Research Article

Effect of Lipid Composition on *In Vitro* Release and Skin Deposition of Curcumin Encapsulated Liposomes

Geethi Pamunuwa,^{1,2} Veranja Karunaratne,^{1,3} and D. Nedra Karunaratne^{1,2}

¹Department of Chemistry, Faculty of Science, University of Peradeniya, 20400 Peradeniya, Sri Lanka

²Postgraduate Institute of Science, University of Peradeniya, P.O. Box 25, 20400 Peradeniya, Sri Lanka

³Sri Lanka Institute of Nanotechnology, Nanotechnology & Science Park, Mahenwatte, Pitipana, 10200 Homagama, Sri Lanka

Correspondence should be addressed to Geethi Pamunuwa; kmgkpamunuwa@gmail.com

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Liposomal encapsulation improves numerous physiochemical and biological properties of curcumin. The aim of this work was to impart slow release and skin delivery of curcumin via liposomal encapsulation. Liposomes were made using egg yolk phosphatidylcholine as the staple lipid while incorporating polysorbate 80 and stearylamine to prepare hybrid liposomes and positively charged liposomes, respectively. Negatively charged liposomes exhibited the highest encapsulation efficiencies ($87.8 \pm 4.3\%$) and loading capacities ($3.4 \pm 0.2\%$). The sizes of all formulations were about 250 nm, while stearylamine increased the polydispersity index. Positively charged liposomes showed lower degradation temperatures than negatively charged liposomes by $10\text{--}15^\circ\text{C}$, attributable to the presence of stearylamine. The melting temperatures of positively charged liposomes ($40\text{--}50^\circ\text{C}$) were much higher than those of negatively charged liposomes ($14\text{--}15^\circ\text{C}$), which may have affected release and skin deposition behavior of liposomes. The positively charged liposomes exhibited the slowest release of curcumin in phosphate buffered saline (pH 6.8) and the release profiles of all liposomal formulations conformed to the Gompertz model. The negatively charged liposomes facilitated the highest skin deposition of curcumin as revealed by studies conducted using excised pig ear skin. Concisely, positively and negatively charged liposomes were optimal for slow release and skin deposition of curcumin, respectively.

1. Introduction

Curcumin is a natural compound that exhibits numerous important bioactivities such as antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic activities [1–4]. However, the poor bioavailability and low stability of this bioactive compound hinder its use as a pharmaceutical ingredient. Among the numerous strategies adopted to overcome these limitations, liposomal encapsulation has been studied extensively [5, 6]. Interestingly, liposomal systems can be used to incorporate important properties such as slow release and skin delivery of bioactive agents in addition to improving bioavailability and stability [7–10].

Liposomal encapsulation has improved numerous important properties of curcumin with potential applications in many different fields. For instance, curcumin encapsulated liposomes have been utilized in developing antimicrobial

surfaces for the food industry [11]. Curcumin nanoliposomes coated with chitosan were considered suitable for applications in the food industry by Shin and coworkers, based on their increased bioavailability, mucoadhesive properties, and storage stability [12]. Research in pharmaceuticals has revealed that liposomal curcumin exhibits higher therapeutic efficacy than free curcumin. For example, increased anticancer, antitumor, and anti-inflammatory properties of liposomal curcumin have been reported [13–15]. Berginc et al. (2012) reported the use of curcumin encapsulated liposomes as permeation enhancers for delivery of curcumin for delivery through artificial and isolated bovine mucus. They found that mucoadhesion increased when liposomes were coated with bioadhesive polymers [16]. Also, it has been demonstrated that the lipid composition of liposomes affects the bioactivity of curcumin encapsulated liposomes [17].

TABLE 1: The amounts of chemicals used in the preparation of curcumin encapsulated liposomes.

Liposomal formulation	PC (mg)	CH (mg)	P80 (mg)	SA (mg)	Curc. (mg)
NL (negatively charged liposomes)	200	50	—	—	10
NHL (negatively charged hybrid liposomes)	200	25	25	—	10
PL (positively charged liposomes)	200	25	—	25	10
PHL (positively charged hybrid liposomes)	200	25	25	25	10

PC: egg yolk phosphatidylcholine; CH: cholesterol; P80: polysorbate 80; SA: stearylamine, Curc.: curcumin.

The lipid composition of liposomes is well documented as one of the main factors that determines the properties of liposomes. Examples of properties of liposomes affected by the lipid composition include size, zeta-potential, stability, encapsulation efficiency, release properties, and skin permeation properties [18–21]. Thus, the lipid composition can be varied to modulate such properties of liposomes to make them well suited for their intended application. Accordingly, this work focused on investigating the effect of lipid composition of liposomes on properties of curcumin encapsulated liposomes.

The present study describes the effect of charge (stearylamine) and surfactants (polysorbate 80) on the properties of curcumin encapsulated liposomes prepared using egg yolk phosphatidylcholine (PC) and cholesterol (CH). The properties of liposomes considered were size, zeta-potential, encapsulation efficiency, loading capacity, *in vitro* release, and skin delivery of curcumin. Also, thermal analyses were carried out to investigate the degradation behavior and phase transition temperatures of curcumin encapsulated liposomes. The expected outcomes of this study were to develop liposomal formulations that facilitate slow release and improved skin delivery of curcumin and to acquire knowledge of the behavior of charged lipids and surfactants in liposomal formulations that will be useful in the future engineering of liposomal systems.

2. Materials and Methods

2.1. Materials. PC (~60%, TLC), CH (purity $\geq$ 99%), P80, SA (assay 90%), curcumin (assay $\geq$ 65%, HPLC), ethanol (HPLC grade), methanol (HPLC grade), phosphoric acid (85% wt.% in water), and dialysis membrane (MWCO 12 000) were purchased from Sigma-Aldrich. Chloroform (reagent grade) was obtained from Fisher Scientific. Deionized water filtered through a 0.2 μ m filter was used throughout the study. Fresh pig ears were obtained from a local slaughter house.

2.2. Methods

2.2.1. Preparation of Liposomes. Thin-film hydration method was used to prepare curcumin encapsulated liposomes. The method described by Aditya and coworkers was followed, with modifications [22]. Briefly, the lipids were dissolved in chloroform (50 mL) in a round bottom flask to which an ethanol solution (10 mL) of 10 mg of curcumin was added. Then, a thin film of lipid soluble components was prepared by evaporating the organic solvent under reduced pressure using a rotary evaporator (Heidolph, Laborota 4000). Any

residual solvent was removed by keeping the round bottom flask with the thin film of lipids in a vacuum oven overnight. Next, the thin film of lipids was hydrated using deionized water (10 mL). The resultant liposomal solution was allowed to stand at 4°C overnight to complete hydration. After that, all types of liposomal solutions were sonicated for 10 min at 4°C using a bath sonicator (Branson 2510). The amounts of the chemicals used are given in Table 1.

2.2.2. Determination of Encapsulation Efficiency (EE) and Loading Capacity (LC). The EE and LC of curcumin encapsulated liposomes were determined using a HPLC method. First, unencapsulated curcumin was removed from liposomes by dialyzing the liposomal solutions against deionized water at 4°C for 3 days. Next, the amount of encapsulated curcumin remaining in the dialysis bag was freeze-dried and, then, determined by HPLC analysis after disrupting the liposomes in ethanol. The formulas used to calculate EE and LC are given in the following:

$$\begin{aligned}
 \text{EE} &= \frac{\text{Mass of curcumin encapsulated in liposomes}}{\text{Mass of curcumin initially introduced}} \\
 &\times 100,
 \end{aligned}
 \tag{1}$$

$$\begin{aligned}
 \text{LC} &= \frac{\text{Mass of curcumin encapsulated in liposomes}}{\text{Total mass of curcumin encapsulated liposomes}} \\
 &\times 100.
 \end{aligned}$$

2.2.3. Determination of Particle Size and Zeta-Potential. Particle sizes and zeta-potentials of liposomes were determined using a Malvern Zetasizer Nano ZS (Malvern instruments, UK) fitted with a red laser of 633 nm, using dynamic light scattering technique and laser Doppler electrophoresis technique, respectively. Liposome suspensions were diluted in deionized water and equilibrated at 25°C prior to analysis [23].

2.2.4. Thermogravimetric Analysis (TGA). TGA was carried out using a thermogravimetric analyzer (TA Instruments SDTQ600). An alumina crucible was used to hold approximately 5 mg of sample. The sample was heated from room temperature to 800°C at a heating rate of 20°C/min under a high purity nitrogen flow of 100 mL/min [10].

2.2.5. Differential Scanning Calorimetry (DSC). DSC was performed on a Q200 DSC. A 4–6 mg portion of the sample was placed in crimped but vented aluminum pans and heated at a rate of 10°C/min in the temperature range of –40°C–+80°C. The sample was purged by a stream of dry nitrogen flowing at 50 mL/min [10].

2.2.6. In Vitro Release Studies. *In vitro* release studies were carried out at 25°C using the dialysis bag method using dialysis tubing of 12 000 MWCO. The release medium was phosphate buffered saline (PBS) of pH 6.8 containing 20% (v/v) ethanol and 0.5% (v/v) P80. Withdrawn aliquots from the release medium at predetermined time intervals were analyzed by HPLC.

Release profiles of different curcumin encapsulated liposomal formulations were fitted to six different drug release models: zero order, first-order, Higuchi, Hixon-Crowell, Korsmeyer-Peppas, and Gompertz. The model that exhibited the adjusted *R*-square closest to unity was selected as the best fit. The functions of the models considered are given in the following [24, 25].

(1) Zero order model is as follows:

$$Q_t = Q_o + K_o t, \quad (2)$$

where Q_t is amount of drug dissolved in time t , Q_o is initial amount of drug in solution, and K_o is zero order release constant.

(2) First-order model is as follows:

$$\log C_t = \log C_o - \frac{Kt}{2.303}, \quad (3)$$

where C_o is initial concentration of drug, C_t is concentration of drug at time t , and K is first-order rate constant.

(3) Higuchi model is as follows:

$$Q = K_H t^{1/2}, \quad (4)$$

where Q is amount of drug released in time t per unit area, K_H is Higuchi dissolution constant, and t is time.

(4) Hixson-Crowell model is as follows:

$$W_0^{1/3} - W_t^{1/3} = \kappa t, \quad (5)$$

where W_o is initial amount of drug in the pharmaceutical dosage form, W_t is remaining amount of drug at time t , and κ is constant incorporating surface-volume relation.

(5) Korsmeyer-Peppas model is as follows:

$$\frac{M_t}{M_\infty} = K t^n, \quad (6)$$

where M_t/M_∞ is fraction of drug released at time t , K is release rate constant, and n is release exponent.

(6) Gompertz model is as follows:

$$X(t) = X_{\max} e^{[-e^{-(\alpha(t-\beta))}]}, \quad (7)$$

where $X(t)$ is percent dissolved at time t , $X_{\max}$ is maximum dissolution, α is coefficient, and β is center.

2.2.7. Ex Vivo Skin Permeation and Skin Deposition of Curcumin. *Ex vivo* skin permeation studies were carried out at 25°C using a Franz diffusion cell using excised full thickness pig ear skin as the barrier membrane under nonocclusive conditions. The receiver compartment of the Franz cell was filled with buffer (PBS, pH 6.8) containing ethanol, 20% (v/v), and P80, 0.5% (v/v). Withdrawn aliquots from the receiver compartment at predetermined time intervals were analyzed by HPLC. After 8 h, pig ear skin used for permeation study was analyzed for skin deposition of curcumin after extracting deposited curcumin into ethanol. Residual curcumin encapsulated liposomes were removed by wiping the skin thoroughly with cotton prior to skin deposition analysis.

2.2.8. HPLC Determination of Curcumin. HPLC determination of curcumin was carried out using a C_{18} column using an Agilent LCMS fitted with a 1100/1200 diode array detector, 1100/1200 quaternary pump, and 1100 autosampler. A phosphate buffer of pH 2.2 and acetonitrile were used for gradient elution and the retention time was 11.5 min. Detection was carried out using a diode array detector at 425 nm.

2.2.9. Statistical Analysis. All data are presented as mean $\pm$ standard deviation (SD) of three parallel experiments ($n = 3$). Microsoft Office Excel 2007 was used for the above calculations. One-way ANOVA was conducted using MINITAB 14 software to compare the results and $P \leq 0.05$ was considered significant. OriginPro9 software was used for curve fitting in determining release kinetics.

3. Results and Discussion

3.1. Encapsulation Efficiency and Loading Capacity. The EE and LC values obtained for each type of liposomes are shown in Table 2. EE values were dependent on the lipid composition of curcumin encapsulated liposomes. Liposomes consisting of only PC and CH in the lipid component showed the highest EE which was $87.8 \pm 4.3\%$. When P80 was incorporated in the lipid bilayer, the EE decreased to $77.8 \pm 5.7\%$. The EE of SA incorporated PL was $57.5 \pm 1.3\%$ showing a pronounced decrease. Consistent with the negatively charged liposomes, incorporation of P80, along with SA, further decreased the EE to $54.5 \pm 2.2\%$. However, this decrease was relatively small, indicating that the effect of P80 on the EE diminishes in the presence of SA.

The LCs ranged from $1.9 \pm 0.1\%$ to $3.4 \pm 0.2\%$ and depended on the lipid composition. Like EEs, LCs decreased upon the incorporation of P80 or SA. Also, the effect of SA superseded that of P80 when both species were present together.

Although P80 and SA decrease the encapsulation of curcumin, it is unreasonable to generalize this effect over all surfactants and positively charged species. Surfactants and positively charged species incorporated in the lipid bilayers show great diversity in their structures so that their effects on the arrangement of lipids in the lipid bilayers also vary widely. Thus, it can be concluded that P80 and SA have a detrimental effect on the EE of curcumin in liposomes made

TABLE 2: Encapsulation efficiencies (EEs) and loading capacities (LCs) of different liposomal formulations. Values are reported as mean $\pm$ SD ($n = 3$). Statistically different values are followed by different superscripts and vice versa, in each column.

Liposomal formulation	EE (%)	LC (%)
NL	87.8 ± 4.3^a	3.4 ± 0.2^a
NHL	77.8 ± 5.7^b	3.0 ± 0.2^b
PL	57.5 ± 1.3^c	2.2 ± 0.1^c
PHL	54.5 ± 2.2^d	1.9 ± 0.1^d

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes; PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

of egg yolk PC and CH, and hence conventional liposomes (NL) are superior in terms of EE and LC in this context.

3.2. Particle Size and Zeta-Potential. The particle size, polydispersity index, and zeta-potential of each type of liposomes are shown in Table 3. The mean diameters span from 225 nm to 285 nm, exhibiting their suitability for, especially, pharmaceutical applications. The statistically different values indicate that the size of curcumin encapsulated liposomes depends on the lipid composition. The types of liposomes in the decreasing order of their size are NL, PHL, PL, and NHL. These results reveal that SA can be used to decrease the size of curcumin encapsulated liposomes. Moreover, P80 decreased the size of negatively charged liposomes while it resulted in an increase in the size of positively charged liposomes. Basically, our results indicate that P80 and SA can be used to modulate the size of curcumin encapsulated liposomes, especially of those intended for pharmaceutical applications if the encapsulation efficiency is not a concern.

Although the incorporation of P80 had a significant effect on the size, it had no effect on the polydispersity index of liposomes. Thus, this surfactant can be used to prepare homogeneous populations of curcumin encapsulated liposomes. According to the polydispersity indices of the four types of liposomes, NLs and NHLs are more homogenous than PLs and PHLs. These results indicate that the incorporation of SA contributes to the formation of more dispersed populations of liposomes. Thus, if the application demands a narrow size distribution of liposomes, either negatively charged liposomes or other positively charged lipids should be utilized instead of SA.

Although the lipids, PC and CH, and the surfactant P80 bear no net charge, liposomes made using those species showed negative charge (NL was -57.4 ± 3.5 mV and NHL was -48.7 ± 1.7 mV). This negative charge may have resulted due to the orientation of the phosphate group of PC towards the external aqueous component of the lipid bilayer, facilitated by CH [26, 27]. Incorporation of SA resulted in positively charged curcumin liposomes with the zeta-potential of $+42.0 \pm 0.3$ mV (PL) and $+48.2 \pm 0.8$ mV (PHL). Upon incorporation of P80 in these liposomes, the zeta-potentials of both negatively charged liposomes and positively charged liposomes increased significantly. This increase may be due to the shielding of the phosphate groups of PC by the ethoxy

moieties of the head groups of P80. Thus, P80 may be used to increase the zeta-potential of both negatively charged and positively charged curcumin encapsulated liposomes.

3.3. Thermogravimetric Analysis. The four types of curcumin encapsulated liposomes and their constituents were subject to TGA and their main degradation temperatures and the corresponding weight losses are given in Table 4.

These results clearly indicate that the degradation of liposomes occur around the degradation temperatures of the staple lipid, phosphatidylcholine. Further, the incorporation of SA in the lipid bilayer of curcumin encapsulated liposomes results in a depression of the main degradation temperature by 10–15°C making the vesicles more susceptible to thermal degradation. In addition, although the main degradation temperature is unaffected by incorporating P80 in negatively charged liposomes, an increase by approximately 5°C was observed in positively charged liposomes. Thus, thermal stability of curcumin encapsulated positively charged liposomes may be increased by incorporating P80.

Despite these subtle variations of the main degradation temperatures, both negatively and positively charged liposomes are unlikely to undergo thermal degradation during manufacturing or storing because manufacturing and storing temperatures of liposomes are much lower than the degradation temperatures exhibited by those liposomal formulations.

3.4. Differential Scanning Calorimetry. The melting temperatures (T_m) and crystallization temperatures (T_c) of the four types of freeze-dried liposomes are shown in Table 5. According to the heating curves, freeze-dried PL and PHL exhibited higher melting temperatures than freeze-dried NL and NHL. The melting temperatures of freeze-dried NL and NHL were 14.8°C and 14.2°C, respectively, while those of freeze-dried PL and PHL were 40.6°C and 50.8°C, respectively. Accordingly, the cooling curves revealed that the crystallization temperatures of the two types of freeze-dried positively charged liposomes were higher than those of the two types of freeze-dried negatively charged liposomes. In fact, the crystallization temperatures of freeze-dried NL and NHL were 9.9°C and 9.7°C, respectively, while those of freeze-dried PL and PHL were 21.0°C and 31.8°C, respectively. These results demonstrate the effect of lipid composition on T_m and T_c of freeze-dried curcumin encapsulated liposomes.

Further, according to our results, as the melting temperatures of freeze-dried NL and NHL are lower than room temperature, the lipid bilayer of negatively charged liposomes may exist mainly in the liquid state at room temperature. However, as the melting temperatures of freeze-dried PL and PHL are higher than room temperature, those liposomes may exist mainly in the solid state at room temperature. Therefore, incorporation of SA into the lipid bilayer may result in the ordering of lipids in the bilayer, thus favoring the solid state. Although the melting temperatures of freeze-dried NL and NHL are quite similar, those of freeze-dried PL and PHL are significantly different. In fact, the melting temperature of freeze-dried PHL is 10°C higher than that of freeze-dried PL. Accordingly, the crystallization temperature of freeze-dried PHL is, also, approximately 10°C higher than

TABLE 3: Diameter, polydispersity index, and zeta-potential of different types of curcumin encapsulated liposomes. Each value represents mean $\pm$ SD ($n = 3$). Statistically different values are followed by different superscripts and vice versa, in each column.

Liposomal formulation	Diameter (nm)	Polydispersity index	Zeta-potential (mV)
NL	284.2 ± 6.3^a	0.296 ± 0.046^a	-57.4 ± 3.5^a
NHL	225.7 ± 5.0^b	0.293 ± 0.020^a	-48.7 ± 1.7^b
PL	226.8 ± 10.9^b	0.399 ± 0.038^b	$+42.0 \pm 0.3^c$
PHL	265.3 ± 3.3^c	$0.363 \pm 0.059^{a,b}$	$+48.2 \pm 0.8^d$

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes.

PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

TABLE 4: The main degradation temperatures and corresponding weight losses of curcumin encapsulated liposomes and their constituents.

Compound/formulation	Degradation temperature ($^{\circ}\text{C}$)	Corresponding weight loss (%)
Phosphatidylcholine	344.63	52.05
	378.84	29.18
Cholesterol	352.03	69.95
	439.90	19.36
Polysorbate 80	413.54	96.92
Stearylamine	262.57	95.88
Curcumin	380.45	58.92
NL	368.29	77.09
NHL	368.06	85.18
PL	353.64	75.59
PHL	359.22	85.76

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes.

PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

TABLE 5: Melting temperatures (T_m) and crystallization temperatures (T_c) of different freeze-dried liposomal formulations.

Liposomal formulation	T_m ($^{\circ}\text{C}$)	T_c ($^{\circ}\text{C}$)
NL	14.8	9.9
NHL	14.2	9.7
PL	40.6	21.0
PHL	50.8	31.8

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes.

PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

that of freeze-dried PL. These features may have a bearing on release properties and interaction of curcumin encapsulated liposomes with the skin.

3.5. In Vitro Release Properties. The ability of liposomes to function as sustained release systems can be improved by incorporating charged lipids into the lipid content during liposome preparation. However, the effect of charge on sustained release properties is dependent on the nature of the encapsulated material [28, 29]. Thus, the effect of charge on the release properties of curcumin from liposomes was evaluated in this study.

Since surfactants usually play significant roles in enhancing skin delivery of encapsulated species of liposomes [30], we evaluated the effect of P80 on the skin delivery of curcumin and its effect on the release properties of curcumin.

The *in vitro* release profiles of the four types of curcumin encapsulated liposomes are shown in Figure 1. The release profiles of curcumin encapsulated liposomes are consistent with the literature. Specifically, Chen and coworkers showed that the release percentage of curcumin at 24 h from liposomes made of egg yolk PC and CH was 45–50% [19]. We obtained a comparable value of 32% for liposomes with a similar lipid composition (see NL of Figure 1). The observed decrease in the release of our study may be due to the lower temperature at which our experiments were carried out [19].

According to the *in vitro* release profiles of different types of liposomes, NL and NHL showed faster average release of curcumin compared to PL and PHL, exhibiting a pronounced effect of charge on the release properties of curcumin encapsulated liposomes. NL released the highest amount of curcumin (32%), for 24 h. NHL exhibited slower average release of curcumin (21% in total) than NL but exhibited faster average release than the other two types of liposomes: PL released approximately 11% while PHL released approximately only 9% of encapsulated curcumin, for 24 h. Thus, incorporation of SA in the lipid bilayer may be considered as a strategy to achieve slow average release of curcumin from liposomes. Indeed, liposomes made of PC and SA have exhibited slower release of encapsulated species than other types of liposomes [20].

The release properties of curcumin encapsulated liposomes appear to correlate with their phase transition temperatures as well as the charge. The fact that the two types of positively charged liposomes (i.e., PL and PHL) exhibited

TABLE 6: Adjusted R -square values of different liposomal formulations of curve fitting for six different drug release models.

Type of liposome	Adjusted R -square					
	Zero order	First-order	Higuchi	Hixson-Crowell	Korsmeyer-Peppas	Gompertz
NL	0.9830	0.6830	0.8817	0.9917	0.9672	0.9987
NHL	0.9917	0.5406	0.8218	0.9919	0.8961	0.9908
PL	0.9832	0.6703	0.7937	0.9837	0.8796	0.9992
PHL	0.9777	0.7740	0.7372	0.9766	0.9425	0.9998

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes.
 PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

FIGURE 1: *In vitro* release profiles of the four types of curcumin encapsulated liposomes in PBS of pH 6.8 ($n = 3$); NL: negatively charged liposomes, NHL: negatively charged hybrid liposomes, PL: positively charged liposomes, and PHL: positively charged hybrid liposomes.

higher melting and crystallizing temperatures than the two types of negatively charged liposomes (i.e., NL and NHL) sheds light on why PL and PHL remain mainly in the more ordered solid state at room temperature while NL and NHL remain in the less ordered liquid state. This greater degree of order in the lipid bilayers of PL and PHL may have contributed to the slower average release exhibited by these types of liposomes [19].

The slower average release of curcumin may be achieved by incorporating P80 in negatively charged liposomes (Figure 1). The effect of P80 on the release of curcumin from positively charged liposomes was less pronounced. Nevertheless, it can be concluded that P80 may be utilized to achieve slow release of curcumin, especially from negatively charged liposomes.

3.5.1. Drug Release Kinetics. Drug release kinetic studies were carried out to select a model that best describes the release behavior of the four different curcumin encapsulated

liposomal formulations. Six different models were tested and their adjusted R -square values are tabulated in Table 6. Accordingly, the Gompertz model, zero order model, or Hixson-Crowell model can be used to describe the release kinetics of the curcumin encapsulated liposomal formulations used in this study. However, the release profiles fit the Gompertz model best in which rapid release is followed by slow release that allows transformation of the release profile into an asymptotic maximal. In general, the two types of negatively charged liposomes (i.e., NL and NHL) exhibit gradual release of curcumin with time while the two types of positively charged liposomes (i.e., PL and PHL) show a more pronounced lag time followed by gradual release of curcumin with time. The parameters for the Gompertz model of release of the four types of liposomal formulations are shown in Table 7. It is evident that $X_{\max}$ is the highest for NL with gradual decrease in $X_{\max}$ for the other liposomes corroborating the decrease in the release profiles. “ α ,” which is indicative of the steepness of the curve, of all liposomal formulations approximated to 0.2. “ β ,” which is the time at which the growth rate is maximal (i.e., inflection point) increased gradually from NL to NHL and from PL to PHL, indicating the suitability of PHL for slow release of curcumin. These parameters (i.e., $X_{\max}$, α , and β) are important in predicting release behavior of curcumin encapsulated liposomes.

Equation for Gompertz model is as follows:

$$X(t) = X_{\max} e^{[-e^{(-\alpha(t-\beta))}]} \quad (8)$$

All liposomal formulations may function as drug depots since all formulations exhibited relatively slow release properties. However, since the two types of positively charged liposomes exhibited much slower average release than the two negatively charged liposomes, positively charged liposomes may be suitable for prolonged release of curcumin. Basically, one can choose a certain type of liposomal formulation depending on the pharmacokinetics desired for the intended application.

3.6. Ex Vivo Skin Permeation and Skin Deposition. According to *ex vivo* skin permeation studies, none of the liposomal formulations used in this study facilitated skin penetration of curcumin during a period of 8 h. However, all types of liposomal formulations facilitated skin deposition of curcumin (Table 8).

In our laboratory, the smaller analogues of curcumin, namely, ferulic acid (FA) and caffeic acid (CA), were found to

TABLE 7: Parameters for Gompertz model of release of curcumin encapsulated liposomes.

Formulation	$X_{\max}$	α	β
NL	26.13	0.19	7.25
NHL	20.58	0.21	8.17
PL	10.77	0.24	8.24
PHL	8.97	0.20	9.60

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes.

PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

TABLE 8: Percentage skin deposition and amount of deposition of curcumin from different types of liposomes at 25°C. Values are indicated as mean $\pm$ SD ($n = 3$).

Liposomal formulation	Percentage of skin deposition (%)	Amount deposited ($\mu\text{g}/\text{cm}^2$)
NL	7.3 ± 0.6	25.7 ± 2.2
NHL	2.9 ± 0.5	8.6 ± 1.4
PL	1.9 ± 0.3	4.4 ± 0.6
PHL	1.1 ± 0.2	2.5 ± 0.5

NL: negatively charged liposomes; NHL: negatively charged hybrid liposomes.

PL: positively charged liposomes; PHL: positively charged hybrid liposomes.

show skin penetration. CA displayed 41% permeation and 2% deposition during 7 h and FA displayed 8–20% permeation and 2–3% deposition during 7 h depending on the charge of the liposomes [31, 32].

Although no skin penetration occurred under the experimental conditions of this study, Chen and coworkers reported skin penetration of curcumin from liposomes made from either egg yolk phospholipids, soybean phospholipids, or hydrogenated phospholipids. In fact, they reported a percentage permeation of approximately 5% at 8 h. The difference in permeation results of our study and the study conducted by Chen et al. may be due to the smaller size of liposomes used by them (100 nm) and the use of rat abdominal skin as compared to the full thickness pig ear skin [19].

Curcumin containing liposomes were monitored by Berginc et al. (2012) for *in vitro* permeation through cow vaginal tissue, rat jejunum, and transwell grown Caco-2 cell monolayers. They found the apparent drug permeability of curcumin to be the lowest among the drugs tested. They used liposomes made of phosphatidylcholine alone in two sizes given as SUV and MLV. In their study, the liposomal curcumin exhibited lower permeability than the free curcumin which they attributed to interaction with the hydrophilic mucus layers on the membranes [16]. We noted the reverse effect in both FA and CA studies which we attribute to interaction with the skin being more favorable to liposomes due to their hydrophobic nature. In addition, the permeation and deposition of curcumin by the MLVs in their study were the lowest and highest, respectively, in comparison to free curcumin and SUVs, possibly due to the larger size of the MLVs. Based on these observations and considering the barrier properties of the stratum corneum of the pig ear skin,

the lack of permeation observed in this study is justifiable. It is also very interesting and supportive of the observations of this study that the permeability coefficient through the Caco-2 cell monolayers in transwells had around 4–6 times higher permeability than the permeability coefficient through cow vaginal tissue and rat jejunum [16].

In general, the negatively charged liposomes exhibited a higher percentage of skin deposition of encapsulated curcumin than the positively charged liposomes. Thus, skin delivery of liposomal curcumin is dependent on the charge of liposomes.

Edge activators and skin-penetration enhancers are substances that improve the skin delivery, including skin deposition, of liposomal bioactive agents, especially when incorporated in liposomes. P80 which is also called Tween 80 has shown to increase skin deposition of many substances [33]. According to the skin deposition results of this study, P80 has a negative effect on the skin delivery of curcumin. Thus, under the experimental conditions of this study, ionic lipids are better than their P80-containing counterparts with respect to skin deposition of curcumin.

4. Conclusion

This study reveals that the charge of liposomes and the presence of surfactants impart a pronounced effect on the properties of curcumin encapsulated liposomes.

Egg yolk PC and CH with or without P80 form negatively charged liposomes. Positively charged liposomes are formed by incorporating SA in liposomes made of egg yolk PC and CH with or without P80.

The EE depends on the lipid composition of curcumin encapsulated liposomes. Negatively charged liposomes were superior in terms of EE and LC to the positively charged liposomes. Moreover, incorporation of P80 resulted in a decrease of these effects.

P80 and SA if incorporated separately result in curcumin encapsulated liposomes of smaller size (approx. 226 nm). Incorporation of P80 results in an increase in the zeta-potential and thus it may be utilized for tuning of zeta-potential of curcumin encapsulated liposomes.

Incorporation of SA influences the liquidity of lipid bilayers of curcumin encapsulated liposomes. This decreased liquidity upon the incorporation of SA may affect properties such as release and skin delivery of liposomal curcumin.

The charge of liposomes has a significant effect on the release properties of liposomal curcumin. Positively charged liposomes show better average slow release properties of curcumin. Incorporation of P80 further decreases the rate of release.

Negatively charged liposomes show better skin deposition of liposomal curcumin than positively charged liposomes. Incorporation of P80 has a detrimental effect on skin delivery of liposomal curcumin.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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