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Increasing bioavailability of silymarin using a buccal liposomal delivery system: Preparation and experimental design investigation

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Abstract

Silymarin is a natural lipotropic agent of low bioavailability from oral products. The aim of our study is to prepare buccal liposomal delivery system of silymarin with higher bioavailability. The effect of lecithin:cholesterol molar ratio on the percentage drug encapsulated was investigated. The influence of fluctuating the amount of added drug was also determined. The effect of additives such as positive charge inducer, negative charge inducer and surfactants was studied using two different 2^3 full factorial designs. Furthermore, additives used to optimize liposomal product were also investigated for their optimal concentrations, release properties and in vitro permeation and absorption through chicken cheek pouch.

Optimal liposomal encapsulation efficiency was found at 7:4 lecithin to cholesterol molar ratio. A decrease in entrapment efficiency with increasing cholesterol content was observed. Tween 20 or Tween 80 beyond 0.5 molar ratio decreased the entrapment efficiency. Positively charged liposomes showed superior entrapment efficiency over neutral and negatively charged liposomes. Release studies as well as permeation and absorption studies showed that hybrid liposomes prepared according to formula 3 containing lecithin, cholesterol, stearyl amine and Tween 20 in 9:1:1:0.5 molar ratio, respectively, gave the best drug absorption and permeation. It showed steady state permeation through chicken cheek pouch for 6 h. This is expected to improve the bioavailability of silymarin in the developed liposomal buccal delivery system, as the results show an increase in drug penetration compared to free drug powder.

Keywords: Liposomes; Silymarin; Hybrid liposomes; Transmucosal delivery system; Experimental factorial design; Bioavailability

1. Introduction

Derivatives of milk thistle (*Silybum marianum*) have been used as herbal remedies for almost 2000 years. They are currently flourished as a reemergening therapy for liver diseases among other natural remedies which become increasingly popular in the United States. Their use has been widespread throughout Europe since preparations became officially available for clinical use in 1969 (Flora et al., 1998).

Silybin (SBN), isosilybin (ISBN), silycristin (SCN), silydianin (SDN) and taxifolin (TXF) are the main active flavonoids commonly found in the dried fruits of *S. marianum*. Concentrations of these compounds, except TXF, are usually expressed together as silymarin content (Campodonico et al., 2001). According to many authors silymarin does not possess high bioavailability (Madaus et al., 1976; Gabetta et al., 1988; Comoglio et al., 1995; Blumenthal et al., 2000; Wachter and Zaeske, 2000; Giacomelli et al., 2002). Silymarin absorption rate levels are between 20 and 50% (Blumenthal et al., 2000). In fact, this poor bioavailability could be attributed to degradation by gastric fluid (Blumenthal et al., 2000), poor enteral absorption (Comoglio et al., 1995; Giacomelli et al., 2002) or its poor water solubility (Madaus et al., 1976; Gabetta et al., 1988; Blumenthal et al., 2000; Wachter and Zaeske, 2000). As a result, silymarin needs to be incorporated in a dosage form that increases its bioavailability. Trials were reported using cyclodextrin (Valcavi et al., 1993), salts of polyhydroxyphenylchromanones (Madaus et al., 1976), soluble derivatives (Giorgi et al., 1989) or complexes with phospholipids (Gabetta et al., 1988) to ameliorate its bioavailability. This work aims at using liposomes incorporated with silymarin in a buccal dosage form in order to enhance its bioavailability.

The oral cavity has been shown to be an attractive site for drug delivery due to ease of administration and avoiding possible

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drug degradation in the gastrointestinal tract as well as first-pass metabolism (Hao and Heng, 2003; Cafaggi et al., 2005). Excellent accessibility, high patient acceptance and compliance are attractive features of buccal mucosa (Senel and Hincal, 2001; McIntyre et al., 2005; Schaff et al., 2005).

Liposomes have been investigated since 1970 as a system for the delivery or targeting of drugs to the specific sites in the body. Some liposomal drug delivery systems exhibit superior pharmacological properties to those observed with conventional formulations (Foradada and Estelrich, 1995). Activity of liposomes as a carrier for drugs depends upon various factors such as charge, rigidity, composition of the liposomal membrane, encapsulating efficiency, stability, release rates and body distribution after administration (Choudhari et al., 1994; Galovic Rengel et al., 2002; Karathanasis et al., 2005; Nii and Ishii, 2005). Liposomes can be formulated from a variety of lipid and lipid mixtures with different compositions (Aranda et al., 2005). They can be modified in particle size, structure and surface charge to obtain desirable physicochemical properties to suit particular needs (Law and Hung, 1998).

Instability of liposomes composed of lipids with low transition temperature as PC (egg phosphatidylcholine) and DMPC (dimyristoyl phosphatidylcholine) in presence of cholate was investigated (Kokkona et al., 2000). It was shown that the addition of cholesterol does not substantially increase the encapsulated molecules retention. Nevertheless, liposomes composed of lipids with high transition temperature, retain significantly higher amounts of encapsulated material (Kokkona et al., 2000). Accordingly, liposomes were prepared in this study using soybean lecithin, containing both low and high transition temperature lipids (Vemuri and Rhodes, 1995), in addition to cholesterol in a buccal liposomal drug delivery system. The introduced buccal formula avoids the instability problems that materialize in the intestinal tract. It is worth mentioning that liposomes show mucoadhesive properties especially the positively charged ones due to the suggested ionic interaction with the negative charge of the mucus layer (Takeuchi et al., 2003).

Hybrid liposomes (HLs) composed of vesicular and micellar molecules have attracted special interests as a safe drug carrier for medical applications (Yamamoto et al., 2002). Furthermore, it has been reported that the hybrid liposomes have no cytotoxicity for normal tissue (Iwamoto et al., 2005). Investigation of this hybrid form in comparison to convential liposomes was carried out in this study using a factorial experimental design.

Accordingly the objective of this study is to improve the bioavailability of silymarin through its incorporation in a liposomal dosage form for buccal administration, using commercially available soybean lecithin. This is expected to avoid instability problems which commonly arouse in the gastrointestinal tract and to improve the poor aqueous solubility of silymarin thus providing a superior dosage form. Also the combination of silymarin with lecithin is intended to increase the permeation of silymarin through the buccal mucosa thus enhancing its bioavailability leading to a high reproducible pharmacological effect.

Thus the first part of this study consisted in investigating the effect of lecithin:cholesterol molar ratio and changing the amount of added drug on the encapsulation efficiency measured by the percentage drug encapsulated. The second part of our study was conducted using double experimental full factorial designs (2^3) for positively and negatively charge inducers separately with other surfactants as Tween 80 and Tween 20. Further investigations were proceeded in order to determine the optimal concentration of certain additives used to optimize liposomal delivery system. In vitro permeation study was carried on using chicken buccal pouch to investigate the permeation properties of certain formulae.

2. Materials and methods

2.1. Materials

Lecithin Soya powder (L) was kindly provided by EIPICo (Cairo, Egypt). Cholesterol (Ch) was purchased from Sigma Chemical Co. (USA). Stearylamine (SA) and dicetylphosphate (DP) were obtained from Fluca Chemical Co. (Germany). Silymarin was kindly supplied by CID Co. (Cairo, Egypt). Tween 20 (T 20), Tween 80 (T 80), methanol, chloroform, diethylether, sodium chloride, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from El-Nasr Chemical Co. (Cairo, Egypt). Spectra/Pore® dialysis membrane (12,000–14,000 molecular weight cut off) was purchased from Spectrum Laboratories Inc. (USA).

2.2. Preparation of liposomes

Silymarin liposomes were prepared using the reverse evaporation technique (Suzoka and Papahadjopoulos, 1978). The lipid components used in the different liposomal products were weighed and dissolved in 10 ml chloroform. The organic solvent was evaporated using a rotary evaporator (Buchi R-110 Rotavapor, Switzerland) to produce a thin lipid film. The lipid film was redissolved in 10 ml ether, and the silymarin solution in 10 ml acetone together with 10 ml distilled water was added. Then the organic solvents were evaporated on the rotary evaporator. The liposomal suspension was kept overnight in the refrigerator. On the following day, the liposomal suspension was filtered through sintered glass filter no. 3 and kept in refrigerator. The drug recovery was well over 95% in all cases.

The formation of liposomes was verified through their morphological aspect, which was examined using transmission electron microscope (ZEISS, EM 10, Germany).

2.3. Determination of silymarin entrapment efficiency in liposomes

The proportion of encapsulated silymarin was determined by centrifugating a certain volume of the collected filtrate of liposomal suspension at 15,000 rpm for 1hr at 4 °C. The liposomes were separated from the supernatant and sonicated with methanol to measure the encapsulated silymarin content at $\lambda = 288$ nm, which is the maximum absorption of silymarin in

methanol (O'Neil et al., 2001)

$$\%E = \frac{\mathrm{ED}}{\mathrm{AD}} \times 100$$

where $\%E$ is the percent encapsulation efficiency, AD the amount of added drug and ED is the amount of encapsulated drug. The percent encapsulation was determined to evaluate:

(a) Effect of lecithin:cholesterol molar ratio on $\%E$ using different molar ratios of L:Ch of 10:0, 9:1, 7:2, 7:4 and 7:6 with constant added silymarin content.
(b) Effect of drug content using molar ratio of L:Ch of 7:4 while increasing amount of added silymarin.
(c) Improving physical properties of redistribution and stability by adding positive charge inducer (stearyl amine), negative charge inducer (dicetylphosphate), Tween 20, and Tween 80 in one molar ratio to the formula L:Ch of 9:1 molar ratio. The effect of such additives was studied through two full 2^3 factorial designs for each type of charge inducer. Results were statistically evaluated. The two levels were considered as presence or absence of the factor.
(d) Different formulae which were prepared to evaluate the effect of changing the molar ratio of certain additives (stearyl amine, dicetyl phosphate, Tween 20).

2.4. In vitro release of silymarin from liposomes

The in vitro release of silymarin from liposomes was measured using dialysis method. Dialysis method was applied using a Spectra/Por® dialysis membrane of 12,000–14,000 MWCO (El-Gazayerly and Hikal, 1997). This membrane assures the permeation of the drug and at the same time retains liposomal forms.

An accurately measured amount of silymarin liposomal dispersion, equivalent to 1 mg silymarin, was transferred to a glass cylinder having the length of 10 cm and diameter of 2.5 cm fitted with presoaked membrane. It was placed in a receiving compartment containing 50 ml phosphate buffer saline (pH 7.4). This volume provides complete sink conditions for the drug as the saturated solubility of silymarin was found to be 0.526 mg/ml in this medium. The whole set is placed on a magnetic stirrer adjusted to a constant speed of 150 rpm at 25 °C. At predetermined time intervals (0.5, 1, 1.5, 2, 3, 4, 5 and 6 h); 4 ml of the release medium were withdrawn for analysis. Withdrawn samples were compensated by phosphate buffer saline (pH 7.4). The samples were measured spectrophotometerically at $\lambda = 326$ nm as this wavelength represents the maximum absorption of silymarin in phosphate buffer saline (pH 7.4) (Maheshwari et al., 2003).

2.5. In vitro drug absorption and permeation

The chicken cheek pouches (Tan et al., 2000; El-Samaligy et al., 2004) were excised and washed in isotonic buffer (pH 7.4). The open cheek pouch mucosa, with the mucosal surface facing up, was placed between two compartments. The upper compartment contains the liposomal product and the lower one contains isotonic buffer. The exposed surface of the mucosa was 1.23 cm^2. Samples of 1 mg of liposomal silymarin formulae or silymarin suspension (composed of pure drug suspended in distilled water) were applied on the mucosal surface in the donor compartment and receiving compartment was 50 ml of isotonic buffer. Sink conditions were assured by frequently taking samples which were replaced with isotonic buffer and by continuous stirring on a magnetic stirrer at 150 rpm. Aliquots (4 ml) were removed from the receiving compartment every 0.5, 1, 1.5, 2, 3, 4, 5 and 6 h. The samples were measured spectrophotometerically at $\lambda = 326$ nm.

Three formulae with the highest $\%E$ were chosen for this study, namely formulae 1–3 composed of L:Ch:SA:T 20 of the molar ratios 9:1:0:0; 9:1:1:0; and 9:1:1:0.5, respectively. For those three formulae, drug suspension was used as a control in order to be able to observe the effect of each formula on the permeation in comparison with the permeation of drug alone in suspension.

2.6. Laser light scattering measurement

The vesicle size distribution was determined using a laser diffraction technique on a Mastersizer X, at 25 °C. The measurements were performed using a 45 mm focus objective and a beam length of 2.4 mm.

2.7. Statistical analysis

A 2^3 factorial design was chosen for a part of this formulation improvement. The formula chosen to be investigated in this part of the study was L:Ch in 9:1 molar ratio. The parameters studied in this design included: (1) presence or absence of charge inducer, (2) presence or absence of Tween 80, (3) presence or absence of Tween 20.

As positively and negatively charge inducers could not be combined in one factorial design, we used two separate designs in order to explore their effects. The used factorial designs are shown in Tables 1a and 1b which show design for positive and negative charge inducers, respectively.

ANOVA-factorial was performed using statview® software to achieve analysis of variance for three factor design with

Table 1a
Qualitative combination of ingredients used to prepare silymarin liposomes according to the first 2^3 factorial design

	Presence of SA (a)		Absence of SA	
	Presence of T 80 (b)	Absence of T 80	Presence of T 80 (b)	Absence of T 80
Presence of T 20 (c)	abc	ac	bc	c
Absence of T 20	ab	a	b	(1)

Table 1b
Qualitative combination of ingredients used to prepare silymarin liposomes according to the second 2^3 factorial design

	Presence of DP (a)		Absence of DP	
	Presence of T 80 (b)	Absence of T 80	Presence of T 80 (b)	Absence of T 80
Presence of T 20 (c)	abc	ac	bc	c
Absence of T 20	ab	a	b	(1)

triple replicates. Difference at $P < 0.05$ was considered to be significant.

All liposomal products prepared in this work were evaluated according to their %*E* or their release percent after 5 h. Differences were evaluated for significance using one-way ANOVA followed by LSD test or independent samples *t*-test using SPSS® software. Difference at $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Factors affecting entrapment efficiency and in vitro release

Fig. 1 shows the produced liposomes prepared by reverse phase evaporation method. As observed, they are well-identified perfect spheres and they exist in disperse and aggregate collection. Drug encapsulation in different molar ratios of L:Ch is shown in Fig. 2. A one-way ANOVA test followed by LSD test showed that there was no significant difference in %*E* between the 10:0, 9:1, 7:2 and 7:4 followed by a decrease in %*E* at the ratio 7:6 ($P = 0.014$). Decreasing entrapment efficiency with increasing cholesterol ratio above a certain limit may be due to the fact that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of the liposomal membrane (New, 1990). Thus, the ratios 10:0, 9:1, 7:2 and 7:4 were the most efficient but by repeating the experiment it could be deduced that the ratios 9:1 and 7:4 were the most reproducible regarding %*E*.

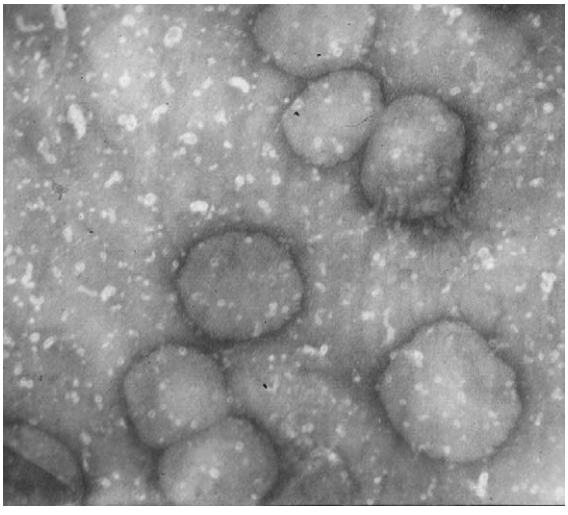

Fig. 1. Transmission electron microphotograph of silymarin liposomes composed of L:Ch:SA:T 20 (9:1:1:0.5) molar ratio. Magnification 30,000×.

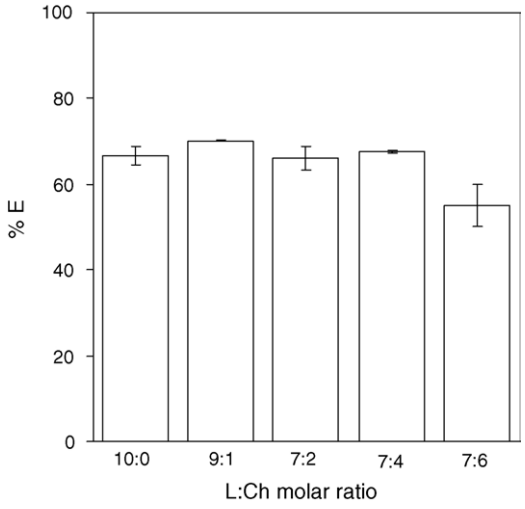

Fig. 2. Effect of cholesterol on silymarin entrapment efficiency (%*E*) ($n = 3$).

Fig. 3 shows that formula composed of 9:1 lecithin: cholesterol molar ratio showed the lowest extent after 5 h followed by 10:0 then 7:2, 7:4 and 7:6 formulae. The decrease in the extent of release in case of 9:1 lecithin:cholesterol molar ratio was statistically significant having $P = 0.011$, 0.002, 0.001 and <0.001 with formulae containing lecithin:cholesterol at 10:0, 7:2, 7:4 and 7:6 molar ratio, respectively. While extent of release after 5 h showed by liposomes composed of lecithin only did not show a significant difference from that shown by 7:2 lecithin:cholesterol molar ratio ($P = 0.065$). In comparison with liposomal formulae having lecithin:cholesterol of 7:4 or 7:6 molar ratios, liposomes composed of lecithin only showed

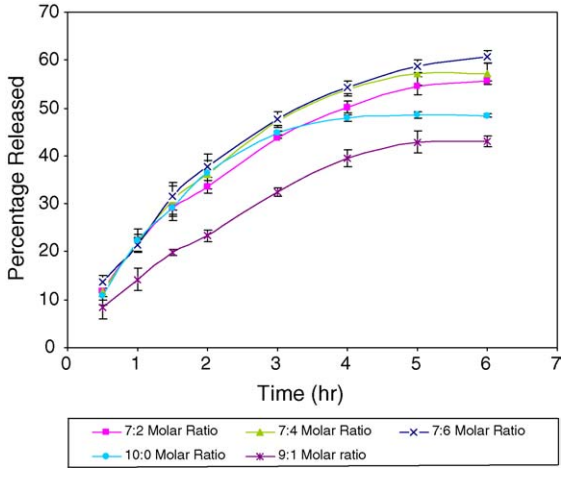

Fig. 3. Effect of cholesterol on release profile of silymarin from liposomal formulae composed of different lecithin:cholesterol molar ratios ($n = 3$).

a significant decrease in release extent ($P = 0.013$ and 0.006, respectively). Lecithin:cholesterol molar ratio of 7:2 did not show a significant difference in release extent when compared with formulae containing 7:4 or 7:6 lecithin:cholesterol molar ratio showing $P = 0.227$ and 0.079, respectively. Furthermore, release extents of formulae lecithin:cholesterol 7:4 and 7:6 was statistically not significant ($P = 0.453$).

Incorporation of cholesterol in a liposomal formulation composed of lecithin:cholesterol (9:1 molar ratio) decreased the release extent of the drug in comparison with liposomes composed only from lecithin. This can be due to the fact that the presence of cholesterol in liposomal preparations reduces the leakage or permeability of encapsulating material by decreasing membrane fluidity (Betageri, 1993). However, increase in the molar ratio of cholesterol significantly increased the release of silymarin and that may be due to the fact that increasing cholesterol beyond a certain concentration can disrupt the regular linear structure of the liposomal membrane (New, 1990). It can be concluded that the liposomal formula composed of lecithin:cholesterol molar ratio of 9:1 is advantageous for further investigation using other surfactants. Cholesterol in this molar ratio produced an optimum hydrophobicity that decreased the formation of the transient hydrophilic holes, by decreasing membrane fluidity, responsible for drug release through liposomal layers (Cocera et al., 2003). This property can help the liposomes to be stable in presence of other surfactants.

Changes in %*E* with increasing the amount of silymarin added are shown in Fig. 4. The amount of entrapped silymarin in mg increased from 5.87 ± 0.49 to 20.27 ± 0.11 to 30.5 ± 1.55 with increasing amount of added silymarin from 10 to 30 to 50 mg in the formulation step. The increased amount of encapsulated silymarin with increasing the amount of silymarin added during the formulation may be due to the increased saturation of the media with the silymarin that forces silymarin to be encapsulated into the liposomes. This increase was found non-significant when computed as percent entrapment ($P = 0.15$).

Evaluation of the effect of different additives on liposomal encapsulation efficiency was based on the results of the two full 2^3 factorial designs (Tables 2a and 3a) and their statistical evaluation (Tables 2b and 3b).

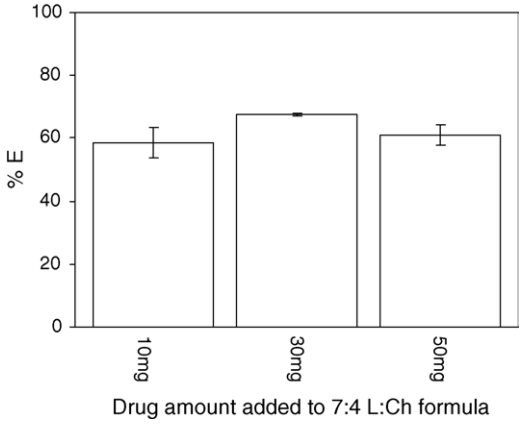

Fig. 4. Effect of silymarin content on silymarin entrapment efficiency (%*E*) ($n = 3$).

Table 2a
Results of 2^3 factorial design using stearyl amine, Tween 80 and Tween 20 for liposomes containing L:Ch in 9:1 molar ratio

Exp.	Molar ratios of ingredients			%*E*
	SA	T 80	T 20	
(1)				70.18 ± 0.21
a	1			58.85 ± 3.18
b		1		37.12 ± 3.18
ab	1	1		48.80 ± 3.58
c			1	43.01 ± 3.31
ac	1		1	56.90 ± 3.51
bc		1	1	22.64 ± 1.36
abc	1	1	1	33.38 ± 0.26

Table 2b
Results of 2^3 factorial design using dicetyl phosphate, Tween 80 and Tween 20 for liposomes containing L:Ch in 9:1 molar ratio

Exp.	Molar ratios of ingredients			%*E*
	DP	T 80	T 20	
(1)				70.18 ± 0.21
a	1			58.63 ± 3.10
b		1		37.12 ± 3.18
ab	1	1		20.28 ± 0.78
c			1	43.01 ± 3.31
ac	1		1	39.68 ± 2.69
bc		1	1	22.64 ± 1.36
abc	1	1	1	18.83 ± 1.60

Evaluation of the effect of different additives on silymarin liposomal encapsulation efficiency through the first full factorial design system shows that addition of positive charge inducer improved the encapsulation of silymarin. This last finding may be due to the interaction between the positively charged phos-

Table 3a
Effect of stearyl amine, Tween 80 and Tween 20 and their interactions on %*E* following 2^3 factorial design

	Effect	*P*
T 20	−14.743	<0.0001
T 80	−21.763	<0.0001
T 20 × T 80	−0.180	0.8957
SA	6.258	0.0017
SA × T 20	6.060	0.0020
SA × T 80	4.970	0.0061
SA × T 80 × T 20	−6.550	0.0013

Table 3b
Effect of dicetyl phosphate, Tween 80 and Tween 20 and their interactions on %*E* following 2^3 factorial design

	Effect	*P*
T 20	−15.500	<0.0001
T 80	−28.170	<0.0001
T 20 × T 80	7.560	0.0002
DP	−8.870	<0.0001
T 20 × DP	5.300	0.0018
T 80 × DP	−1.430	0.2525
T 20 × T 80 × DP	1.190	0.3348

pholipids and the negative centers in the drug (El-Gazayerly and Hikal, 1997). Presence of either Tween 80 or Tween 20 in one molar ratio produced a decrease in drug encapsulation which may be due to mixed micelles formation that leads to vesicles destruction (Maza and Parra, 1997). Presence of stearyl amine molecules within the phospholipids vesicles could be more preferred than Tween molecules due to the advantageous similarity between the structure of phospholipids and stearyl amine than that between it and Tween molecules (Vemuri and Rhodes, 1995; Uchegbu and Vyas, 1998). Therefore, according to the structure of hybrid liposomes (Yamamoto et al., 2002), presence of positively charge inducer (stearyl amine) with the negatively charged drug (silymarin) can pack the layers of hybrid liposomes in a better way. This packing may reject the presence of large amount of assembled Tween 20 or Tween 80 molecules within the phospholipids vesicles. This rejection could explain the interaction between SA and T 20 or between SA and T 80 that causes significant increase in encapsulation efficiency. The interaction between SA and T 20 and T 80 could be explained as a result of the presence of two molar ratio of Tween (1 mol T 20 and 1 mol T 80) that force their monomers to be incorporated into the liposomal structure and hence disrupt its vesicles and decreasing the encapsulation efficiency.

In the second factorial, dicetyl phosphate introduced negative charge to the lipid layers that produces a repulsion force with the drug causing a decrease in entrapment efficiency (Khalil et al., 1992). Presence of Tween 80 or Tween 20 produced a decrease in the entrapment efficiency as it was also observed in the first factorial. The significant interaction between T 20 and T 80 may be explained by that the monomers of both surfactants reached their critical micelle concentration (CMC) and so their molecules were associated in a vesicular structures. These vesicular structures cause an entrapment of some drug molecules that showed the effect of increasing the encapsulation efficiency (Carafa et al., 1998). Tween 20, instead of being incorporated into the micelles, forms vesicles in the presence of dicetyl phosphate and cholesterol (Carafa et al., 1998; Uchegbu and Vyas, 1998). These vesicles may incorporate phospholipids molecules (Choudhari et al., 1994) and increase entrapment efficiency revealed by the interaction between T 20 and DP. A comparison between neutral, positively and negatively charged liposomes in %*E* is shown in Fig. 5.

Table 4 results were analyzed using one way ANOVA followed by LSD test or independent samples *t*-test using SPSS® software to show the effect of additives molar ratios on %*E* in the formulae prepared using L:Ch (9:1) molar ratio. Increasing the molar ratio of stearyl amine or dicetyl phosphate from 0.5 to 1 molar ratio did not produce a significant change in %*E* ($P = 0.88$ and 0.902, respectively). Significant decrease in %*E* was observed upon raising the molar ratio of stearyl amine or dicetyl phosphate to 2 molar ratio ($P = 0.012$ and 0.003, respectively). The decrease in entrapment efficiency on raising the molar ratio of dicetyl phosphate to 2 molar ratio may be explained by the increase in the repulsion between the negatively charged phospholipids and the negative centers in the drug structure. The decrease in silymarin encapsulation efficiency upon increasing stearyl amine to 2 molar ratio could be explained by the destruction of liposomal vesicles on increasing surfactant concentration.

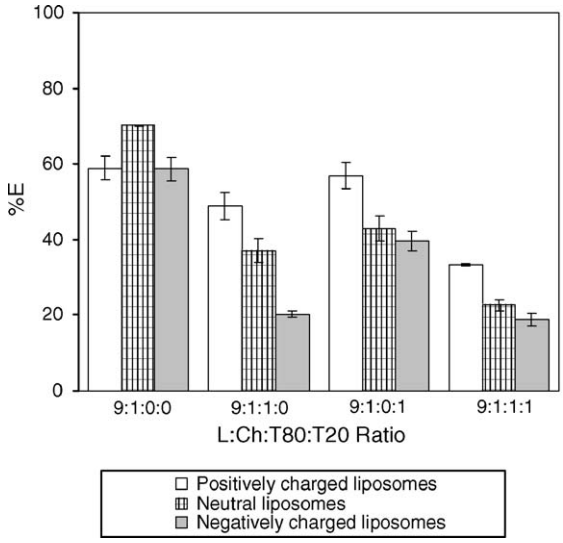

Fig. 5. Effect of charge on silymarin entrapment efficiency (%*E*) ($n = 3$).

Decreasing the molar ratio of Tween 20 from 1 to 0.5 molar ratio in the formula L:Ch:SA (9:1:1) produced a significant increase in %*E* ($P = 0.045$). This increase in the encapsulation efficiency could be attributed to reacquiring the ability to form vesicles to produce hybrid liposomes. On the other hand, decreasing the molar ratio of Tween 20 by the same extent in the formula L:Ch:DP (9:1:1) did not produce a significant effect in %*E* ($P = 0.237$).

Figs. 6 and 7 show the effect of change in charge inducing agent on the release profile of some silymarin liposomal formulae. Increasing molar ratio of dicetyl phosphate from 0.5 to 1 significantly decreased drug release extent after 5 h ($P = 0.015$). Further increase of dicetyl phosphate to 2 molar ratio significantly increased the rate and extent of silymarin released ($P = 0.001$). Increasing molar ratio of stearyl amine from 0.5 to 1 mole did not significantly affect the release extent ($P = 0.082$). Meanwhile further increase in stearyl amine to reach 2 mol significantly increased release extent ($P = 0.004$).

Table 4
Effect of additives in different molar ratios on liposomal encapsulation efficiency ($n = 3$)

Liposomal products having L:Ch 9:1 molar ratios containing			%*E*
DP[a]	SA[a]	T 20[a]	
2.0			30.48 ± 0.2
1.0			58.63 ± 3.5
0.5			59.05 ± 4.5
	2.0		39.02 ± 3.3
	1.0		58.87 ± 2.8
	0.5		59.05 ± 4.5
	1.0	1.0	56.90 ± 3.5
	1.0	0.5	69.22 ± 0.6
1.0		1.0	39.68 ± 2.9
1.0		0.5	34.83 ± 3.0

[a] Molar ratios.

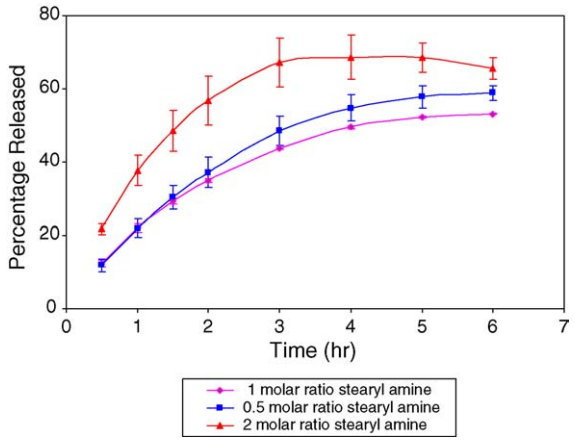

Fig. 6. Release profile of silymarin from liposomal formulae composed of L:Ch (9:1) at different molar ratios of stearyl amine ($n = 3$).

Decreasing the drug release extent on increasing molar ratio of dicetyl phosphate from 0.5 to 1 molar ratio could be due to decreased ability of the drug possessing negative centers, to cross the negatively charged phospholipids layers. However, increasing stearyl amine or dicetyl phosphate to 2 molar ratio increased silymarin release extent. This could be attributed to the layers instability upon addition of large amounts of charge inducer that may cause repulsion among charged layers (Kulkarni et al., 1997).

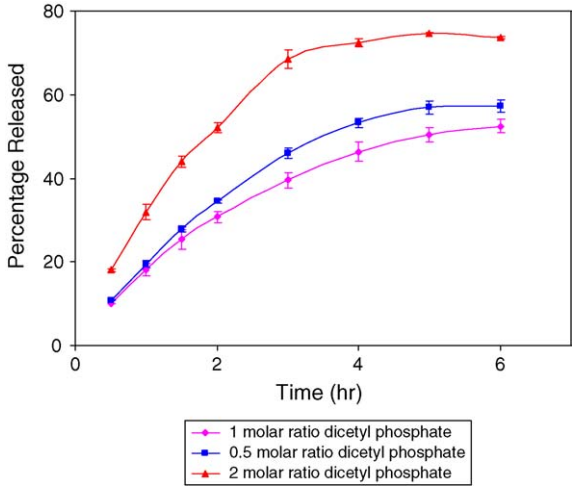

Fig. 7. Release profile of silymarin from liposomal formulae composed of L:Ch (9:1) at different molar ratios of dicetyl phosphate ($n = 3$).

3.2. In vitro drug absorption and permeation

Permeation profile of silymarin from its suspension relative to liposomal formulae 1–3 through chicken cheek pouch are represented in Fig. 8. The extent of drug permeation after 6 h from products of formulae 1 and 2 was not significantly different relative to that from silymarin suspension ($P = 0.072$ and 0.131, respectively). Formula 3 produced a significant increase in the permeation of silymarin through chicken pouch ($P = 0.013$).

Incorporation of silymarin into neutral (formula 1) or positive liposomes (formula 2) did not produce an enhancement effect

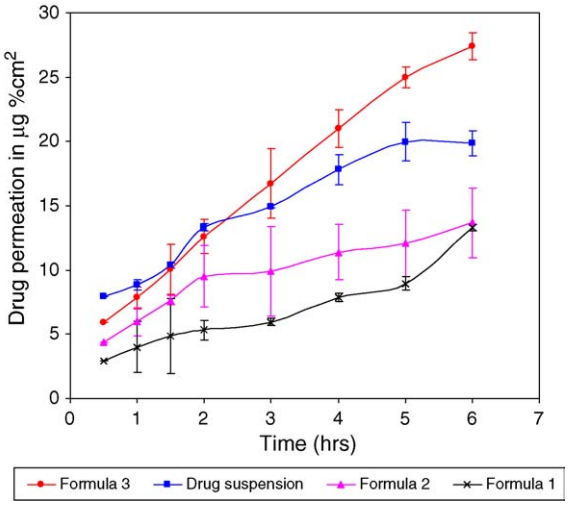

Fig. 8. Permeation profile of silymarin through chicken cheek pouch from liposomal formulae 1–3 in comparison to its suspension ($n = 3$).

on the penetration through excised buccal pouch. This may be explained by the fact that dead buccal pouch cells are not capable of undergoing the endocytosis process which could enhance the permeation of liposomes in vivo (Chen and Langer, 1998). Incorporation of Tween 20 into liposomes produced an enhancement effect and increased the permeation of silymarin. Hence hybrid liposomes composed of lecithin, cholesterol, stearyl amine and Tween 20 are expected to produce better bioavailability than convential liposomes in vivo.

3.3. Laser light scattering measurement

The hybrid liposomal formula of choice composed of L:Ch:SA:T 20 having 9:1:1:0.5 molar ratio showed a mean particle size of 800 nm and a span of 2.031 which is larger than that observed with convential liposomes (formula 2) prepared by the same method (mean particle size of 710 nm and span = 3.619). On the other hand these hybrid liposomes containing Tween as edge activator were able to be ultradeformable enabling them to penetrate the buccal membrane and enhance silymarin penetration (Essa et al., 2002).

4. Conclusion

This study of silymarin encapsulated liposomes revealed an amelioration in the encapsulation efficiency upon increasing amount of added drug in the preparation. Addition of cholesterol beyond a certain limit produced a decrease in encapsulation efficiency. Studying the effect of certain additives and their interactions using two full 2^3 factorial designs enabled the determination of certain enhancement or decrease in encapsulation efficiency according to the additive. Addition of stearyl amine as a positively charge inducer was capable of enhancing the encapsulation efficiency. Tween 20, Tween 80 and dicetyl phophate in one molar ratio decreased the encapsulation efficiency. Molar ratios of some ingredients were explored to determine best encapsulation efficiency. In vitro permeation study

through chicken cheek pouch of hybrid liposomes containing L:Ch:SA:T 20 of 9:1:1:0.5 molar ratio showed superior permeation results compared with neutral or positively charged liposomes.

Liposomes were able to incorporate silymarin as a full spectrum product containing the different components of the extract. Formulation of silymarin in a liposomal dosage form intended to be used buccaly could provide the desired requirement of higher bioavailability and consequently lower dose. This formula, containing safe ingredients, being used in a buccal spray liposomal dosage form, can supply the need of silymarin to children who suffer from liver diseases.

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