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Development and characterization of liposomes containing glycols as carriers for diclofenac

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ABSTRACT

This paper focuses on the preparation and characterization of new, stable phospholipid formulations as carriers for dermal delivery of diclofenac (DCF). To prepare these vesicles two water miscible permeation enhancers (PE) with glycol group: diethyleneglycol monoethyl ether (Transcutol®, Trc) and propylene glycol (PG), were added at different concentrations (10%, 20%, 30%, 40%, 50%) during the preparation of diclofenac loaded soy lecithin (SL) liposomes. We added Transcutol® or propylene glycol to the hydrophilic phase in order to obtain new systems able to enhance the skin delivery of diclofenac thanks to the synergic effect of glycols and phospholipids. Permeation enhancing vesicles (PEVs) were characterised by means of transmission electron microscopy (TEM), zeta potential, entrapment efficiency (*E*%) and reological properties. TEM micrographies showed that vesicle morphology was irregular and an ovoidal shape was predominant. Vesicular size decreased in the presence of Transcutol® (up to 20%) and propylene glycol (from 10% to 50%). *E*% increased in vesicles prepared with 10% and 20% of Trc with respect to SL liposomes and PEVs containing the same amount of PG. Vesicle dispersions were slowly frozen and unfrozen to break the vesicle bilayer and the rheological measurements of defrosted samples were carried out without stirring to avoid vesicle reconstitution. The shear stress values were measured for a given rate of shear. Results showed that the presence of Trc or PG in vesicle dispersions facilitated the vesicle segregation from lamellar phase allowing a reduction of hysteresis loop area and apparent viscosity.

1. Introduction

2-[(2,6-Dichlorophenyl)amino]benzene acetic acid (diclofenac acid, DCF) is a non-steroidal anti-inflammatory drug (NSAID). Its commercially available form is the anhydrous sodium salt. It is used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and also for a variety of non-rheumatic inflammatory conditions [1]. Diclofenac is usually administered orally but topical dosage forms are desirable for chronic treatment of regional rheumatic symptoms, in order to decrease the well known local mucosal irritation. Moreover, with topical application is possible to avoid the first passage metabolism that occurs in liver and leads to a partial inactivation of the drug. Diclofenac is topically administered in the form of a gel as 1% sodium salt. Several diclofenac-carrier formulations have been developed to improve the cutaneous delivery of this drug and their effects have been studied in vivo and in vitro [2–5]. Liposomes potentially could enhance diclofenac penetration into the stratum corneum, maintain drug release in the epidermis, and localize the drug within the deep skin layers with a reduction of the amount of drug absorbed into the systemic circulation [6–9]. Liposomes are also able to form occlusive films, which lead to increased skin hydration and thus drug penetration into the stratum corneum. Moreover, as they are composed of phospholipids, natural components of the cell membranes, they act as non-irritating moisturizing agents. Recent approaches in modulating drug delivery through the skin have resulted in the design of two novel vesicular carriers, ethosomes and deformable liposomes.

Ethosomes are composed of phospholipids, water and ethanol [10–12]. Ethanol is water miscible but it is also able to intercalate into the lipid bilayers allowing a better deformability of phospholipid membranes and conferring a net negative charge to the liposome surface, which causes a decrease of vesicle size [10]. In ethosomes, due to the presence of ethanol, the solubility of many drugs increases [13] and high encapsulation efficiency values for a wide range of molecules, including lipophilic drugs, can be obtained. Ethanol may provide vesicles with soft flexible characteristics, which allow them to penetrate more easily into the deeper layers of the skin.

Deformable liposomes are the first generation of elastic liposomes and have been reported to be able to penetrate intact skin carrying therapeutic concentration of drugs when applied in non-occlusive conditions [14–19]. These liposomes are liquid-state

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vesicles, characterised by a highly deformable membrane. They are prepared by incorporating an edge activator that is able to destabilise the vesicular lipid bilayer and to increase its elasticity thanks to redistribution of the amphiphilic lipids [20,21]. Examples of edge activators include surfactants: sodium cholate, sodium deoxycholate, Tween-80 and Span-80.

This paper describes preparation and characterization of vesicular systems obtained by the addition of a permeation enhancer (PE) during phospholipid vesicle preparation. The aim of this study was to obtain new, stable vesicular carriers for skin delivery of therapeutic amount of diclofenac. Two water miscible permeation enhancers with a glycol group, diethyleneglycol monoethyl ether (Transcutol®, Trc) and propylene glycol (PG), were used to prepare these permeation enhancer stabilized vesicles (PEVs). In particular, diclofenac loaded soy lecithin (SL) liposomes were prepared at first. Then PEVs were obtained spontaneously by increasing permeation enhancer concentration in the water phase (Trc or PG 10%, 20%, 30%, 40%, 50%). This paper focuses on the preparation and characterization of PEV formulations as DCF delivery system.

2. Materials and methods

2.1. Materials

Soy lecithin (SL) and propylene glycol (PG) were purchased from Galeno (Prato, Italy). Transcutol® (Trc) was a gift of Gattefossé SA (Saint Priest, France). Phosphate buffer solution (PBS) pH 7 was obtained from Carlo Erba Reagents (Rodano, Italy). All the other products were of analytical grade and were purchased from Aldrich, Milan, Italy. Diclofenac free acid (DCF) was obtained from diclofenac sodium salt (Sigma–Aldrich) by precipitating it with HCl. The obtained diclofenac free acid was filtered and dried at room temperature under vacuum.

2.2. Vesicle preparation

Multilamellar vesicles (MLVs) were prepared according to the thin film hydration method [22]. SL (180 mg) and DCF (10 mg) in chloroform solution were mixed. The lipid mixture was deposited as a thin film in a round-bottom flask by roto-evaporating the chloroform under vacuum. The film was hydrated with 1 ml of phosphate buffered saline solution at pH 7.0 (PBS) or with a solution of Trc or PG in PBS at different concentration (10%, 20%, 30%, 40%, 50% v/v). Hydration was carried out by mechanical stirring for 1 h at room temperature (25 °C). Each vesicle suspension was purified from non-incorporated drug by exhaustive dialysis for 4 h in distilled water at 5 °C using Spectra-por® membranes (12–14,000 cut-off 3 nm pore size, Spectrum laboratories, Inc., USA). Incorporation efficiencies (*E*%), expressed as a percentage of the total amount of DCF found in the studied formulations at the end of the preparation procedure, were determined by HPLC after disruption of vesicles with Triton X-100. Diclofenac content of samples was analysed at 227 nm using a liquid chromatograph Alliance 2690 (Waters), equipped with a photodiode array detector and a computer integrating apparatus (Millennium 32). The column was a Nova-Pack C18 (60 Å, 4 μm, Waters). The mobile phase was a mixture of methanol and PBS (60:40, v/v); delivered at a flow rate of 1.2 ml/min. The lipid recovery after vesicle purification was calculated by means of a colorimetric assay, which is based on the formation of a complex between phospholipids and ammonium ferrothiocyanate [23].

2.3. Vesicle characterization

Vesicles were characterised by transmission electron microscopy (TEM), optical and light polarized microscopy for vesicle formation and morphology and by Zetasiser for mean size, polydispersivity index and zeta potential.

To obtain TEM images, a drop of vesicle dispersion was applied to a carbon film-covered copper grid and was stained with a 1% phosphotungstic acid. Then samples were examined and photographed with a Jeol Jem1010 transmission electron microscope at an accelerating voltage of 80 kV. Optical and light polarized micrographs were obtained with an optical microscope Zeiss Axioplan 2, at 25 °C.

Average diameter and polydispersity index (PI) of all the samples were determined by photon correlation spectroscopy (PCS) using a Zetasizer 3 apparatus (Malvern, UK). Instrument detects the scattering information at 173 and 25 °C. This is known as backscatter detection. It is important to note that this mean size is an intensity mean. The cumulant analysis is actually the fit of a polynomial to the log of the correlation function of the scattered light intensity and it was carried out with the ALV-correlator V3.0 software. Sample mean size was detected as bimodal distribution of sizes. Vesicle diameter was converted by inputting the dispersant viscosity and the application of the Smoluchowski or Huckel theories. The polydispersity index (PI) was used as a measurement of the width of the size distribution. PI less than 0.4 indicates a homogenous and monodisperse population. Zeta potential was measured as the particle electrophoretic mobility means of laser microelectrophoresis in a thermostated cell. All the samples were analysed 24 h after their preparation.

2.4. Vesicle dispersion viscosity measurement

Soy lecithin dispersions were frozen and unfrozen to break vesicle bilayer without stirring in order to avoid their reconstitution before viscosity measurement. The rheology study was carried out on a Bohlin Visco 88 rheometer (Bohlin Instruments, Cranbury, NJ) equipped with eight rotational speed settings in geometric progression from 20 to 1000 rpm and a coaxial cylinder C-30 geometry. Temperature control of the samples was done at 25 ± 1 °C. The shear stress against shear rate as well as the viscosity against shear rate plots were made by "ViscoSoft" software. The apparent viscosity measurements were made at a constant shear rate of 402 s^{-1}. All the viscosity measurements were repeated three times.

2.5. Vesicle stability evaluation

Vesicle stability was studied during four weeks. Liposome dispersions were stored at 4 ± 1 °C and size, polydispersity index and zeta potential were measured at 1st, 2nd, 3rd, 7th, 14th, 21st and 28th day. Mean values were used for the analysis of the data.

2.6. Statistical analysis of data

Data analysis was carried out with the software package Microsoft Excel, version 2003. Results are expressed as mean ± standard deviation (3 independent samples). Statistically significant difference was determined using Student's *t*-test or analysis of variance (ANOVA) to substantiate statistical differences between groups, with $P = 0.05$ as a minimal level of significance.

3. Results and discussion

In the present work Transcutol®-PBS or propylene glycol-PBS mixtures were used as hydrophilic phase to obtain potential stable and small vesicles able to entrap a high amount of diclofenac acid. Moreover, thanks to the synergic enhancing effect of phospholipid vesicles and glycols the DCF dermal delivery can be facilitate [24–27].

At first, conventional soy lecithin liposomes were prepared and their ability to load 1% of diclofenac acid was evaluated. One of