Colloids and Surfaces B: Biointerfaces 123 (2014) 207–212

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Niosomes containing hydroxyl additives as percutaneous penetration enhancers: Effect on the transdermal delivery of sulfadiazine sodium salt

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ARTICLE INFO

Article history:
Received 5 June 2014
Received in revised form 1 September 2014
Accepted 8 September 2014
Available online 16 September 2014

Keywords:
Niosomes
Ethanol
Propylene glycol
Glycerol
Sulfadiazine sodium
Skin permeation

ABSTRACT

The aim of this study was to improve the transdermal permeation of sulfadiazine sodium, employing synergistic combination of surfactants (in the form of niosomes) and additives with different number of hydroxylic groups, (following referred to as "alcohol"), as component of the bilayer. In particular the effect of different concentration of each alcohol (ethanol, propylene glycol or glycerol, from 5%, to 40% v/v) on niosomes size and distribution, drug entrapment efficiencies and *ex vivo* drug percutaneous permeation were evaluated, identifying formulations giving the best performances. The findings revealed that the presence of alcohol critically affect the physico-chemical properties of niosomes, with regards to dimensions, drug encapsulation and permeation. Vesicular size increased with the amount of alcohol and at the same alcohol concentration, follow the sequence ethanol > propylene glycol > glycerol. Loaded niosomes were larger than empty ones. Low *E*% values were found for ethanol, even less in propylene glycol and glycerol based samples, confirming that the chemical structure of the alcohol and its physico-chemical properties, affected the sulfadiazine entrapment efficiency. The comparative evaluation of percutaneous permeation profiles showed that the cumulative amount of permeated drug increases with alcohol concentration up to 20% v/v. Higher concentration (40% v/v) resulted in a strong decrease of the potential skin permeation. Best performances were obtained with glycerol. In all cases *ex vivo* sulfadiazine percutaneous permeations are controlled and improved respect to the corresponding free drug solutions and traditional niosomes used as controls.

1. Introduction

Transdermal administration of many drugs is generally a problem because of the stratum corneum barrier. To reduce this diffusional barrier, penetration enhancers, pharmacologically inert and having an immediate but reversible effect on the sc, can be added to formulations. In general, permeation enhancers may contain a wide variety of different chemical functional groups and act by a multiplicity of mechanisms to enhance transport of small molecules as well as large drugs across the skin. In addition they also show an effect on the solubility of the drug in the vehicle [1]. Permeation enhancers can directly exert their effect on skin structure by acting on intercellular lipids or corneocytes. In particular, they can extract lipids from the skin, thereby creating diffusion pathways for the drug to permeate through or they can partition themselves into the lipid bilayers thus disrupting the highly ordered lipid lamellae and causing their fluidization [1].

Alcohols including alkanols, alkenols, glycols, polyglycols and glycerols are frequently used as vehicles, solvents, or penetration enhancers to improve transdermal delivery of drugs. In particular, alcohols can enhance skin permeation by extraction of lipids and proteins, swelling of the stratum corneum, improvement of drug partitioning into the skin or drug solubility in the formulation [2,3]. Obviously, the alkyl chain length of the alcohol is an important parameter in permeation enhancement [4]: lower molecular weight molecules act as solvents, enhancing the solubility of drugs in the matrix of the sc, while disruption of its integrity through extraction of biochemicals, occurs with the more hydrophobic

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http://dx.doi.org/10.1016/j.colsurfb.2014.09.017
0927-7765/

alcohols [5]. Often, the use of alcohol in pharmaceutical formulations is limited by the accompanying skin irritation, and for these reasons the possibility to use niosomes (vesicular structures composed of surfactant molecules assembled into bilayers, acting themselves as percutaneous permeation enhancers) [6], containing a certain amount of alcohol in their formulations, have been proposed to reduce side effects and promote the drug percutaneous permeation by synergistic combination [7].

In these lights ethosomes are relatively new types of vesicle systems, primarily composed of water, ethanol and phospholipids, that have been reported to be effective at delivering molecules to and through the skin; ethanol, in fact, may provide vesicles with soft flexible characteristics, which allow them to penetrate more easily into the deeper layers of the skin [8]. In ethosomes, due to the presence of ethanol, the solubility of many drugs increases and high encapsulation efficiency values for a wide range of molecules are obtained [9]. Furthermore, transfersomes consist of phospholipids and an edge activator, that increases the deformability of the bilayers, conferring vesicles ultradeformable properties suitable for drug transport across the skin [10]. Recently Barichello et al. studied the combined effect of vesiculation and addition of glycerol on the transdermal delivery of isosorbide 5-nitrate, confirming that glycerol action on sc is useful to facilitate skin permeation and accumulation of drugs formulated in vesicles [11].

To our knowledge, there are no comparative studies concerning the influence of alcohols with different chemical structure on vesicles physico-chemical properties and drug permeation. In this report we investigated the effect of ethanol, propylene glycol and glycerol as component of niosomes, on the transdermal delivery of sulfadiazine sodium, an antibiotic usually used for the topical cure of infected burns. In particular, the effects of different amounts of alcohol in the formulations were evaluated. All formulations were compared in terms of dimensions, morphology and polydispersity index (PI), while *ex vivo* percutaneous permeation profiles were investigated by using the Franz diffusion cells.

2. Materials and methods

2.1. Chemicals

2.1.1. Preparation of niosomes

Multilamellar niosomal vesicles (MLVs) were prepared by the film hydration method [12]. Accurately weighed amounts of Tween 60 were dissolved in chloroform in a round-bottom flask. After mixing, the solvent was evaporated under reduced pressure and constant rotation to form a thin lipid film. The lipid film was then hydrated with 10 mL of sodium sulfadiazine aqueous solution (1.46×10^{-3} M) or sodium sulfadiazine hydroalcoholic solutions (additive/water 5%, 10%, 20% and 40% v/v) at 60 °C for 30 min to form large multilamellar vesicles (MLV), at 1×10^{-2} M of total lipid concentration (Table 1). After preparation, the dispersion was left to equilibrate at 25 °C overnight, to allow complete annealing and partitioning of the drug between the lipid bilayer and the aqueous phase. Small unilamellar vesicles (SUV) were prepared starting from MLV by sonication in an ultrasonic bath for 30 min at 60 °C. The purification of niosomes was carried out by exhaustive dialysis for 4 h, using Visking tubing (Spectra/Por®, cut-off 12–14 kD), manipulated before use in according to Fenton's method [13]. After purification, niosomes were stored at 4 °C and in the dark until used in subsequent experiments.

2.2. Zeta-potential and size distribution analysis

The *Z*-potential of the formulations was measured with the laser Doppler electrophoretic mobility measurements using the Zetasizer ZS (Malvern Instruments Ltd., Malvern, U.K.), at 25.0 ± 0.1 °C. All analyses were done in triplicate. *Z*-potential values and standard deviations were elaborated directly from the instrument.

The niosomes size and distribution were determined by Dynamic Light Scattering (DLS) analyses using Zetasizer ZS (Malvern Instruments Ltd., Malvern, U.K.), at 25.0 ± 0.1 °C by measuring the autocorrelation function at 90°. The laser was operating at 658 nm. The distribution size was directly obtained from the instrument fitting data by the inverse "Laplace transformation" method [14]. The PI was used as a measure of the width of size distribution. Polydispersity index less than 0.3 indicates a homogenous population for colloidal systems [15]. Each sample was measured three times and the results are expressed as mean ± standard deviation.

2.3. Transmission electron microscopy (TEM)

The morphological analysis of vesicles was carried out by transmission electron microscopy (TEM), using a ZEISS EM 900 unit working at an accelerating voltage of 80 kV. A drop of the vesicular formulation was placed on a carbon-coated copper grid, and the sample in excess was removed using a piece of filter paper. A drop of 2% (w/v) PTA (phosphotungstic acid solution) was then stratified on the carbon grid and left to stay for 2 min. Once the excess of staining agent was removed by filter paper, the samples were air-dried and the thin film of stained niosomes was observed. Each experiment was carried out in triplicate.

2.4. Drug entrapment efficiency

Drug encapsulation efficiency was determined using the dialysis technique for separating the non-entrapped drug from niosomes [16]. According to this method, 3 mL of drug-loaded niosomal dispersion were dropped into a dialysis bag immersed in 100 mL of distilled water and magnetically stirred (Visking tubing, Spectra/Por®, cut-off 12–14 kD). Free drug was dialyzed for 30 min each time and the dialysis was complete when no drug was detectable in the recipient solution. The percentage of encapsulation efficiency (*E*%) was expressed as the percentage of the drug entrapped into niosomes referred to the total amount of drug present in non-dialyzed samples. It was determined by diluting 1 mL of dialyzed and 1 mL of non-dialyzed niosomes in 25 mL of methanol, and by measuring the absorbance of these solutions at 270 nm. This procedure was necessary to break the niosomal membrane. Absorption spectra were recorded with a UV ± vis JASCO V-530 spectrometer using 1 cm quartz cells. Each experiment was carried out in triplicate and the results are expressed as mean ± standard deviation.

2.5. Ex vivo permeation studies

The experiments were carried out in the vertical Franz diffusion cells for 24 h at 37 °C, through rabbit ear skin obtained from a local slaughterhouse. The skin, previously frozen at −18 °C, was pre-equilibrated in physiological solution at room temperature for 2 h before the experiments. A circular piece of this skin was sandwiched securely between the receptor and donor compartments with the dermal side in contact with the receiver medium and the epidermis side in contact with the donor chamber (contact area = 0.416 cm^2). The donor compartment was charged with an appropriate volume of sample to keep constant the drug moles and the receptor compartment was filled with 5.5 mL of distilled water. At regular intervals up to 24 h, the medium in the receiver compartment was removed and replaced with an equal volume of pre-thermostated (37 ± 0.5 °C) fresh medium [17]. The content of