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Innovative topical formulations from diclofenac sodium used as surfadrug: The birth of *Diclosomes*

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ABSTRACT

Hypothesis: Due to the well-know surfactant-like properties of diclofenac sodium (DS), vesicular systems consisting exclusively of DS, named *diclosomes*, were designed with the aim to minimize or avoid the use of other excipients and to improve the formulation biocompatibility.
Experiments: Diclosomes were designed and characterized in terms of dimensions, polydispersity index, ξ-potential, drug retained, stability as a function of storage time and *ex-vivo* percutaneous permeation profiles. Additionally, *diclosomes* were incorporated into gel dosage forms and their performance in terms of permeation enhancement were evaluated.
Findings: DS was found to form nanosized vesicular systems, both alone and in presence of cholesterol. Increasing hydrophobicity (due to the presence of cholesterol) resulted in smaller vesicles, always spherical and homogeneous in shape. Permeation of DS from free solution was found to be lower respect to ones obtained for all diclosomal formulations, allowing these aggregates to be considered as percutaneous permeation enhancers. DS permeated from diclosomal gels was higher than that obtained with traditional niosomal gel, DS plain gel and commercial specialty Voltaren Emulgel® 1%, while containing a considerably lower drug amount.

1. Introduction

Drug delivery systems (DDS) are a class of nanodevices able to provide enhanced efficacy and to reduce adverse side effects of therapeutics agents [1]. DDS are typically inert and they only transport drugs by forming a carrier able to encapsulate hydrophobic or hydrophlilic drugs [2]. Moreover, they have been reported to target these compounds at desired site, with definite rate and timing. Unfortunately, despite the variety of nanocarriers developed to treat diseases (*i.e.* nanoparticles, liposomes, polymers and proteins), success is limited to just a few formulations [3]. The most important drawback of these nanocarriers is their low drug loading capacity [4,5]. To overcome this limitation, the direct use of drug molecules as components of nanocarriers has been recently proposed by several researchers, with the aim to substantially increase the drug loading content, to minimize the use of inactive materials, and suppress drug premature burst release [6].

Many pharmacologically active compounds are amphiphilic molecules, which tend to self-associate and to interact with biological membranes likewise classical surfactants, whereby they have been defined as *surfadrug* (blend of *surface active drug*) [7]. Using an amphiphilic drug in the design of DDS would minimize or avoid the use of other excipients, improving the formulation safety and thus facilitate their clinical translation [8,9]. Thus, DDS made of *sulfadrugs* could represent a great innovation in the pharmaceutical field, because of their dual function: one related to the pharmacological nature of the molecule and the other related to the technological properties of the obtained carriers [10]. Additionally, these nanodevices may also serve as carriers of other drugs, to achieve combination therapy [10].

Classes of amphiphilic drugs including analgesics, tranquilizers, antibiotics, local anesthetics, non-steroidal antiinflammatory and chemotherapics have been extensively reviewed [8]. The structural features of amphiphilic drug molecules influence their association pattern in aqueous solution and consequently their interaction with biological membranes. Generally, *sulfadrugs* contain one or more flexible and hydrophobic aromatic nuclei, to which an ester group or a charge-bearing N atom is directly attached or which include a pyridine-like N atom [11]. The flexibility of the aromatic ring leads to these drugs may resemble typical surfactants in their association behaviour. In aqueous medium, *surfadrugs* can exist as monomers or can aggregate into micelles, bilayers and mesophases,

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depending on the concentration, hydrophilic-hydrophobic balance and, obviously, method of preparation [11]. Additionally, the different structures can be interconverted as a function of pH, temperature, ionic strength and *surfadrug* concentration [12,13].

Since macromolecular aggregates obtained from *surfadrugs* have been reported to act as effective drug delivery systems [14], we decided to test the aggregation properties of a widely used amphihilic drug, the diclofenac sodium. The vehiculation of DS has been studied in depth by the scientific community and also our research group contributed to increase knowledge in this field, investigating the effect of its compartimentalization and vehiculation into different macromolecular drug delivery systems [15–17]. Considering the well-know surfactant-like properties of diclofenac sodium, we hypotized that its direct use to form *diclosomes* (blend of diclofenac sodium-based niosomes), could avoid the use of other excipients in the preparation of DDS and increase the amount of loaded drug. In this context, pure *diclosomes* were compared with *diclosomes* contained different mole ratio of drug and cholesterol in terms of dimensions, polydispersity index, ξ-potential, retained DS and *ex-vivo* percutaneous permeation profiles. Additionally, to make *diclosomes* more exploitable for a direct application onto the skin, we incorporated vesicles into a gel dosage form and we evaluated its performance in terms of permeation enhancement, comparing with commercial specialty (Voltaren Emulgel® 1%).

2. Materials and methods

2.1. Chemicals

Diclofenac sodium (DS), cholesterol (Ch), Span 60 and carboxymethyl cellulose were purchased from Fluka (Sigma-Aldrich, Milan, Italy, 98% purity). All organic solvents were supplied from Sigma-Aldrich (Milan, Italy) and are of high performance liquid chromatography grade. Pharmaceutical specialty Voltaren Emulgel® 1% (Novartis FarmaSpA, Italy) was obtained commercially.

2.2. Preparation of vesicular systems and gels

Diclosomes were prepared by the hydration of lipidic film method. Accurately weighed amounts of DS and Ch were dissolved in ethanol in a round-bottom flask. After mixing, solvent was evaporated under reduced pressure and constant rotation to form a thin lipid film. This film was then hydrated with 10 mL of distilled water at 60 °C for 30 min, to obtain multilamellar vesicles (MLV). After preparation, the dispersions were left to equilibrate at 25 °C overnight. Small unilamellar vesicles were obtained from MLV by sonication in an ultrasonic bath for 30 min at 60 °C. The purification of *diclosomes* from untrapped materials was carried out by exhaustive dialysis for 4 h (details are reported in Section 2.3.3), using Visking tubing (Spectra/Por®, cut-off 12–14 kDa), manipulated before use in according to Fenton's method [18]. After purification, *diclosomes* were immediately used in subsequent experiments.

Additionally, to make *diclosomes* more exploitable for a direct application onto the skin, we incorporated vesicles into gel dosage forms. Diclosomal gel and diclosomal gel 55 were prepared incorporating *diclosomes* obtaining from DS alone and DS/cholesterol in ratio 50:50 into the gel matrices, respectively. In DS plain gel, DS was incorporated directly into the gel, while DS niosomal gel were prepared incorporating Span 60-niosomes into the polymeric matrix.

Details on the preparation procedures were reported below.

Diclosomal gels formulations were prepared adding 5 mL of diclosomal solutions to 0.150 g of carboxymethyl cellulose and magnetically stirring up to 3 h, to get homogeneous opalescent gels.

DS plain gel formulation was prepared according to the same procedure, dissolving DS in 5 mL of distilled water and adding this solution to 0.150 g of carboxymethyl cellulose. This formulation was realized with a DS content of 1% w/w, to compare with the commercial specialty Voltaren Emulgel® 1%.

DS niosomal gel was prepared as follow. Firstly, traditional niosomes were prepared dissolving 43 mg of Span 60 in ethanol, evaporating the solvent to obtain a thin lipid film and then hydrating with 10 mL of DC aqueous solution (1.25×10^{-3} M corresponding to 3.94 mg of DS). A certain amount of niosomal suspension was lyophilized and 0.71 g of this powder (containing about 50 mg of DS) were added to 5 mL of distilled water, to obtain a DS content of 1% w/w. Afterwards, DS niosomal gel was obtained by adding this suspension to 0.150 g of carboxymethyl cellulose and proceeding as above reported.

2.3. Characterization of niosomes

2.3.1. Size distribution and ξ-potential analysis

Vesicles diameter and size distribution were determined by dynamic light scattering (DLS), using a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation, New York, USA) at 25.0 ± 0.1 °C. The autocorrelation function was measured at 90°, while the laser beam was operating at 658 nm. The polydispersity index (P.I.) was used as a measure of the size distribution. It was directly obtained from the instrumental data fitting procedures by the inverse "Laplace transformation" and by Contin methods [19]. P.I. values ≤ 0.3 indicate homogenous and monodisperse populations in the case of colloidal systems. The ξ-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. ξ-potential values were calculated by the instrument software, using Helmholtz–Smoluchosky equation. All analyses were done in triplicate and expressed as mean ± standard deviation.

2.3.2. Morphology

The morphology of *diclosomes* was examined by Transmission Electron Microscopy (TEM) and the images were obtained with a TEM Jeol 1400 Plus electron microscope, operating at an acceleration voltage of 80 kV. A droplet of the vesicles suspension was placed on a Formvar/Carbon coated copper grid, forming a thin liquid film. Water in excess was removed by a piece of filter paper, followed by air-drying.

2.3.3. Evaluation of DS content into vesicles

The amount of DS retained in the vesicles was determined by exhaustive dialysis. 3 mL of *diclosomes* dispersion were dropped into a dialysis bag, immersed in 100 mL of distilled water and magnetically stirred. Samples were dialyzed for 60 min each time until no drug was detectable by UV–vis spectrometry. The percentage of DS retained into the vesicles (E%) was expressed as the percentage of the drug retained in the purified sample, referred to the total amount of drug present in the non-purified one. E% was determined by diluting 1 mL of purified and 1 mL of non-purified *diclosomes* in 25 mL of ethanol, followed by the measurement of maximum absorbance of these solutions at 276 nm, corresponding to the DS wavelength. Ethanol allows the breaking of vesicular membranes and the solubilizing of DS. 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.