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Research Article
Size, Entrapment Efficiency and Stability of Curcumin Niosomes Prepared at Different pH Conditions

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Abstract

Background and Objective: The pH has been shown to affect curcumin stability. However, no studies regarding the effects of pH of dispersion system during curcumin niosomes construction have been reported. This study investigates the influence of pH value during the incorporation of curcumin into niosomes on the properties of vesicles. **Materials and Methods:** Niosomes were prepared using Span 60/Tween 60 at 1:1 mole ratio with the addition of dicetylphosphate and cholesterol via thin film hydration method. Curcumin incorporation into niosomes was conducted at pH 3, 7 and 9. The size of niosomes was reduced using extrusion. **Results:** Spherical niosomes was obtained at pH 3 and pH 7, while preparation at pH 9 produced irregular shaped niosomes. The entrapment efficiency of niosomes prepared at all pH was more than 60%, in which the highest was for niosomes prepared at pH 3 (75.23±2.85%). The initial size of niosomes (>376 nm) was reduced to <160 nm using extrusion. Although extruded niosomes have zeta potentials less negative than -30 mV, the niosomes showed minimal increase in size due to aggregation after 28 days of storage at all conditions. **Conclusion:** The pH during hydration process does not significantly affect the size of the vesicles before storage, but influences its stability with respect to their rigidity structure during storage. The high entrapment efficiency of niosomes prepared at pH 3 suggested that it is more favorable to incorporate curcumin into vesicles in acidic medium.

Key words: Curcumin, niosomes, non-ionic surfactants, drug delivery, thin film hydration method

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Niosomes are vesicles that consist of a bilayer of non-ionic surfactant which are formed on the hydration of the mixtures of non-ionic surfactants, cholesterol and phosphate in an aqueous media[1]. They have the ability to entrap hydrophobic and hydrophilic drugs in between the bilayers and in their core, respectively. Niosomes could be synthesized at a low cost using numerous non-ionic surfactants and require no special conditions of handling and storage. Moreover, non-ionic surfactants are known to be biocompatible, biodegradable and non-immunogenic to the human body[2]. Thus, niosomes are very suitable as carrier for drugs that require specific condition to achieve the target such as delivering hydrophobic drug through hydrophilic or aqueous system.

Curcumin is a hydrophobic polyphenol which benefits includes anti-inflammatory[3], anticancer[4] and wound healing properties[5]. However, the application of curcumin as a therapeutic agent is restricted by its poor water solubility and high susceptibility to decomposition in buffer solutions with neutral-basic pH conditions at 37°C[6]. Therefore, suitable carriers are needed for curcumin to ensure site-specific delivery with improved bioavailability, where the use of niosomes is promising for this purpose.

The physicochemical properties of niosomes have obvious implication on the stability and bioactivity of loaded drug. Thus, studies examining the influence of parameters (e.g., composition, membrane surface charge, temperature of dispersion medium and pH of dispersion system) responsible for physicochemical behavior of vesicles are warranted. Previous study by Belkacemi *et al.*[7] shows that pH has a great influence on curcumin stability. In acidic pH (1-6), the curcumin is stable with very minimal degradation. However, another study showed that curcumin is not stable at both neutral and basic pH and undergoes degradation of up to 90% within 30 min, forming trans-6-(4-hydroxy-3-methoxyphenyl)-2,4-dioxo-5-hexanal as a major product while the minor products are ferulic acid, feruloylmethane and vanillin[6]. To our knowledge, no studies regarding the effects of pH values of dispersion system during curcumin niosomes construction on the properties of the niosomes have been reported. Therefore, in this study, the effects of pH variation during curcumin niosome self-assembly on the properties of vesicles were investigated. The properties of curcumin niosomes were characterized in terms of encapsulation efficiency, size, structure and surface charge. Stability studies were also performed by storing the samples under different temperature for 28 days to observe the changes in the properties of niosomes.

MATERIALS AND METHODS

Curcumin (Sigma-Aldrich, ≥65% purity), cholesterol (Chol, Sigma-Aldrich, ≥90% purity), dicetylphosphate (DCP, Sigma-Aldrich, >99% purity), sorbitan monostearate (Span 60, R and M Chemicals), polyoxyethylene sorbitan monostearate (Tween 60, R and M Chemicals), chloroform (Merck, 99.8% purity) and methanol (Merck, 99.9% purity) were used as received.

Preparation of curcumin niosomes: The research project was conducted at the School of Chemical Engineering, Universiti Sains Malaysia. In this project, curcumin niosomes were prepared by utilizing thin film hydration method[8,9]. A solution of curcumin in methanol (0.40 mg mL^{-1}) was added to Span 60/Tween 60 mixtures in the ratio of 1:1 to Chol in chloroform/methanol 4:1 (v/v) to dilute the final niosomal dispersion concentration to 50 mg mL^{-1}. The ratio of Span 60/Tween 60 was kept constant at 2:1. The solvent was then evaporated by a rotary evaporator (Buchi) to obtain a thin film deposit. This was followed by the hydration of the lipid-curcumin film with 10 mL of phosphate buffered saline (PBS) solution at pH 7 and swirling of hydrated film at 60°C for 60 min in water bath. After completion of hydration, the resulting vesicles were ultrasonicated at 60°C for 30 min using a bath sonicator (Elmasonic Type S30) to minimize the size. The experiments were repeated with acidic and alkaline pH of PBS solution during the hydration step. The pH of PBS solution was measured by using a pH meter at 25°C. The adjustment of pH of PBS solution was carried out by titrating 0.1 M HCl and/or 0.1 M NaOH to attain the desired pH 3 for acidic condition and pH 9 for the basic condition during the hydration step. Prepared curcumin niosomes were also subjected thrice to extrusion at 60°C using a polycarbonate membrane with 200 nm pore diameter by an extruder device (Avanti Mini Extruder).

Determination of morphology: The niosomes morphology was observed by transmission electron microscopy (TEM). An electronic-microscope grid was used as a cover with collodion as an electron-transparent support film. A drop of niosomes suspension was positioned on the grid and left for 1 min to allow some vesicles to attach. A piece of filter paper was used to remove the excess niosomes followed by the addition of a drop of 2% uranyl acetate solution to the attached niosomes. The remaining solution was then removed after 1 min and the sample was examined using TEM after completely dried[10].

Size of niosomes: The size of niosomes and polydispersity index (PDI) was analyzed by using Dynamic light scattering (DLS) technique. Dilution of 50 µL niosomal suspensions in 1 mL distilled water were conducted and 500 µL of diluted sample were injected into the disposable folded capillary cell. Independent samples were taken from each of the dispersion and particle size was measured in triplicates at 25°C for about 120 sec. An average dimensional distribution can be calculated by referring to the mode.

Efficiency of drug entrapment: The efficiency of drug entrapment (EE %) was used to determine the ability of niosomes to entrap curcumin and the formula is shown in Eq. 1:

$$EE\ (\%) = \frac{\text{Amount of entrapped curcumin}}{\text{Total amount of curcumin}} \times 100 \quad (1)$$

Initially, free curcumin was removed by centrifuging the niosomal dispersion and the pellet was collected and diluted with 5 mL distilled water and vortexed. This was followed by the addition of 99% methanol (5 mL) to the diluted samples. Sample was then vortexed and centrifuged. The supernatant was separated and assayed accordingly using UV-VIS spectrophotometer (Agilent Technologies) at 420 nm using methanol as blank. The remaining pellet was diluted with 1 mL methanol and estimation of the encapsulated amount was conducted using UV-VIS spectrophotometer at a similar wavelength.

Zeta potential: The electrophoretic mobility of the curcumin niosomes was obtained by using Zetasizer Nano ZS (Malvern Instruments). Sample (50 µL) was diluted in distilled water (1 mL). Then, 500 µL of the diluted sample was dropped into the zetasizer electrophoretic cell. Measurement was conducted at 25°C in distilled water at field strength of 20 V cm^{-1} and based on the measurement of the electrophoresis mobility of the charged particle. Charge of the particles and the mean values for zeta potential with standard deviation were acquired directly from the obtained data.

Stability test: The stability of curcumin niosomes after extrusion process was studied at various temperature by storing the samples in air-tight sealed vials at 3 different conditions; room temperature, refrigerator (4°C) and freezer (-4°C) for 28 days. Samples from each vial were analyzed every 7 days to observe the variation of PDI, size and zeta potential.

RESULTS AND DISCUSSION

Appearance of curcumin-loaded niosome prepared at different pH: Figure 1 shows the hydrated medium containing curcumin niosome. At pH 3, it appears to have bright yellow colour while colour fading occurs at pH 7 together with cloudy suspension and dark orange colour forming at pH 9. Curcumin was found to be basically insoluble in neutral and acidic pH but soluble in basic condition. The characteristic is expected to influence the formation of

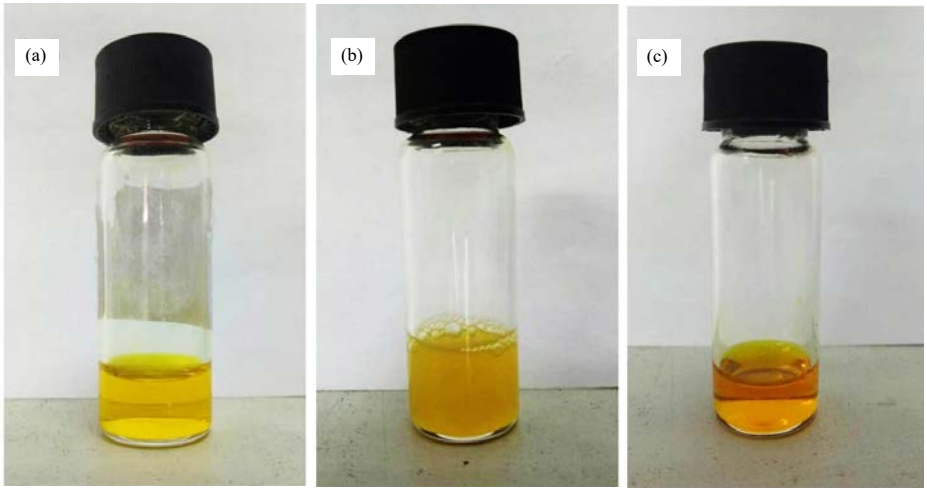

Fig. 1(a-c): Physical appearance of curcumin-loaded niosomes formulated at conditions of (a) pH 3, (b) pH 7 and (c) pH 9, prior to extrusion

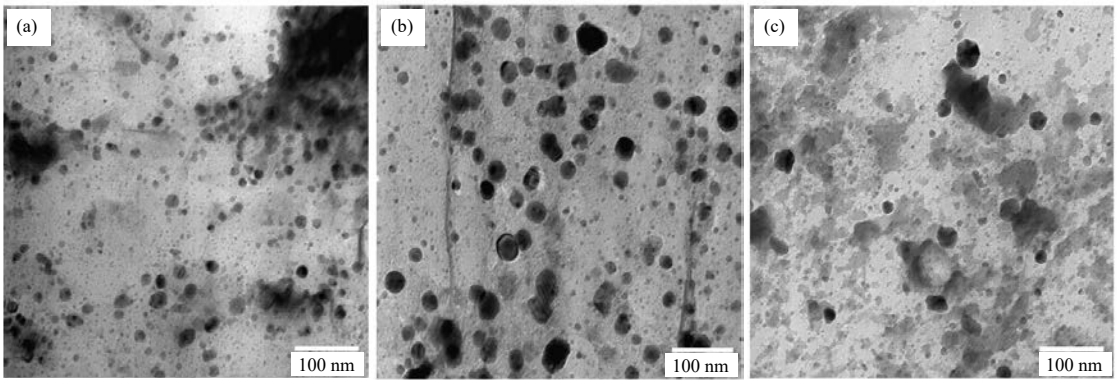

Fig. 2(a-c): Transmission electron microscope images for curcumin niosome formulated at conditions of (a) pH 3, (b) pH 7 and (c) pH 9, after extrusion

Table 1: Mean vesicle size, polydispersity index, zeta potential and entrapment efficiency of curcumin-loaded niosome at different formulations

		pH of hydration formulation		
Samples	Properties	3	7	9
Before extrusion	Z-ave (nm)	556.37±28.44	453.60±12.23	394.07±17.35
	PDI	0.60±0.02	0.54±0.02	0.53±0.04
	Zeta (mV)	-13.70±0.90	-31.00±0.90	-29.90±0.36
	EE (%)	75.23±2.85	72.98±3.24	64.79±2.83
After extrusion	Z-ave (nm)	152.73±2.94	150.30±2.29	156.30±2.17
	PDI	0.08±0.01	0.08±0.01	0.08±0.01
	Zeta (mV)	-24.10±0.49	-14.83±0.54	-29.70±0.75

Mean±SD, n = 3

curcumin niosomes due to the pH effects during hydration. Visual observation after hydration with different pH of PBS buffer showed immediate and distinct colour changes. Curcumin is more stable in acidic conditions due to the conjugated diene structure. However, adjusting to neutral-alkaline condition causes deprotonation of phenolic groups within curcumin which makes the molecule unstable but more soluble due to increased polarity[11-13]. Dark orange colour formation at high pH value is due to the low stability of the curcumin as it is highly prone to degradation.

Morphology of curcumin-loaded niosome: Observation by TEM (Fig. 2) revealed that niosome formulation at pH 3 (Fig. 2a) and pH 7 (Fig. 2b) have spherically shaped vesicles while pH 9 (Fig. 2c) showed irregular shape vesicles, which might be caused by leakage of curcumin from the niosome during preparation or extrusion. This suggested that the niosome formulation at pH 9 produces vesicles with low rigidity bilayer structure.

Properties of curcumin-loaded niosome prepared at different formulation: Table 1 presents the values of mean vesicle size (Z-ave), PDI, zeta potential (mV) and entrapment efficiency that represent all of pH formulations samples. The PDI values of these niosomes were between 0.45-0.65 for all pH conditions, indicating large size distribution and less homogenous system. The presence of cholesterol could be the contributing factor to the larger size vesicles[14,15]. Another possible reason for the larger size is due to the formulation itself as Span 60 has longer saturated alkyl chain and increasing hydrophobicity of surfactant decreases the surface free energy[16]. Moreover, the volume of the aqueous compartment would increase with the inclusion of charge inducing agents such as DCP due to the interaction between surfactant head and charged group that increases mutual repulsion[17].

Extrusion process was used to produce small niosomes with homogeneous size and dispersity and to reduce aggregation. After extrusion, the size of the curcumin niosome was found to be reduced to <160 nm for all formulations with PDI value of 0.08±0.01. This clearly indicates that extrusion is an effective post size reduction technique.

Zeta potential relates the mobility and interaction of colloidal particle which indicates the stability of the system. Since the addition of DCP has direct relationship towards the charge of niosome, it is expected to have negatively charged zeta potential value due to ionization of the acidic ($-HPO_4$) group of DCP. The addition of DCP causes the niosome particles to repel each other and this prevents aggregation which in turn increases its stability. Generally, a stable colloid system will have zeta potential value of more than 30 mV (either positive or negative)[18].

From Table 1, the zeta potential for all the freshly prepared curcumin niosomes at different pH were below -30 mV, indicating none of the systems were stable and have higher tendency to aggregate over time. Ideally, with the addition of 0.05 mol of DCP, it is expected to have zeta

Table 2: Mean vesicle size, polydispersity index and zeta potential of curcumin-loaded niosome stored for 28 days after subjected to extrusion process

		Storage condition		
pH	Properties	Room temperature	4°C	-4°C
3	Z-ave (nm)	161.57±1.84	173.30±0.87	171.35±2.42
	PDI	0.24±0.01	0.12±0.01	0.14±0.01
	Zeta (mV)	-11.60±0.46	-8.37±0.35	-9.04±1.32
7	Z-ave (nm)	159.73±1.62	173.13±3.35	135.23±1.78
	PDI	0.14±0.01	0.16±0.03	0.16±0.02
	Zeta (mV)	-19.00±2.05	-17.67±0.35	-31.93±0.32
9	Z-ave (nm)	159.67±2.27	174.93±2.80	151.63±4.97
	PDI	0.12±0.01	0.16±0.01	0.28±0.01
	Zeta (mV)	-17.37±0.74	-13.83±0.42	-22.50±1.06

Mean±SD, n = 3

potential more than -30 mV since DCP increases the negative charge on curcumin niosome's surface. Meanwhile, the formulations of curcumin niosomes at pH 7 shows the lowest value of zeta potential, which indicate a lack of surface charge transfer system occurred in the neutral dispersion medium.

The highest EE (%) was recorded for preparation at pH 3 with 75.23±2.85% while sample formulation at pH 9 has the lowest EE (%) at 64.79±2.83%. This low EE (%) is probably due to the vesicle's leakage caused by low stability bilayer structure of vesicles formed in the alkaline hydration medium. This finding is supported by TEM morphology analysis where few whole niosomes could be observed.

Stability studies: Size changes (Table 2) were recorded during storage at 4 and -4°C, which contradicted with the findings from previous research where larger particles will eventually form at high temperature due to greater aggregation[19]. Higher temperature will increase the bilayer fluidity since the electrostatic repulsive forces among the vesicle and repulsive entropic forces of the surfactant head groups have decreased, in which curcumin might have been leaked[20,21] which leads to smaller vesicles. The size of curcumin niosomes produced at pH 7 and pH 9 decreased slightly from the initial size (Table 1) when stored at -4°C, probably due to rigidization of the vesicles at low temperature. Nevertheless, all the formulations showed minimal increase in size (<25 nm) and minor change in PDI ($\leq$0.20), suggesting that curcumin-loaded niosomes were stable over 28 days of storage.

Most of the formulation experienced a decrease in zeta potential during storage, as aggregated particles tend to have slower movement resulting in the increase of zeta potential during storage[22]. It is clearly shown that the sample formulation at pH 3 had the lowest zeta potential after 28 days of storage. This is due to the fact that negative charge was introduced onto the surface of niosomes by DCP and at higher pH, the extent of ionization increased (pKa of DCP 4.5). When acidic buffer pH 3 is used, H^+ ions tends to accumulate on the surface of niosomes in order to neutralize the particle which results in lower zeta potential across the storage time. At pH 7 and 9, the process of dissociation is complete and accumulation of OH^- ions on the niosome surface contributed to the higher zeta potential.

CONCLUSION

In this study, the pH during hydration process does not significantly affect the size of niosomes before storage. However, it has more influences on the stability of the prepared curcumin niosomes especially on their rigidity. With high EE (%) of curcumin niosomes prepared at pH 3, curcumin is much more favourable to be incorporated into niosome in acidic medium.

SIGNIFICANCE STATEMENT

This discovery about the influence of pH during synthesis of curcumin niosome on the efficiency of curcumin entrapment and niosomes properties upon storage is beneficial for the formulation of an ideal delivery system for curcumin. This study will help other researchers to uncover the critical areas of curcumin delivery and bioavailability that many researchers were not able to explore.

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