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Short Communication

Effect of formulation variables on drug release from bilosomes; effect of cholesterol concentration

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ABSTRACT

Bilosomes are bile salts-containing vesicles that have recently drawn attention as a novel nanocarrier for drugs. Compared to traditional nanocarriers, bilosomes have the advantage of being able to withstand disruption caused by physiological bile salts normally secreted in the gastrointestinal tract. In addition to nonionic surfactants and bile salts, cholesterol is one important ingredient in bilosomes composition. This work investigated the effect of cholesterol concentration on the release rate of the entrapped drug. Tamoxifen was used as a model drug that suffers from poor oral bioavailability due to poor solubility and extensive pre-systemic degradation. Bilosomes composed of Span 60, cholesterol, and bile salts were prepared. Cholesterol was used at two different concentrations of 0.4% and 0.8% w/v, producing formulations BiL1 and BiL2, respectively. The entrapment efficiency and in vitro drug release were evaluated using Franz diffusion cells. Increasing cholesterol concentration reduced drug release. The release efficiency values after 24 hours of release study were 9.7 and 6.8% for BiL1 and BiL2, respectively. This indicates that increasing cholesterol concentration increased the rigidity of the bilosomal membrane and enhanced drug encapsulation. Reduced release would indicate that the vesicles retain the encapsulated drug, which is advantageous, taken into consideration the lymphatic absorption of the vesicles.

Keywords: Nanocarriers, lymph drainage, bile salts, pre-systemic metabolism.

The oral route of drug delivery is one of the most convenient routes. However, the technology of oral drug delivery faces many problems, such as poor drug solubility and drug degradation through first-pass metabolism. Pre-systemic degradation can be overcome by employing the intestinal lymphatic pathway as the major route for drug delivery[1]. Nanocarrier systems, like liposomes, niosomes, etc., can be used as a useful tool for drug transport through the intestinal lymphatic system. The lymphatic system delivers the absorbed entities to the systemic circulation, bypassing, therefore, the extensive hepatic first-past degradation of drugs. This strategy was a successful tool for increasing the bioavailability of many drugs that suffered from pre-systemic metabolism problem[s].

Bile salt-containing vesicles (bilosomes) is a novel form of the vesicular nanocarrier. Due to its amphoteric nature, bile salt molecules are embedded within the lipidic (in case of liposomes) or nonionic surfactant (in case of niosomes) bilayers[3]. As bile salts are naturally produced (i.e., endogenous) surfactants in the human body, their incorporation in bilosomes imparts many benefits to the vesicles, such as biodegradability and minimal toxicity.

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Compared to other traditional nanocarriers such as niosomes or liposomes, bilosomes can improve the oral bioavailability of many drugs due to their ability to withstand destruction by gastrointestinal tract bile salts[4].

Nonionic surfactants constitute the main ingredient of bilosomes. Span 60 (Sorbitan monostearate) is one of the commonly used surfactants in niosomes/bilosomes preparation as it provides physical stability under a wide range of temperatures owing to its relatively high phase transition temperature. Besides nonionic surfactant, cholesterol is an important component of bilosomes. Cholesterol content is important to control membrane permeability by increasing its rigidity, therefore reducing the incidence of vesicular aggregation and fusion processes[5]. It was reported that cholesterol content in the lipid nanocarriers should be carefully optimized based on the physico-chemical properties of the used surfactants as well as the loaded drugs[6].

Tamoxifen is a selective estrogen receptor modulator commonly indicated for the treatment of breast cancer. It is also prescribed for women as a prophylactic against the same disease. Unfortunately, the oral bioavailability of Tamoxifen is too low (about 20-30%) due to its low water solubility as it is categorized under Class II drugs by the Biopharmaceutical Classification System[7]. Importantly, pre-systemic metabolism is another major reason for poor oral bioavailability[8]. Therefore, it would be useful to try to improve Tamoxifen's oral bioavailability by encapsulating it into nanocarriers. Due to its stability against endogenous bile salts, bilosomes were considered the most suitable vesicular carrier that would reserve the entrapped drug. Therefore, the aim of this work was to prepare Tamoxifen bilosomal system and evaluate the effect of cholesterol concentration on the characteristics of the produced bilosomes.

The analysis of tamoxifen was performed using a UV spectrophotometric assay at 237nm. Tamoxifen was dissolved in ethanol to prepare a stock solution of 1mg/mL, from which series concentrations ranging from 5 to 25 µg/mL were prepared. The calibration curve was constructed by plotting absorbances as a function of concentrations **(Figure 1)**. The drug obeyed Beer-Lambert law with 'R^2' value of 0.997.

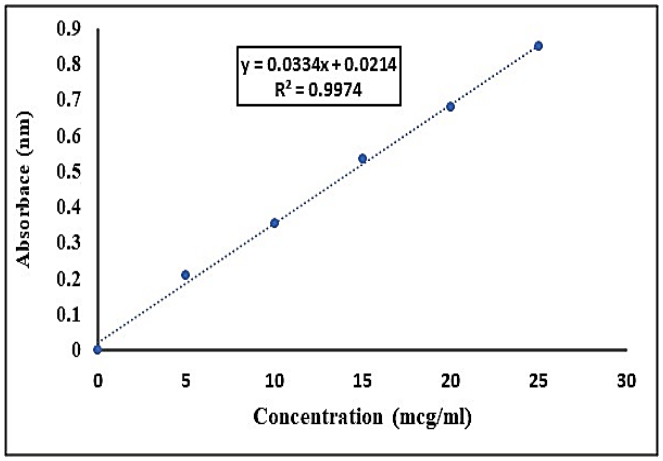

Figure 1: Calibration curve of Tamoxifen.

The main components of the bilosomes were Span 60, bile salts, and cholesterol at weight ratio 6:2:1 and 6:2:2, respectively **(Table 1)**. Vesicles were prepared employing a previously described technique by Sultan and coworkers[9]. Briefly, all ingredients were mixed and heated at 80°C till the clear liquid melted. Then, 25mg of Tamoxifen powder (enough to produce 1mg/mL concentration of the final colloidal dispersion) was mixed into the melted lipid phase. Cholesterol was used to give a concentration of either 0.4% w/v (BiL1) or 0.8%w/v (BiL2) of the final dispersion. Purified water (volume equal to that of ethanol) was then added while heating and thoroughly mixed until a homogenous dispersion was obtained. This produces a concentrated bilosomal dispersion, which is then hydrated with the remaining volume of water that is enough to produce 25 mL of bilosome dispersion. After overnight standing, the now swelled vesicles were downsized by bath sonication for 30 minutes (Elmasonic S 60H, Elma Schmidbauer GmbH, Gottlieb- Daimler, Germany).

The entrapment efficiency of Tamoxifen (i.e. amount of drug encapsulated within the vesicles) was determined indirectly by computing the unentrapped drug in the dispersion medium. In short, about 2 mL of each colloidal dispersion was centrifuged at 20,000 rpm for 90 minutes (SIGMA 3-30K centrifuge, Germany). The centrifugal temperature was adjusted to 26ºC (to avoid overheating). The supernatant was collected, and the concentration of free unentrapped Tamoxifen was determined spectrophotometrically[10]. The percentage of drug entrapped was calculated by subtracting the unentrapped drug from the total drug concentration, then dividing the outcome by the total drug concentration[9]. Entrapment efficiency was found to be 93.5±0.4 for formula BiL1. Duplication of cholesterol concentration in BiL2 slightly, but nonsignificant ($P>0.05$ using student t-test), increased drug entrapped. This coincides with previous research that reported increased enoxacin[11] and flurbiprofen[6] entrapment with increasing cholesterol content in niosomes.

The cumulative amount of Tamoxifen released for 24 hours was investigated employing Franz diffusion cells having a diffusional area of 2.27 cm^2. The previously soaked semipermeable membrane (MW cutoff 14000, Sigma Aldrich, USA) was mounted between the donner and receptor parts of each cell. After filling the receptor compartment with 0.02 N HCl (release medium), 2.0 mL of each bilosomal dispersion was placed in the donor part under occlusion (using aluminum foil). The cells were maintained at 37ºC using a thermos-stated water bath, and samples of 5 mL were withdrawn from the receptor liquid at scheduled time intervals for 24 hours. The withdrawn liquid was compensated by fresh

Table 1. Composition of bilosomes, together with release parameters.

	Span 60 (mg)	Cholesterol (mg)	Bile salts (mg)	Ethanol (mL)	Q2	Q24	RE (%)
BiL 1	600	100	200	1.0	2.3±0.10	16.9±0.9	9.7
BiL 2	600	200	200	1.0	1.4±0.14	12.8±1.4	6.8

These amounts are enough to produce 25 mL of bilosomes dispersion

medium to keep the same volume. The amount of Tamoxifen released was analyzed spectrophotometrically at 237 nm. The release profiles of formulations BiL1 and BiL2 were then obtained by plotting the cumulative drug released (expressed as a percentage) versus time **(Figure 2A)**.

The release parameters were selected as the amount released after 2 hours (Q2) and 24 hours (Q24). The efficiency of Tamoxifen released (RE) was calculated for the two profiles corresponding to the method described by Khan[12]. Release parameters are presented in (**Table 1)** and graphically represented as a histogram in (**Figure 2B)**.

Formula BiL1 containing 0.1 cholesterol released 2.3±0.1 of the loaded dose after 2 hours, with a total release of 16.9±0.9 at the end of the release study. The computed RE (%) was 9.7%. This could be due to the lipophilic nature of Tamoxifen, which dictates its preferential retention in the lipid bilayer of the vesicles. Increasing cholesterol concentration to 0.2 (BiL2) significantly ($P < 0.05$) reduced drug release.

This could be due to the membrane-stabilization effect of cholesterol. It was suggested that cholesterol molecules form hydrogen bonds with the alkyl chains of the surfactant molecules via their hydroxyl groups. This improves the stability of the vesicular bilayers with increased rigidity and, consequently, decreasing the release of the encapsulated dr ug[13].

This is advantageous, knowing that the prepared bilosomes are intended for oral administration with intact vesicular absorption in mind. Reduced drug release reflects the fact that most of the drug payload will be reserved within the vesicles while in the gastrointestinal tract. Additionally, the presence of bile salts is one of the main components that protects the vesicles against the degradation effect. The release kinetics were computed by fitting the release profiles to different kinetic models, and the R2 value reflected

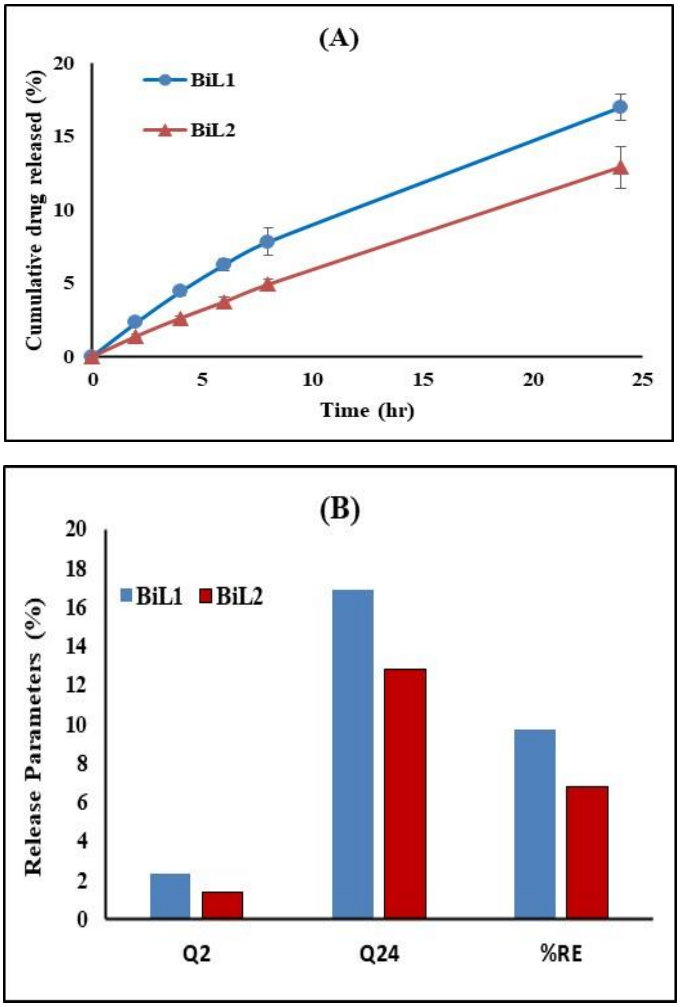

Figure 2: (A) Cumulative amount of Tamoxifen released from different bilosomes formulations (B) Release parameters represented as amount released after 2 hours (Q2), 24 hours (Q24), and percentage drug release efficiency (%RE). Detailed formulations are in (**Table 1)**.

by considering the multilayer structure of the obtained vesicles through which the entrapped drug may slowly diffuse, similar to diffusion out from the matrix system. Comparable results were formerly reported and similarly explained[14].

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