anti-microbial and anti-tumor agent with low toxicity [2,3]. Curcumin is also used as chemopreventive agents in various types of cancer, such as leukemia and lymphoma, gastrointestinal cancer, genitaurinary cancer, nerve cancer and sarcoma, breast cancer and lung cancer [4]. Despite these broad applications, the therapeutic functions are hindered by low bioavailability [5]. The low bioavailability of curcumin is instigated by low level of solubility in aqueous media, low stability at basic pH, and easy elimination from the body [6]. Proper and careful design of a delivery system can significantly increase bioavailability.

Various delivery methods of active ingredients have been developed, and one of them is liposomes. Liposomes are chosen as a delivery system due to their sustainable, biodegradable, nontoxic and nonimmunogenic nature [7]. Liposomes are widely used as a delivery system to stabilize drugs and overcome problems in bioavailability [8]. Liposomes are microscopic bilayer vesicles made from phospholipids dispersed in aqueous media. Exploration on liposomes from various natural resources has been conducted to acquire the desired results [9–14]. At present, an ongoing exploration of natural liposomes is the cocoliposome, which is made from CocoPLs [15–17].

Research on cocoliposome includes its ability to encapsulate a variety of active materials involving vitamin C, beta-carotene, cinnamic acid and galangal extract [18,19]. Although further research is needed, cocoliposome has indeed shown prospects as delivery systems for both polar and nonpolar substances. To know better about the nature of cocoliposome as delivering tool for various active matters, in this study, we explored the cocoliposome for encapsulating curcumin. Encapsulation of curcumin in cocoliposome (CCL) formulation was carried out with the desire of

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increasing its bioavailability, biocompatibility and stability. We scrutinized the CCL parameters in simulated intestinal Fluid (SIF) and simulated gastric fluid (SGF) environments separately.

Furthermore, cholesterol is an important component in liposomal formulations. Cholesterol affects liposome stability, drug encapsulation efficiency and control liposome release. It plays an important role in controlling physical and chemical properties of liposome, such as membrane flexibility and rigidity [20,21]. In relation to the effectiveness of liposomes as a delivery system for curcumin, the composition of the liposomal membrane is very important. It is known that the presence of cholesterol in the liposome membrane is very essential [20,22,23]. It is related to the physical properties of the membrane, its permeability and the ability of the liposome as a carrier system of curcumin. To comprehend how cholesterol influences the curcumin encapsulation in cocoliposome, we added cholesterol in various compositions to the formulations. We also explored the scavenging activity of CCL preparations. To our knowledge, this is the first report presenting the *in vitro* study of cocoliposome encapsulation ability for curcumin. The present discovery provides evidence on the encapsulation capability of cocoliposome especially for polyphenolic compounds such as curcumin.

The relationship between the liposome composition, the environment where the liposome will be used, and the encapsulation parameters can be delicate and unforeseen. It can also influence the intended effect of the encapsulated material. The aim of this research was to further characterize the encapsulation of curcumin in cocoliposome in both SIF and SGF environments by exercising important parameters in encapsulation process and curcumin antioxidant property.

2 Methods

2.1 Preparation of coconut phospholipid (CocoPLs)

Coconut phospholipid (CocoPLs) was isolated in-house using Hudiyanti's procedure (1,2). Concisely, CocoPLs extraction procedure is divided into extraction by soaking dry coconut powder in chloroform/methanol (2/1) mixture followed by solvent partition of the liquid extract in hexane and ethanol 87% solvent. Finally, the ethanol layer was evaporated to obtain CocoPLs. CocoPLs obtained was used to prepared liposomes.

2.2 Simulated intestinal fluid (SIF) solution preparation

Two homogeneous solutions of 7.5 g of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (0.05 M) and 3.9 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.05 M) in 500 mL of demineralized water were prepared, respectively. About 9.5 mL of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.05 M) and 40.5 mL of $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ (0.05 M) solutions were mixed. The solution was diluted to 100 mL and adjusted to pH 7.4.

2.3 Preparation of simulated gastric fluid (SGF) solution

Two grams of NaCl was dissolved in 800 mL of demineralized water. A solution of 4.5 mL of 37% HCl was added to the NaCl solution drop by drop, followed by demineralized water to reach 1 L in volume. The pH was adjusted to 1.2.

2.4 Preparation of curcumin standard curve

Two milligrams of curcumin was dissolved in 100 mL ethanol to obtain 50 ppm curcumin stock solution. An array of curcumin solution with concentrations 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 ppm was made from the stock solution. The λ_{max} (426 nm) of the curcumin was determined using a UV-Vis spectrophotometer, and the standard curve was established from the array of curcumin solutions.

2.5 Preparation of curcumin loaded coconut liposomes (CCL)

CCL was prepared in five formulations composed of CocoPLs, cholesterol (Chol) and curcumin (Cur). The formulation is presented in Table 1. Each formula was dissolved in 100 mL of chloroform/methanol (9:1, v/v). A 10 mL solution of LCO was placed in a reaction tube and nitrogen gas was streamed into the tube until all solvent evaporated leaving a thin film at the bottom of the tube. Then, 10 mL SIF solution was poured into the tube. The tube was then exposed to freeze-thawing cycles at 4°C cooling and 45°C heating until the thin film was dispersed. The procedure of freeze-thawing cycles was adapted from the study by Hudiyanti et al. [15,18,19].

Table 1: Formulation of curcumin-loaded coconut liposomes (CCL) dispersions

CCL formulation	Composition (w/w/w) in mg		
	CocoPLs	Chol	Cur
LC0	125	0	1
LC10	125	12.5	1
LC20	125	25	1
LC30	125	37.5	1
LC40	125	50	1

The dispersion was then sonicated at 27°C for 30 min. This process was repeated for every formula in Table 1 with SIF and SGF solutions. There were 10 diverse dispersions under 2 simulated conditions, alkaline for SIF (pH 7.4) and acidic for SGF (pH 1.2) solution, respectively.

2.6 Functional group analysis by FTIR spectrometry

CocoPLs, cholesterol, curcumin and CCL were analyzed to determine changes and interactions that might occur during encapsulation through changes in the functional groups using Fourier Transform Infrared Spectrophotometer (FTIR, Perkin Elmer Model Frontier FT-IR, USA), range of 5,500–435 cm^{-1} , resolution 4.0 cm^{-1} , number of scans 3.

2.7 Encapsulation efficiency (EE) and loading capacity (LC)

The ability of cocoliposome to load curcumin was denoted by encapsulation efficiency (EE) and loading capacity (LC) of cocoliposome. Each CCL dispersion previously prepared was dissolved in absolute ethanol with ratio 1:5 (v/v). Afterward, the solution was centrifuged at $3,461 \times g$ for 40 min followed by measuring curcumin concentration in the supernatant by UV–Vis spectrophotometer at 426 nm. This technique was carried out for every dispersion formulated in Tables 1. The encapsulation efficiency (EE) of cocoliposome for curcumin was calculated using equation 1 in which the encapsulated curcumin (C_{en}) was divided by the initial amount of curcumin (C_{in}). The LC of cocoliposome for curcumin was calculated using equation 2 in which the encapsulated curcumin (C_{en}) was divided by the CocoPLs amount in the preparation (C_{CocoPLs}).

$$\text{EE} = \frac{C_{\text{en}}}{C_{\text{in}}} \times 100\% \quad (1)$$

$$\text{LC} = \frac{C_{\text{en}}}{C_{\text{CocoPLs}}} \times 100\% \quad (2)$$

2.8 Release rate of curcumin

Average release rate ($\overline{\text{RR}}$) of curcumin from the formulation was done by monitoring curcumin released from liposome into the liquid medium, i.e., SIF and SGF solutions until all curcumin was released. The analysis was performed using a UV–Vis spectrophotometer to determine the concentration of released curcumin. Release rate was calculated as the encapsulated curcumin (C_{en}) divided by the duration of total release (T_{tot}), equation 3.

$$\overline{\text{RR}} = \frac{C_{\text{en}} \text{ ppm}}{T_{\text{tot}} \text{ day}} \quad (3)$$

2.9 DPPH free radical scavenging assay

1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was conducted following the method by Blois [24]. Briefly, 1 mL of each CCL dispersion was mixed with 3 mL of DPPH solution (40 $\mu\text{g}/\text{mL}$). The mixtures were incubated at room temperature without exposure to light for 30 min. Later, the mixture's absorbance was analyzed using UV–Vis spectrophotometry at λ_{max} (516 nm). The radical scavenging activity was calculated as DPPH scavenging activity (IR) in the following equation:

$$\text{IR} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100\% \quad (4)$$

where IR is the DPPH scavenging activity; A_0 is the absorbance of DPPH solution without sample addition; A_1 is the absorption of DPPH solution with sample after 30 min incubation.

2.10 Statistical analysis

Data were collected in duplicate. Statistical analysis was executed with ANOVA. Data were expressed as means $\pm$ standard deviation (SD). Results with P value < 0.1 were considered statistically significant.

Ethical approval: The conducted research is not related to human or animal use.

3 Results and discussion

3.1 Functional group analysis by FTIR spectrometry

Functional group analysis using Fourier Transform Infrared (FTIR) spectroscopy was performed to verify the encapsulation of curcumin in the cocoliposome. FTIR can disclose the chemical alterations by generating an infrared absorption spectrum. Figures 1–4 present the FTIR spectra of curcumin, cholesterol, CocoPLs and the CCL. The characteristic peaks for phospholipids were found at $\sim 1,228$ and $\sim 1,085$ cm^{-1} for PO_2^- stretching; $\sim 1,047$ cm^{-1} indicating C–O–P stretching; ~ 819 cm^{-1} denoting P–O stretching; $\sim 1,738$ cm^{-1} denoting C=O stretching in esters; $\sim 2,925$ and $\sim 2,854$ cm^{-1} indicating CH_2 stretching of acyl chains; $\sim 2,971$ and $\sim 2,855$ cm^{-1} denoting CH_3 stretching of acyl chains; $\sim 1,457$ and $\sim 1,366$ cm^{-1} signifying CH_3 bending and $\sim 1,598$ cm^{-1} denoting =C–H stretch [17,22,25]. The major bands for cholesterol molecule were found at several positions as follows: the peaks between 2,800 and 3,000 cm^{-1} signifying stretching vibrations of CH_2 and CH_3 groups. The wide and intense band at $\sim 3,400$ cm^{-1} was assigned to OH stretching. The distinctive strong band at 2,933 cm^{-1} was assigned to CH_2 stretching vibration. The double bond (C=C) in the second ring was shown at 1,667 cm^{-1} . The band at 1,465 cm^{-1} was assigned to asymmetric stretching vibrations of CH_2 as well as CH_3 groups. The peak at 1,377 cm^{-1} was assigned to the CH_2 and CH_3 bending vibration. The peak at 1,054 cm^{-1} was assigned to ring deformation of cholesterol. The peak at 839 cm^{-1} was assigned to the C–C–C stretching. The peaks between 900 and 675 cm^{-1} were assigned to the C–H out-of-plane bending, which were the distinctive of the aromatic replacement configuration [23]. The spectrum of curcumin displayed a sharp absorption peak at 3,510 cm^{-1} , signifying the existence of the phenolic –OH stretching vibration. The

Figure 1: Fourier transform infrared spectra of coconut phospholipids (CocoPLs).

Figure 2: Fourier transform infrared spectra of cholesterol (Chol).

strong peak at 1,628 cm^{-1} signified primarily fused C=O and C=C groups, and 1,599 cm^{-1} was attributed to the symmetric aromatic stretching vibration. The sharp peak at 1,508 cm^{-1} was attributed to C=C vibrations. The sharp peak at 1,455 cm^{-1} was from the phenolic C–O, while the enolic C–O peak was appeared at 1,278 cm^{-1} . The peak at 1,025 cm^{-1} was attributed to the asymmetric stretching of C–O–C. The peak at 721 cm^{-1} was the C–H vibration of aromatic ring [26,27]. The FT-IR spectra of the curcumin, cholesterol and CocoPLs was found to have merged in CCL. All the sharp peaks of curcumin, cholesterol and CocoPLs were observed. However, no observable new characteristic peaks were found in the FTIR spectra of CCL. Therefore, it could be predicted that no chemical reaction occurs in the process of curcumin encapsulation in the cocoliposome. The interactions that occur were thought to be non-covalent interactions.

3.2 Encapsulation efficiency

The EE of CCL in both SIF and SGF solutions were presented in Figures 5 and 6.

Figure 3: Fourier transform infrared spectra of curcumin (Cur).

Figure 4: Fourier transform infrared spectra of CCL.

In SIF environments, Figure 5, the addition of cholesterol in the cocoliposome composition decreased the efficiency of encapsulation. These data indicated that the composition of cholesterol in the cocoliposome membrane exerted great influence on the encapsulation efficiency. Data showed that the efficiency of encapsulation remains high, 90.47%, when the composition of cholesterol is 10%. The same results were obtained in the SGF milieu, Figure 6, the efficiency of encapsulation decreased with increasing cholesterol concentration in the cocoliposome membrane. The data revealed that in the SGF environment, cholesterol also had a significant effect on the efficiency of the curcumin encapsulation system. The best cholesterol composition to get high EE value in SGF solution was 10% with the EE value around 90.63%. These data specified that cholesterol modified the efficiency of cocoliposome encapsulation against curcumin. The effect of cholesterol was quite small at low concentrations, 10%. Statistical analysis of the data disclosed that EE was significantly affected by cholesterol concentration. However, the encapsulation efficiency in SIF condition was not significantly different from SGF.

Figure 5: Encapsulation efficiency of Cur in cocoliposome in SIF solution as a function of cholesterol.

Figure 6: Encapsulation efficiency of Cur in cocoliposome in SGF solution as a function of cholesterol.

3.3 Loading capacity

The LC calculates the amount of curcumin successfully encapsulated in cocoliposome per unit weight (gram) of CocoPLs in the liposome. It provides an overview of how the preparations can be used practically. The LC of CCL in both SIF and SGF solutions is presented in Figures 7 and 8. Figure 7 presents the LC of curcumin in SIF environment. The LC of curcumin in the cocoliposome preparations without addition of cholesterol was 7.47 mg/g. The data disclosed a decrease in LC value when cholesterol concentration is increased. When the cholesterol concentration is 10%, the LC was still close to the initial value of 7.24 mg/g. Figure 8 described the LC in SGF solution. Similar changes occurred when cholesterol concentration was increased and the LC was decreased. The LC of curcumin in the cocoliposome preparations without the addition of cholesterol was 8.17 mg/g. The LC was still close to the initial value of 7.79 mg/g when the cholesterol concentration is 10%. The information from both the SIF and SGF conditions indicated that cholesterol altered the LC value of curcumin in the cocoliposome

Figure 7: Loading capacity of Cur in cocoliposome in SIF solution as a function of cholesterol.

Figure 8: Loading capacity of Cur in cocoliposome in SGF solution as a function of cholesterol.

preparations. However, the data suggested that the cholesterol concentration of 10% still gave a fairly high LC. The value of LC was affected significantly by cholesterol concentration and environmental conditions (SIF and SGF).

3.4 Release rate (RR)

RR of active agent carriers such as liposomes or other forms of carrier is an important feature related to the therapeutic activity of active agents [28]. Therefore, we studied the release rate of curcumin from our prepared CCL. The RR of CCL in both SIF and SGF solutions were presented in Figures 9 and 10. Data presented that in SIF condition, Figure 9, generally the addition of cholesterol increased the rate of release of curcumin. The RR of CCL without cholesterol (non-cholesterol CCL) in the liposome membrane was 0.78 mg/L day. The lowest RR, 0.70 mg/L day, was obtained when the cholesterol concentration was 10%. For higher concentration of cholesterol, the RR increased above the RR of non-cholesterol CCL. The RR in SGF condition, Figure 10, typically ascended with an increase in cholesterol concentration in the cocoliposome

Figure 9: Release rate of Cur from cocoliposome in SIF solution as a function of cholesterol.

Figure 10: Release rate of Cur from cocoliposome in SGF solution as a function of cholesterol.

membrane. The most promising composition in SGF condition to attain a low release rate was at 10% of cholesterol with the release rate of 0.75 mg/L day. The data revealed that either in SIF or SGF solution, 10% cholesterol concentration would give the lowest RR. The RR was influenced significantly by cholesterol concentration and environmental condition. This discovery was in accordance to the EE and LC data.

3.5 DPPH scavenging activity assay

The antioxidant activity of encapsulated curcumin was analyzed by DPPH scavenging activity assay. Antioxidant activity will be high if the DPPH scavenging activity is high. DPPH scavenging activity of CCL in both SIF and SGF solutions were presented in Figures 11 and 12. Prior to the analysis, we measured DPPH scavenging activity of CocoPLs. We found that the DPPH scavenging activity was low and insignificant compared with curcumin. Therefore, we deliberated that the DPPH scavenging activity of CCL preparations came from curcumin. DPPH scavenging

Figure 11: DPPH scavenging activity of CCL in SIF solution as a function of cholesterol.

Figure 12: DPPH scavenging activity of CCL in SGF solution as a function of cholesterol.

activity of the preparations in SIF milieu, Figure 11, revealed that increasing cholesterol concentration would reduce the DPPH scavenging activity. In SGF setting, an increase in cholesterol composition also decreased the DPPH scavenging activity, Figure 12. Both graphs exposed that the trend of DPPH scavenging activity was still decreasing when the cholesterol concentration reached 40%. Besides the DPPH scavenging activity in SIF milieu were higher than that in SGF. At cholesterol concentration of 10%, the scavenging activity in SIF and SGF was 80.99 and 67.70%, respectively. The scavenging activity in SGF was significantly affected by the cholesterol concentration. Moreover, the scavenging activity was induced significantly by environmental conditions (SIF and SGF).

3.6 Curcumin encapsulation in cocoliposome

3.6.1 Cholesterol effect

As predicted in the FTIR analysis results, it is estimated that the interactions that occur in the curcumin encapsulation in the cocoliposome are non-covalent interactions. The molecular structure of curcumin shows that the curcumin molecule, has two hydrogen bond donor and six hydrogen bond acceptor, with a topological polar surface area of $93.1 \text{ \AA}^2$ [29]. Therefore, the possible interaction is a hydrogen bond between the curcumin and phospholipid molecules. Curcumin has a large polar surface area, but the polar area is geometrically spread across the length of the molecule so that the position of the curcumin tends to be laterally located inside the liposome membrane close to the polar area of the membrane. The large polar surface area and the number of sites for hydrogen bond formation explain the ease of curcumin to be encapsulated in the

liposome membrane. This is manifested in the high EE and LC values for liposome formulations without cholesterol.

Meanwhile, cholesterol has an amphiphilic structure with one hydrogen bond donor and acceptor group. As a result, the presence of cholesterol in liposome formulations will interfere with non-covalent interactions between curcumin and phospholipids. There is competition for the formation of hydrogen bonds with phospholipids between curcumin and cholesterol. The relatively small size of polar portion of the cholesterol and its position at the tip of the molecule boost the chance of cholesterol to form hydrogen bond with phospholipids. The cholesterol affinity on hydrogen bonding formation with phospholipids is higher than curcumin. It is evident from the decrease in EE and LC of curcumin when there is cholesterol in the formulations. The greater the cholesterol concentration, the greater the decrease is. The same rationalizes also justified how cholesterol causes RR to increase when there is cholesterol in the liposomal formulations. In the case of RR, it is also necessary to pay attention to the packing of molecules in the liposome membrane. Similar RRs for formulations with 0 and 20% cholesterol concentrations are thought to be associated with the molecular packing of phospholipid, cholesterol and curcumin molecules in the liposome membrane. The packing of molecules which then sterically affects the diffusion and movement of curcumin in the membrane will ultimately affects the release of curcumin from the membrane as well. Cholesterol at a concentration of 20% is believed to provide a molecular packing and steric effect similar to that of liposome membranes without cholesterol. The data indicated that the liposome formulation to obtain the most optimum EE, LC and RR was the formulation with a cholesterol concentration of 10%.

DPPH scavenging activity of curcumin in liposome preparations is related to the amount of encapsulated curcumin in CCL. It is then represented by the EE and LC values. Data show that DPPH scavenging activity of CCL decreases with increasing cholesterol concentration. Thus, the reduction of DPPH scavenging activity of the CCL preparations is in accordance with the EE and LC data.

3.6.2 Effect pH (SIF and SGF) conditions

Encapsulation of curcumin in cocoliposome was thought to involve non-covalent interactions, such as hydrogen bond interactions. The hydrogen bond interactions

depend on pH values. Changes of pH affected protonation of phosphate group in phospholipids. Meanwhile in acidic and neutral conditions, curcumin molecules appeared in protonated form where curcumin played as a potent hydrogen donor [30]. However, in basic situations where the enolate form of the heptadienone chains prevailed, curcumin operated instead as an electron donor. These properties were thought to cause discrepancies in the value of the encapsulation parameters and scavenging activity under acidic (SGF, pH = 1.2) and neutral (SIF, pH = 7.4). The scavenging activity at 10% cholesterol composition was 82.13 and 67.70% in SIF and SGF condition, respectively. In view of the scavenging activity, SIF condition was more suitable for CCL formulation. This result is in line with the poor solubility of curcumin under acidic conditions [31].

4 Conclusion

Encapsulation of curcumin in cocoliposome was affected by cholesterol and pH conditions. Under both the SIF and SGF conditions, when the cholesterol concentration increased then the encapsulation efficiency, loading capacity and DPPH scavenging activity decreased, but the release rate increased. The formulation to obtain the optimum encapsulation parameters value was at a cholesterol concentration of 10%. The DPPH free radical scavenging activity at 10% cholesterol composition of CCL was 82.13 and 67.70% in SIF and SGF surroundings, respectively. The CCL formulation thrived better under SIF conditions for free radical scavenging activities.

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