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Effect of Formulation Variables and Bile Salts Addition on Entrapment Efficiency of Curcumin Loaded Niosomes

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ABSTRACT

Background: Curcumin is an important natural compound that has been extensively studied for its multifunctional pharmacological activities. Different nanotechnological techniques have been applied for improving its poor solubility and bioavailability. **Objectives:** This research aimed to study the effect of formulation variables on the entrapment efficiency of curcumin-loaded niosomes (curcusomes) made by a thin-film hydration technique. **Methods:** Curcumin-loaded noisome were prepared using the thin-film hydration technique using Span 40 and 60 as surfactants, in addition to bile salts and cholesterol. The surfactant and cholesterol were added to curcumin in different ratios, with/without the addition of 10% of bile salts. **Results:** Eighteen formulae were obtained from the addition of bile salts and spans. Results showed that increased surfactant concentration and low cholesterol ratio enhanced the %EE of formulae. **Conclusion**: The formulation of curcumin-loaded niosomes with the thin-film hydration method where span 40 or span 60 are used as surfactant enhances the %EE of curcusomes which in turn improves the poor solubility and bioavailability of curcumin. The addition of bile salts enhanced the %EE which will be investigated furtherly for more *in vitro* and *in vivo* studies.

Keywords: *Curcumin, Niosomes; Entrapment Efficiency; Cholesterol; Bile salts.*

INTRODUCTION

For drug administration, oral delivery is the most convenient route, especially for chronic illness, with high patient compliance, ease of administration, cost-effectiveness, and other benefits [1]. Although high oral bioavailability is highly desirable, many important drugs in the clinic suffer from poor oral bioavailability and highly variable exposure. This can be due to various factors, including low solubility, limited permeability, first-pass metabolism, and drug efflux [2]. To reach the bloodstream, a drug should first dissolve in the gastrointestinal (GI) fluid. Thus, dissolution may be the rate-limiting step in oral administration of poorly water-soluble drugs [3], resulting in erratic absorption and low oral bioavailability. Intestinal and hepatic first-pass metabolism can also restrict oral bioavailability to a significant extent [4]. In the efforts to enhance oral bioavailability, various approaches have been employed, such as solid dispersions [5], salt forms [6], nanosizing, and micronization [7]. In the last two decades, there has been increased interest in studying colloidal particulate

carriers such as liposomes, niosomes, and micelles, as well as polymeric and lipidic nanoparticles. The Curcumin ($C_{21}H_{20}O_6$) is a natural yellow compound typically found in *Curcuma longa* L. that is regarded as a natural polyphenolic antioxidant presented in many kinds of herbs [8]. Curcumin has been exhibited multiple therapeutic relevance including anticancer, anti-inflammatory, antioxidant, antimicrobial, antirheumatic, and hepatoprotective activities [9–13]. Its anticancer, anti-inflammatory, antiangiogenic, antineoplastic, and chemo sensitizing effects make it a potent candidate in the treatment for multiple types of cancer [14,15]. In spite of the well-received pharmacological properties, the therapeutic application of curcumin has been impeded by its shortcomings such as low aqueous solubility at acidic and physiological pH and its degradability in alkaline conditions [8,16,17]. In addition, poor absorption and rapid metabolism of curcumin severely limit its bioavailability [17]. For this purpose, researchers have been exploring methods for the effective delivery of curcumin with novel formulations including liposomes [18,19], micelles [20,21], conjugates [22], nanoparticles [23], and nano globules[24]. These have shown distinct advantages over conventional dosage forms in oral drug delivery [25]. In addition to enhanced solubility and dissolution rates, these carriers provide a powerful means to avoid first-pass metabolism through stimulation of lymphatic transport, leading to improved bioavailability [26,27]. Niosomes are nonionic surfactant vesicles with a bilayer structure, which have been used to deliver various drug elements including chemotherapeutic agents, genes, hormones, antigens, and peptides [28,29]. Niosomes share some similarities with liposomes but are composed of nonionic surfactants such as Span 80, Span 60, Span 40, Span 20, Tween 80, and Pluronic 188 instead of phospholipids used in liposomes[30,31]. Typically, niosomes are produced from two main components: nonionic surfactants and additives [32]. While nonionic surfactants serve as the vesicular layer, the additives such as cholesterol act to enhance the rigidity of the bilayer. Bile salts has been recognized as natural biosurfactants with crucial roles in endogenous organotropism[33]. Their extraordinary emulsifying and solubilizing properties have led to their utilization as delivery systems for medicines and cosmetics as well [34,35]. In pharmaceutical field, bile salts have been reported to enhance hydrophilicity of water-insoluble active pharmaceutical ingredients mainly by the wetting effect [36–38]. Furthermore, bile salts have been employed as permeation enhancers in topical dosage forms including buccal, ocular, nasal, and transdermal routes of administration [39–42]. Moreover, bile salts are known by their stability in the acidic stomach medium, their adaptability to dynamic pH variations and the presence of selective uptake transporters in the intestine, bile acid-based therapeutic systems may be suitable for oral drug delivery [43,44]. Therefore, the present study examines the delivery of curcumin effectively through encapsulation of curcumin in niosomes composed of nonionic surfactant Span 40 and Span 60 to improve the solubility and the therapeutic effects of curcumin. In addition, evaluate the efficiency of the addition of bile salts to formed curcusomes in constant percentage.

MATERIAL AND METHODS

Material

Curcumin was purchased from Sigma-Aldrich Co (St Louis, MO, USA). Span 40 (Sorbitan monopalmitate), Span 60 (Sorbian monostearate) and Cholesterol (CH) were purchased from Loba-Chemie (Mumbai, India). Sodium Deoxycholate (SDC) was purchased from Alfa Aesar (Karlsruhe, Germany. Methanol, Chloroform, and Isopropyl alcohol were supplied as analytical grades by Fisher Scientific (Loughborough, UK). Double-distilled water was used throughout the study.

Preparation of standard solution of Curcumin for UV Visible Spectroscopy

A 10 mg of curcumin was accurately weighed and transferred in a 100 ml volumetric flask. Methanol was then added up to the mark to obtain a concentration of 100 µg/ml of Stock solution. From Stock solution, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, 0.5, 0.55, 0.6, and 0.65 ml were withdrawn and diluted to 10 ml with Isopropyl alcohol to obtain concentrations of 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5 µg/ml, respectively.

Determination of maximum wavelength by UV-visible spectroscopy:

A six µg/ml of curcumin solution was scanned using UV spectrophotometer (Specord 210 plus (Analytik Jena AG, Germany) in the range of 200-600 nm where isopropyl alcohol was used as blank.

Preparation of standard calibration curve of curcumin by UV-visible spectroscopy:

The standard calibration curve of curcumin was obtained through measuring the absorbance of curcumin solution in a ranged concentration (1-6.5 µg/ml). the measurements were implied for samples prepared from the stock solution in isopropyl alcohol in tri-replicates. Calibration curve of curcumin was then plotted with absorbance rate on y-axis and curcumin concentration on x-axis.

Preparation of curcumin-loaded niosomes (Curcusomes)

The thin-film hydration technique [45] was employed to formulate the curcusomes, depending on the

variation in the amount of lipids used for the formation of bilayer (surfactant and CH), and the number of surfactant parts (surfactant to CH ratio). In the present study the effect of surfactant type (span 40, 60), lipid to drug molar ratio (10:1, 20:1, and 40:1), and Molar ratio of surfactant to cholesterol (90:10, 80:20 and 70:30) was investigated. In a round-bottomed flask, a specified amount of curcumin, 3.68 mg and an accurately weighed amounts of lipid mixture (span/CH) were dissolved in 10 ml of chloroform: methanol mixture (7:3 v/v). The obtained clear organic solution was evaporated slowly at 60°C using a rotary evaporator (Heidolph-GI Germany) under reduced pressure for 30 min. at 90 rpm. After evaporation, a thin film on the wall of the flask which was then hydrated 10 mL of deionized water by rotating the flask in a water bath maintained at temp 60°C for Span 60 and 55° for span 40 for 45 min at 120 rpm using the same apparatus under normal pressure to form niosomal dispersion of curcumin. The formed suspension was sonicated in a water bath for 10 min (bath sonicator, LBS 2-10 FALC, Italy). The hydration step took place in the presence of glass beads to increase the yield of the formed nanovesicles and to reduce size [46,47]. During film hydration, the temperature was maintained at 60°C, above the gel-liquid crystal transition temperature (Tc) of the used surfactants, to allow hydration of lipids in their fluid phase. The resulted dispersion was allowed to stand for 2 hrs at ambient temperature for complete hydration then left to equilibrate overnight at 4°C for further investigation.

Preparation of curcumin loaded bile salts reached niosomes

Following the same preparation procedures mentioned in the previous section, the bilosomes were prepared by the addition of 10% of sodium deoxycholate (SDC) (bile salt) to the ionized water at the hydration step.

Determination of Entrapment efficiency of curcumin-loaded niosomes and bile salts-reached niosomes

Isopropyl Alcohol was selected as an appropriate solvent for disrupting the prepared vesicles according to literature. Total drug content (free + entrapped) of the prepared niosomal dispersion was determined spectrophotometrically by measuring the absorbance at the wavelength of 427 nm in isopropyl alcohol. To calculate % EE, it was estimated either by direct or indirect method for accurate quantification of the drug. The free curcumin was separated from the prepared niosomes by ultracentrifugation at 15000 rpm at 4°C for 1 hour using a cooling centrifuge (XC-HR20, Bio Lion, and USA).

Direct determination of %EE:

The separated vesicles were disrupted by isopropyl alcohol. The concentration of the entrapped drug was determined spectrophotometrically by measuring the absorbance of the clear solution at the wavelength of 427 nm in isopropyl alcohol. The %EE of entrapped drugs was calculated using the following equation:

$$\%EE = \frac{The\ amount\ of\ entaraped\ drug}{Total\ drug\ amount\ (free + entrapped)} \times 100$$

Indirect determination of %EE

By calculating the difference between the total amount of curcumin in the prepared niosomal dispersion and the free (unentrapped) curcumin in an aqueous medium. Curcumin content in the resultant supernatant was determined spectrophotometrically by measuring the absorbance at the wavelength of 427 nm in isopropyl alcohol. Drug EE% was determined according to the following equation:

$$\%EE = \frac{Total\ drug\ content\ (free + entrapped) - unentrapped\ drug}{Total\ drug\ amount\ (free + entrapped)} \times 100$$

Statistical analysis

The resulted data were statistically examined through SPSS 16.0 program (SPSS Inc., Chicago, IL, USA) with paired sample T-test. The level of significance was at $P < 0.05$.

RESULTS AND DISCUSSION

Determination of maximum wavelength by UV-visible spectroscopy

According to the results shown in **Figure 1**, Wavelength corresponding to maximum absorbance of curcumin in isopropyl alcohol was observed at 427nm.

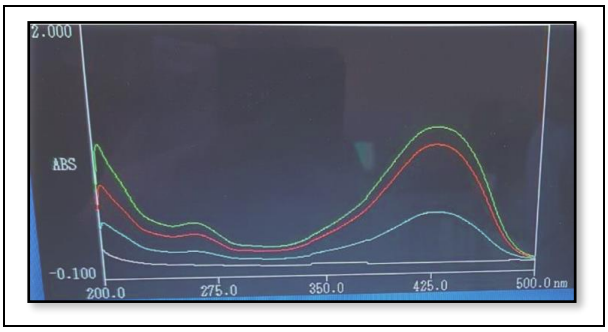

Figure 1. Maximum absorbance of curcumin in isopropyl alcohol

Standard calibration curve of curcumin by UV-visible spectroscopy

The standard calibration curves of curcumin in isopropyl alcohol at the predetermined λ_{max} were presented in **Figure 2** and **Table 1**. The results of the

figure showed that Beer's law was obeyed in the concentration range 1-6.5 µg/ml. The linear regression of absorbance and concentrations of curcumin illustrated a straight line passing through the origin with correlation coefficient 0.999. The values of slope were recorded as 0.143 which will be furtherly used in calculation of amount of drug entrapped in curcusomes. The Percent error values did not exceed 2% which indicated the validity of the assay method.

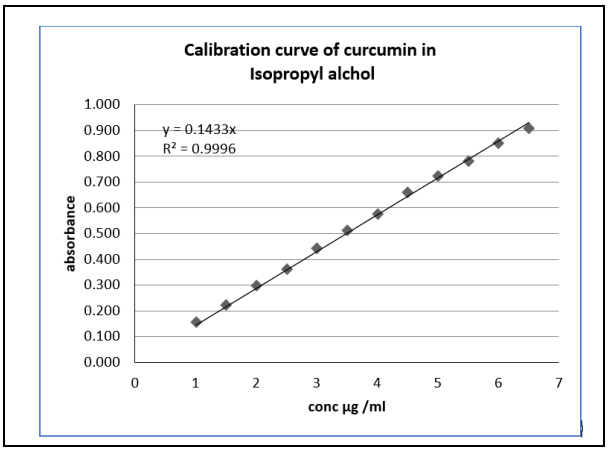

Figure 2. Calibration curve of curcumin in isopropyl alcohol.

Table 1. Calibration of curcumin by UV-VIS spectrophotometry

Curcumin concentration (µg/ml)	Absorbance rate (Mean ± S. D.)
1	0.154 ± 0.006
1.5	0.223 ± 0.007
2	0.301 ± 0.03
2.5	0.362 ± 0.006
3	0.441 ± 0.006
3.5	0.515 ± 0.009
4	0.577 ± 0.01
4.5	0.659 ± 0.009
5	0.722 ± 0.005
5.5	0.783 ± 0.001
6	0.850 ± 0.005
6.5	0.908 ± 0.01

Preparation of curcumin-loaded niosomes

The screening results demonstrated that this solvent mixture produced clear continuous film when evaporated for 30 min at 90 rpm [48]. The temperature was maintained at 60°C during film hydration had a significant impact on the shape and size of the vesicles and surfactants assembly into them [49].

Effect of formulation variables on entrapment efficiency of prepared curcusomes

Using the nonionic surfactant; Span 40 and Span 60, were the most suitable surfactant as they exhibited high Tc and optimum HLB (4-8) so that suitable for vesicle preparation [50]. The Span 60 was favored over the Span 40, in this study, as it produced vesicles with relatively high %EE due to its higher lipophilicity (HLB 4.7), compared to span 40 (HLB 6.7) which may result in decreasing the mass transfer within the droplets with consequent lower growth of nuclei and smaller particle size [48,51].

Table 2 presented the composition of different niosomal formulations coded from 1 to 18 and their corresponding determined %EE. The results showed that the total Lipid/Drug molar ratio (L/D) has an impact on drug loading. Altering the L/D molar ratio from 10:1 to 20:1 while keeping other factors invariant significantly augmented the %EE of curcumin from 80 ± 1.9 for formula 1 to 89.2 ± 1.91 for formula 4 as shown in **Table 2**. This could be obviously explained that increasing the concentration of bilayer-forming materials, which in turn increases the number of vesicles in a given volume, can increase the amount of drug entrapped in the vesicles. Although enhancement of the L/D molar ratio leads to higher EE, that EE% decreased significantly by increasing lipid amount as shown in formula 18 (%EE was 59 ± 1.26). This may be due to higher amount of CH would reduce drug entrapment by competing with the drug for the bilayer, thus preventing incorporation of the lipophilic drug into the vesicles.

It has been reported that the presence of CH enhanced the curcumin-loaded niosomes bi-layer cohesion and rigidity [52] led to a reduction in the size of niosomes. It was also reported that more cholesterol contents in niosomes can lead to a smaller diameter for niosomes [53]. It has been shows that CH influenced the membrane permeation and EE%, thus led to the less permeable niosomes [54,55].

Mokhtar et al. [56] have evaluated the effects of formulation variables like cholesterol contents of niosomes on the flurbiprofen encapsulation and showed that %EE enhanced as CH: surfactant ratio increased. Rahman and Manggau[57] have loaded curcumin into niosomes through reserve phase evaporation technique using various concentrations of span and cholesterol. Comparing EE% of their study with the current study

Table 2. The schematic data for the preparation of curcumin-loaded niosomes using the thin-film hydration procedure

Formula	L/D ratio	Type of span	Span to cholesterol molar ratio	%EE ± S. D.
1	10:1	40	90:10	80 ± 1.9
2	10:1	40	80:20	81 ± 1.93
3	10:1	40	70:30	84.4 ± 1.77
4	20:1	40	90:10	89.2 ± 1.91
5	20:1	40	80:20	91.5 ± 1.96
6	20:1	40	70:30	87.6 ± 1.88
7	40:1	40	90:10	81.5 ± 1.68
8	40:1	40	80:20	88.1 ± 1.85
9	40:1	40	70:30	85.4 ± 1.82
10	10:1	60	90:10	78.1 ± 1.76
11	10:1	60	80:20	70.4 ± 1.63
12	10:1	60	70:30	69.6 ± 1.59
13	20:1	60	90:10	90.8 ± 1.94
14	20:1	60	80:20	88.6 ± 1.89
15	20:1	60	70:30	83.5 ± 1.86
16	40:1	60	90:10	77.6 ± 1.66
17	40:1	60	80:20	68.3 ± 1.49
18	40:1	60	70:30	59 ± 1.26

Table 3. The schematic data for the preparation of bile salts-enriched niosomes (bilosomes) using the thin-film hydration procedure after the addition of 10% of bile salts

Formula	L/D molar ratio	Type of span	Span to cholesterol molar ratio	%EE ± S. D.
1	10:1	40	90:10	90.4±2.66
2	10:1	40	80:20	91.8±1.79
3	10:1	40	70:30	88.8±3.1
4	20:1	40	90:10	91.6±3.2
5	20:1	40	80:20	92.4±3.58
6	20:1	40	70:30	90.8±2.66
7	40:1	40	90:10	89.9±4.53
8	40:1	40	80:20	90.2±2.48
9	40:1	40	70:30	86.3±3.83
10	10:1	60	90:10	91.9±3.4
11	10:1	60	80:20	91.2±3.66
12	10:1	60	70:30	80 ±2.69
13	20:1	60	90:10	92.2±3.56
14	20:1	60	80:20	91.5 ±2.87
15	20:1	60	70:30	87.3±4.22
16	40:1	60	90:10	84.2±4.9
17	40:1	60	80:20	83.7±3.95
18	40:1	60	70:30	81.6±5.1

showed that EE% was much lower (61%) than the EE% obtained in the current study (90%).

As shown also in **Table 2**, incorporation of CH into span 60 niosomes (90:10 & 80:20) for span 40 considerably enhanced curcumin EE. However, beyond this amount (70:30), a decrease in EE was observed. The increase in EE can be rationalized by proposing that CH increases the microviscosity of the membrane by abolishing the gel-to-liquid phase transition of the surfactant bilayer, resulting in a more stable and hydrophobic bilayer that retards permeation and prevents leakage of hydrophobic drugs entrapped in the bilayer. In contrast, subsequent intercalation of CH would reduce drug entrapment by competing with the drug for the bilayer, thus preventing incorporation of the amphiphilic or lipophilic drug into the vesicles [55].

Preparation of curcumin loaded bile salts reached niosomes

The addition of 10% of SDC (bile salt) has successfully produced bilosomes. The %EE of the produced formulae were listed in **Table 3**. According to obtained data, the addition of 10% of SDC enhanced the %EE to 92.4%, when the L/D ratio was 20, and span to cholesterol molar ratio was 80:20 in case of span 40, while the %EE was enhanced to 92.2% when span 60 was employed, the L/D ratio was 20, and span to cholesterol molar ratio was 90:10. The enhancement of entrapment efficiency could be attributed to the formation of a more favorable hydrophobic region for the highly lipophilic curcumin. Further studies will be conducted for characterization of formed bilosomes.

Overall, as shown in **Table 3**, two formulations of curcumin-loaded niosomes gave a suitably high %EE. *Formula 5* composed of 20:1 lipid to drug molar ratio (span 40), 90:10 lipid to CH molar ratio and 10% bile salt and *Formula 13* composed of 10:1 lipid (span 60), 80: 20 Lipid: CH with 10% bile salt.

CONCLUSION

From all obtained results, we can conclude that the formulation of curcumin-loaded niosomes with the thin-film hydration method where span 40 or span 60 are used as surfactant enhances the %EE of curcusomes which in turn improves the poor solubility and bioavailability of curcumin. Moreover, the addition of bile salts enhanced the %EE which will be discussed furtherly. It is highly recommended to discuss the effect of bilosomes on enhancing oral delivery of curcumin.

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Conflict of interest:

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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