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Investigating the potential of employing bilosomes as a novel vesicular carrier for transdermal delivery of tenoxicam

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ABSTRACT

Bilosomes represent an evolving vesicular carrier that have been explored for oral vaccines delivery based on its ability to resist enzymes and bile salts in the gastrointestinal tract (GIT). Bilosomes vesicles are formed of bilayer membrane of non-ionic surfactant molecules encompassing bile salts. Although, bilosomes have not been proposed for transdermal drug delivery, this carrier seems to have promising potential in this regard. Accordingly, the aim of this investigation was to assess the capability and safety of utilizing bilosomes for transdermal delivery of tenoxicam (TX) as a model drug. A $3^1 2^2$ full factorial design was adopted to study the effects of different formulation parameters on bilosomes properties and select the optimal formulation using Design-Expert® software. The selected formulation displayed nano-sized spherical vesicles (242.5 ± 6.43 nm) with reasonable entrapment efficiency percent ($68.33 \pm 2.33\%$). Confocal laser scanning microscopy confirmed the capability of the flourolabeled bilosomes to penetrate deep within the skin. Both, *ex vivo* permeation and *in vivo* skin deposition studies confirmed the superiority of bilosomes over drug solution in delivering TX transdermally. In addition, *in vivo* histopathological study proved the safety of topically applied bilosomes. In summary, the highlighted results confirmed that bilosomes can be further adopted for delivering drugs transdermally.

1. Introduction

Recently, there has been an increasing interest toward delivering non-steroidal anti-inflammatory drug (NSAIDs) transdermally using various vesicular carriers such as liposomes (Jain et al., 2005; Szura et al., 2014), niosomes (El-Menshawe and Hussein, 2013), transferosomes (Duangjit et al., 2013, 2014) and ethosomes (Bragagni et al., 2012; Ghanbarzadeh and Arami, 2013) to circumvent the problems associated with their oral administration. Several mechanisms were reported to explain the ability of the aforementioned colloidal vesicles to enhance transdermal drug delivery. Since the stratum corneum (SC) is the key barrier for drug permeation and absorption into skin, its structure modification by vesicular carriers is one of the likely reasons for enhancing drugs permeation. It is reported that the SC intercellular lipid barrier becomes more loose and permeable when treated by these colloidal vesicles (Honeywell-Nguyen and Bouwstra, 2005), thereby, allowing the drug to easily diffuse across the SC, followed by systemic uptake *via* superficial dermal capillaries. Also, the lipid components encompassed in the constructs of these vesicles act as penetration enhancers due to their proven fluidizing effect on the SC (Kirjavainen et al., 1996; Benson, 2006; Singh et al., 2014).

Bilosomes (bile salts containing niosomes) represent a new form of vesicular carriers that were first described in the pioneering work published by Conacher et al. (2001). Typically, bilosomes are closed bilayer vesicles of non-ionic amphiphiles similar to niosomes but integrating bile salts. Different researches have confirmed the potential of employing bilosomes to enable successful oral delivery of vaccines (Aburahma, 2014; Mann et al., 2006; Shukla et al., 2008, 2011; Jain et al., 2014; Wilkhu et al., 2013). Bile salts present in the lipid bilayers of bilosomes make them more resistant against gastro-intestinal (GI) bile salts and

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enzymes, therefore, offer protection for the entrapped vaccine against the hostile environment of the GI tract (Aburahma, 2014; Mann et al., 2006; Shukla et al., 2008, 2011). Although, bilosomes have not been yet investigated for transdermal drug delivery, their nano-sized vesicles associated with the presence of surfactants and bile salts in their constructs suggest promising prospects for employing them transdermally.

Tenoxicam (TX) is a long-acting NSAID belonging to the oxicam family that is characterized by potent anti-inflammatory, antipyretic and analgesic effect (El-Gazayerly, 2000). At present, TX is widely used for management of rheumatic diseases (Gonzalez and Todd, 1987). TX adverse effects' profile is comparable to that of other NSAIDs as it mainly affects the GI tract initiating epigastric pain, indigestion, dyspepsia, vomiting, and GI ulceration (Gonzalez and Todd, 1987). Further, it has been reported that transdermal penetration of TX is very poor limiting its use transdermally (Gwak and Chun 2001; Negi et al., 2013). Accordingly, the aim of the present work was to investigate the ability of bilosomes to increase the transdermal transport of TX, thereby, avoid unnecessary GI side effects associated with oral administration. To achieve this purpose, different variables influencing bilosomes characteristics were examined employing a full factorial design to identify the optimal bilosomes formulation. *Ex vivo* permeation study of the selected bilosomes applying donor/receiver experiments in comparison to drug solution was carried out to assess TX permeation from this carrier. Confocal laser scanning microscopy was employed to visualize the permeation profile of bilosomes through skin layers. Further, *in vivo* skin deposition of TX and histopathological studies of both the optimal bilosomes in comparison with TX solution were conducted in rats.

2. Materials and methods

2.1. Materials

Tenoxicam (TX) was kindly provided by Epico Co. (10th of Ramadan City, Egypt). Cholesterol was obtained from ITX Biomedicals (Santa Ana California, USA). Span 40 (sorbitan monopalmitate), Span 60 (sorbitan monostearate), Span 80 (sorbitan monooleate), methanol (HPLC grade), acetonitrile (HPLC grade) and Rhodamine B isothiocyanate were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Missouri, USA). Sodium deoxycholate was purchased from BASF Co. (Florham Park, New Jersey, USA). All other chemicals and solvents were of analytical grade and were used as received.

2.2. Preparation of TX loaded bilosomes

TX loaded bilosomes were prepared according to thin film hydration technique (Dai et al., 2013) but with slight modification. Briefly, TX (20 mg), the surfactant (Span 40, Span 60, or Span 80) and cholesterol were dissolved in 10 mL chloroform in a round bottom flask using an ultrasonic bath sonicator (Ultrasonic bath sonicator, Model SH 150-41; USA) for 10 min. The obtained organic solution was evaporated at 65 °C under reduced pressure using a rotary evaporator (Rotavapor, Heidolph VV 2000; Heidolph Instruments, Kehlheim, Germany) for 30 min until a thin, completely dry film was formed. The latter was left overnight to ensure complete evaporation of residual organic solvent. The obtained dry film was then hydrated with 10 mL distilled water containing the bile salt, sodium deoxycholate, for 1 h to form a crude dispersion of TX loaded bilosomes. The particle size of the formed bilosomes dispersion was further reduced by sonication for 5 min in a bath sonicator at 25 °C. The formed opalescent dispersion of bilosomes was stored at 4 °C until use.

2.3. Characterization and optimization of TX loaded bilosomes

2.3.1. Determination of TX entrapment efficiency percent (EE%)

The EE% of TX in bilosomes was estimated indirectly by determining the free TX (non-entrapped drug) in the dispersion medium. 1 mL of bilosomes dispersion was centrifuged using a cooling centrifuge (3K30, Sigma, Germany) at 15,000 rpm for 2 h at 4 °C. The supernatant was separated, diluted, and the concentration of TX was determined spectrophotometrically (Shimadzu, model UV-1601 PC, Kyoto, Japan) by measuring the UV absorbance at λ_{max} 368 nm.

Drug EE% was determined according to the following equation:

$$EE(\%) = \left[\frac{(\text{total amount of TX} - \text{total amount of free TX})}{\text{total amount of TX}}\right] \times 100 \quad (1)$$

2.3.2. Determination of particle size (PS), zeta potential (ZP), and polydispersity index (PDI)

The average PS, ZP, and PDI of the prepared bilosomes were determined by Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) using the dynamic light-scattering method employing a helium-neon laser with a wavelength of 633 nm at temperature of 25 ± 2 °C. The bilosomes dispersions were diluted 10 fold using distilled water before the measurements to make sure that the light scattering intensity was inside the instrument's sensitivity range. All measurements were performed in triplicate and the mean values obtained were reported.

2.3.3. Studying the influence of different formulation parameters using $3^1 2^2$ full factorial design

A complete $3^1 2^2$ factorial design was used to estimate and optimize the influence of different variables on the properties of TX loaded bilosomes dispersions using the least number of experiments (Myers and Montgomery, 2002). In the utilized design, 3 factors were assessed, one with three levels (X_1: type of surfactant) while the others with 2 levels (X_2: surfactant to cholesterol molar ratio) and (X_3: bile salt molar concentration) (Table 1). The experimental trials were performed with all possible combinations for preparing TX loaded bilosomes (Table 2). The EE% (Y_1), PS (Y_2), PDI (Y_3), and ZP (Y_4) were designated as dependent variables.

Design-Expert® software version 8 (Stat-Ease, Inc., Minneapolis, Minnesota, USA) was used to analyze experimental results to source independently the main effects of these factors followed by analysis of variance (ANOVA) to determine the significance of each factor.

2.3.4. Optimization of TX loaded bilosomes

For choosing the optimal formulation to be subjected for further investigations, the desirability function that syndicates all the

Table 1
Full factorial design $3^1 2^2$ used for optimization of the bilosomes formulations.

Factors (independent variables)	Levels		
X_1: type of Span	Span 40	Span 60	Span 80
X_2: surfactant:cholesterol molar ratio	5:1		5:3
X_3: bile salt molar concentration	0.5 M		0.25 M
Responses (dependent variables)	**Desirability constraints**		
Y_1: EE (%)	Maximize		
Y_2: PS (nm)	Minimize		
Y_3: PDI	Minimize		
Y_4: ZP (mV)	Maximize		

Abbreviations: SDC, sodium deoxycholate; PS, particle size; PDI, polydispersity index; ZP, zeta potential.