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Research paper

A new approach for the evaluation of niosomes as effective transdermal drug delivery systems

Rita Muzzalupo *, Lorena Tavano, Roberta Cassano, Sonia Trombino, Teresa Ferrarelli, Nevio Picci

Department of Pharmaceutical Sciences, Calabria University, Rende, Italy

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ABSTRACT

The central motivation for this study was to evaluate if the increased hydrophilic drug permeation across the skin, which is always observed in presence of vesicular systems, is dependent on the structural organization of niosomes, that are used to transport the active molecules, or if it is only dependent on the surfactant dual nature. To answer this question, non-ionic surfactants belonging to the class of Pluronic and sucrose esters were used both as components of niosomal systems or in the form of sub-micellar solutions. The obtained niosomes were characterized by their entrapment efficiency, size and morphology.

The enhancing effect of niosomes on the *ex vivo* percutaneous penetration of a model drug was investigated using a Franz-type diffusion chamber and compared to that obtained by using sub-micellar solution of surfactant or achieving pretreatment of the skin with surfactants' sub-micellar solution or empty niosomes.

The results suggest that the surfactants used in this study could be considered as percutaneous permeation enhancers only when they are in the form of drug-loaded vesicular systems: no percutaneous promotion was achieved by using sub-micellar solution containing free Sulfadiazine sodium salt or performing pretreatment with empty niosomes or sub-micellar solutions of the surfactant. In our experiments, only niosomes act as effective transdermal drug delivery systems.

1. Introduction

Transdermal drug delivery is the controlled release of drugs through the skin to obtain therapeutic levels systematically [1]. The skin forms an attractive and accessible route of delivery for systemic drugs because of the problems associated with other methods of administration, such as oral and parenteral. It provides the advantage of avoidance of the first-pass effect, ease of use, withdrawal of side effects and better patient compliance [2]. One of the major disadvantages in transdermal drug delivery is the low penetration rate of hydrophilic substances through the skin. The diffusional barrier for most substances is localized in the upper layer of the skin, the stratum corneum (sc), which consists of corneocytes embedded in a lipid matrix [3].

In the recent decades, several physical (sonophoresis, iontophoresis, electro-osmosis, electroporation and temperature) [4,5] and chemical technological advances have been made to enhance percutaneous drug penetration, including the co-administration of absorption enhancers or delivery agents and the encapsulation of bioactive molecules in particles. Currently, the most widely used approach to drug permeation across the sc barrier is the use of chemical penetration enhancers (sorption promoters and accelerants). Penetration enhancers are substances that facilitate the absorption of a molecule through the skin by temporarily diminishing its impermeability, decreasing mucus viscosity, the leakage of proteins through membranes and the opening of tight junctions [6]. Ideally, these materials should be pharmacologically inert, non-toxic, non-irritating, non-allergenic, compatible with the drug and excipients, odorless, colorless, cheaper and they should have good solvent properties.

Different classes of compounds have been tested for their enhancer action [7], and different approaches to enhancement include the use of enzymes, natural oils, phospholipid micelles, liposomes [8], niosomes [9], polymers, lyotropic liquid crystals [10] and surfactants [11]. Among these strategies, special formulation approaches based mainly on the use of surfactant solutions or vesicles (niosomes) are the most promising [12].

Surfactants contribute to the overall penetration enhancement of compounds primarily by adsorption at interfaces, by interacting with biological membranes and by alteration of the barrier function of the sc, as result of reversible lipid modification [13].

Vesicular systems have attracted a great deal of attention in the transdermal delivery field because of many advantages, like biodegradability, non-toxicity, amphiphilic nature and possibility to modulate drug bioavailability [14]. Moreover, they can be modified

* Corresponding author. Department of Pharmaceutical Sciences, Calabria University, Ponte P. Bucci, Ed. Polifunzionale, 87030 Rende, Italy. Tel.: +39 0984493173; fax: +39 0984493298.
E-mail address: rita.muzzalupo@unical.it (R. Muzzalupo).

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in their structural characteristics like size, shape and lamellae nature modifying their composition [15]. They may serve as a solubilizing matrix, local depot for sustained release or permeation enhancers of dermally active compounds or as a rate-limiting membrane for the modulation of systemic absorption of drugs via the skin [16].

In this study, we achieved the preparation of vesicular systems (niosomes) obtained from non-ionic surfactants, belonging to the class of Pluronic (P105 and L64) and the class of sucrose esters (Tegosoft LSE 65K®) for the percutaneous release of Sulfadiazine sodium salt. We also decided to change the Pluronic surfactants' polar head increasing their hydrophilicity, by the oxidation of the terminal —CH_2OH into —COOH, to assess changes in physical–chemical properties of vesicles and consequently the permeation of the drug. The obtained niosomes were characterized by their entrapment efficiency, size and morphology.

The final aim of our study was to evaluate if the increased hydrophilic drug permeation across the skin, which is always observed with vesicular systems, is dependent on the typical structural organization of niosomes, that are used to transport the active molecules, or if it is only dependent on the surfactant dual nature.

The desire to answer this question was the central motivation for this study, in which the non-ionic surfactants were used both as components of niosomal systems and in the form of sub-micellar solution. In fact, we designed experiments to examine what was the real role of the surfactant molecule and the influence of its structural organization on the ex vivo permeation of a hydrophilic drug.

For these reasons, the percutaneous permeation profiles of Sulfadiazine sodium salt obtained by the following were compared:

- niosomes;
- sub-micellar solutions of each surfactant;
- drug aqueous solution after skin pretreatment with empty niosomes;
- drug aqueous solution after skin pretreatment with sub-micellar solutions of each surfactant.

2. Materials and methods

2.1. Chemicals and instruments

Pluronic L64 and P105 were kindly donated by BASF (Mount Olive, NJ, USA); sucrose cocoate (TEGOSOFT LSE 65K®) was obtained from A.C.E.F. s.p.a.

All reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA). Chromium trioxide (CrO_3), sulfuric acid (H_2SO_4) potassium hydroxide (KOH) and phenolphthalein indicator were used with no further purifications. The used drug was Sulfadiazine sodium salt and it was purchased from Sigma–Aldrich (St. Louis, MO, USA). The solvents are of high-performance liquid chromatography grade.

To ensure the synthetic quality, IR spectra were recorded with a FT-IR JASCO 4200 spectrometer, and ^{13}C NMR and ^{1}H NMR spectra were recorded with a Bruker 300 ACP NMR spectrometer. The content of drug in the release studies was analyzed by UV ± VIS JASCO V-530 spectrometer using 1-cm quartz cells at wavelength of 264 nm, typical of Sulfadiazine sodium salt. Franz static cells were used to perform the percutaneous permeation studies.

2.2. Preparation of L64ox and P105ox

The L64ox and P105ox were synthesized according the procedure shown in the Fig. 1 and reported in literature for poly(ethyleneglycol) [17].

In particular, 3.45×10^{-3} mol of Pluronic surfactant was placed in 150 mL of acetone. The content of the flask was heated to obtain a clear and homogeneous solution. The solution was allowed to achieve room temperature and then 2.05 mL of Jone's Reagent (containing 7 mmol of CrO_3) was added by stirring for 16 h. Finally, the reaction was quenched by adding 1.7 mL of isopropyl alcohol (free radical scavenger). To remove the chromium salts, 1 g of activated charcoal was added to the suspension and stirred for 2 h. The suspension was filtered to obtain a colorless, clear acetone solution. At last, it was dried under reduced pressure for 24 h. The obtained L64ox and P105ox dicarboxylic acids were quite viscous ivory-like liquids, and the yield of reactions was about 84 and 80%, respectively. IR values (on KBr) are 3400 and 1738 cm^{-1}. These peaks are significant for the oxidation of L64 and P105, in fact 3400 cm^{-1} is the stretching of —OH and 1738 cm^{-1} is the stretching C=O of the acid (Fig. 2a and b).

The significant chemical shift values for L64ox and P105ox surfactant protons and carbons (in ppm) performed in acetone, at 300 MHz, are the following:

^{1}H NMR (acetone d_6) δ (ppm): 4.3 δ singlet (—O—CH_2— vicinal to —COOH), 4.8 δ (—O—CH_2— vicinal to —COOH), 8.2 δ singlet (—COOH), 8.9 δ singlet (—COOH) for L64ox and P105ox, respectively.

^{13}C NMR (acetone d_6) δ (ppm): 174.6 (—COOH) and 178.3 (—COOH) for L64ox and P105ox, respectively.

The estimation of acid groups of L64ox and P105ox was performed by dissolving 1 g of L64ox or P105ox in 10 mL of distilled water. The solution was titrated against 0.01 N KOH solution, in the presence of phenolphthalein as indicator. The acid value was calculated in terms of mg of KOH required to neutralize 1 g of dicarboxylic acid. The experimental acid values, indicated as millimoles carboxyl groups/g, were 0.53 and 0.16, while the theoretical ones were 0.42 and 0.10 for L64ox and P105ox, respectively.

2.3. Preparation of vesicles

Multilamellar vesicles (MLVs) were prepared by a modification of the hydration of lipidic film method [18]. Surfactants were completely dissolved in about 10 mL of chloroform (1×10^{-2} M). The organic solvent was vacuum-evaporated for 4-8 h at room temperature. The obtained film was hydrated, under mechanical stirring at 30 °C for 30 min, with 10 mL of distilled water (empty niosomes) or with a Sulfadiazine sodium salt aqueous solution (2×10^{-3} mol/L, loaded niosomes). 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

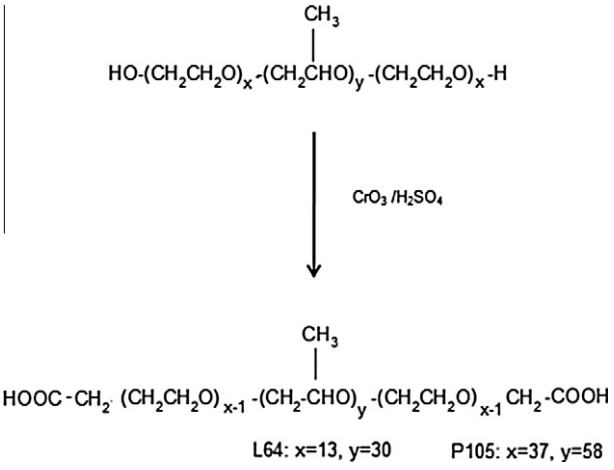

Fig. 1. Schematic representation of the synthesis of L64ox and P105ox.

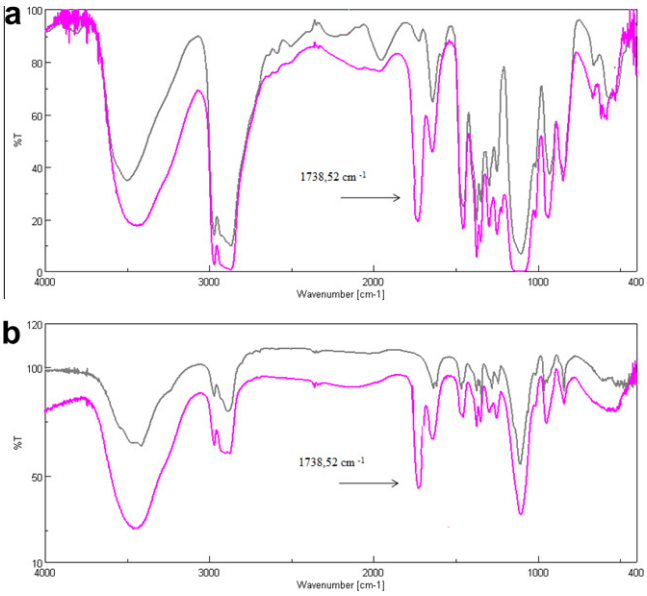

Fig. 2. FT-IR spectra of (a) L64ox and (b) P105ox: pink line = modified surfactant and gray line = commercial surfactant. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

phase. Small unilamellar vesicles (SUV) were prepared starting from MLV by sonication in an ultrasonic bath for 30 min at 30 °C. The purification of niosomes was carried out by exhaustive dialysis for 4 h, using Visking tubing (20/30), manipulated before use in according to Fenton's method [19].

2.4. Size and distribution analysis

The particle sizes and polydispersity index of the niosomes were measured by dynamic light scattering (DLS, 90 Plus Particle Size Analyzer, Brookhaven Instruments Corporation, New York, USA) at 25.0 ± 0.1 °C by measuring the autocorrelation function at 90°. The laser was operating at 658 nm. The mean size and standard deviation (±S.D.) was directly obtained from the instrument fitting data by the inverse "Laplace transformation" method and by CONTIN [20,21].

The polydispersity index discloses the quality of the dispersion from values lower than 0.3 for suitable measurements and good quality of the colloidal suspensions. Each experiment was carried out in triplicate.

2.5. Transmission electron microscopy (TEM)

The morphology of hydrated niosome dispersions was examined by TEM. A drop of dispersion was stratified onto a carbon-coated copper grid and left to adhere on the carbon substrate for about 1 min. The dispersion in excess was removed by a piece of filter paper. A drop of 2% phosphotungstic acid solution was stratified and, again, the solution in excess was removed by a tip of filter paper. The sample was air-dried and observed under a ZEISS EM 900 electron microscope at an accelerating voltage of 80 kV.

2.6. Drug entrapment efficiency

Drug encapsulation efficiency was determined using the dialysis technique for separating the non-entrapped drug from niosomes [22]. According to this method, 3 mL of drug-loaded niosomal dispersion was dropped into a dialysis bag (Spectra/Por, MW cut-off 12,000, Spectrum, Canada) immersed in 100 mL of distilled water and magnetically stirred. Free drug was dialyzed for 30 min each time, and the dialysis was complete when no drug was detectable in the recipient solution. The percent of encapsulation efficiency (*E*%) was expressed as the percentage of the drug entrapped into niosomes and referred to the total amount of drug that is present in the non-dialyzed sample. It was determined by diluting 1 mL of dialyzed and 1 mL of non-dialyzed niosomes in 25 mL of methanol, followed by measurement of absorbance of these solutions at the Sulfadiazine wavelength. This procedure is 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.

2.7. Transdermal permeation study

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, and the receptor compartment was filled with 5.5 mL of distilled water. During the study, the donor chamber was covered by parafilm. At regular intervals up to 24 h, the medium in the receiver compartment was removed and replaced with an equal volume of prethermostated (37 ± 0.5 °C) fresh distilled water. The complete substitution of the medium was needed to ensure sink conditions and quantitative determination of the small amounts of drug permeated. The content of drug in the samples was analyzed by UV–Vis spectrometry. Each experiment was carried out in triplicate, and the results were in agreement within ±4% standard error.

3. Results and discussion

Therefore, the aim of this work was to prepare niosomes, intended for topical drug delivery, containing Sulfadiazine sodium salt in the aqueous core, with the purpose of evaluating if the surfactants act as percutaneous permeation enhancer only in the form of vesicular systems or also as sub-micellar solutions.

In this light, we prepared niosomes by thin-layer evaporation method starting from non-ionic surfactants, such as TEGOSOFT LSE 65K®, Pluronic L64 and P105 and the corresponding synthesized carboxylic acids (L64ox and P105ox). Pluronics are polyethylene oxide (PEO)-polypropylene oxide (PPO)-polyethylene oxide tri-block co-polymers of different molecular weights. The hydrophobic PPO group in the middle links the two hydrophilic PEO groups. The amphiphilic nature of the Pluronic and the variation of their molecular characteristics (PPO/PEO ratio, molecular weight) allow these macromolecules to have optimum properties that meet specific requirements in different areas. Since the dynamic PEO chains prevent particles opsonization and render them 'unrecognizable' by reticulo-endothelial system (RES), several studies have been carried out regarding the interaction of Pluronic with vesicles [23] with the aim to reduce their uptake by the RES, thus prolonging their circulation half-life considerably [24] and sterically stabilize them [25]. Recently, our research group studied the ability of Pluronic L64 surfactant and its acrylate derivatives to give niosomes useful for the transdermal release of diclofenac [26].

Sucrose cocoate is a mixture of fatty acid sucrose esters, produced through the chemical esterification of coconut oil with

sucrose. Coconut oil contains fatty acid chains of different lengths as reported elsewhere [9]. It is an emulsifier employed in emollient, skin-moisturizing formulations and it has been used extensively as pharmaceutical excipient in cosmetic and dermatological products. Sucrose cocoate was used in drug delivery field in order to enhance nasal and ocular insulin release (as solution) [27] or to obtain niosomal vesicles for transdermal applications.

All obtained niosomes were characterized for particle size, morphology and encapsulation efficiency, using dynamic light scattering (DLS), transmission electron microscopy techniques (TEM) and UV-spectroscopy, respectively.

We found that L64, P105 and P105ox were able to form vesicles without the addiction of membrane additives, while L64 ox needs cholesterol to give niosomes. All formulations are stable at room temperature over 12 months. No sedimentation, creaming or flocculation can be inferred.

Vesicles size and polydispersity index (PI) are reported in Table 1. PI of the samples ranged from 0.2 to 0.3, and the size distribution of the niosomal formulations, as measured by DLS, shows one narrow peak, indicating that the vesicles population is relatively homogenous in size.

The same niosomal formulations were prepared loading Sulfadiazine sodium salt as model drug. As reported in Table 2, vesicle sizes were affected by the inclusion of the drug.

Table 1
Hydrodynamic diameter (nm) and polydispersity index of empty vesicular systems at 25 °C. Values represent mean ± S.D. (n = 3).

Formulation name	Surfactant (g)	Cholesterol (g)	Diameter (nm)	Polydispersity Index
L64	0.290	–	413.5 ± 15	0.261
L64ox	0.234	0.008	455.7 ± 17	0.283
P105	0.650	–	420.3 ± 17	0.254
P105ox	0.653	–	625.8 ± 20	0.271
Tegosoft	0.037	–	341.2 ± 15	0.262

Table 2
Hydrodynamic diameter (nm) and entrapment efficiency of drug-loaded vesicular systems at 25 °C. Values represent mean ± S.D. (n = 3).

Formulation name	Drug	Diameter (nm)	Polydispersity index	*E*%
L64	Sulfadiazine	366 ± 12	0.238	10.33 ± 2.90
L64ox	Sulfadiazine	399 ± 15	0.259	42.41 ± 1.50
P105	Sulfadiazine	330 ± 10	0.246	26.46 ± 2.10
P105ox	Sulfadiazine	599 ± 20	0.261	41.88 ± 1.50
Tegosoft	Sulfadiazine	335 ± 10	0.177	13.60 ± 2.70

In fact, Sulfadiazine-loaded niosomes were smaller than the corresponding empty ones. Probably, the reductions in size may be due to the favorable interaction between drug and niosomal matrix that result in an increased cohesion among the polar portions of the bilayer [28,29]. This reduction was most evident in P105-based formulation.

The encapsulation efficiencies of Sulfadiazine in our vesicular systems are summarized in Table 2. Niosomes based on L64 and P105 exhibited a moderate encapsulation efficiency that became more relevant when the oxidized surfactants were used for the preparation.

In addition, the different *E*% achieved for niosomes based on L64 and P105 (about 10% and 26%, respectively) could be dependent on the length of the surfactant: long chain produces high entrapment, as reported in the literature [30].

In the case of Tegosoft, the entrapment efficiency was similar to that obtained by L64 and P105, probably this is due to the low affinity of the niosomal matrix for the Sulfadiazine.

Micrographs of niosomes, observed by TEM, showed vesicles spherical in shape (Fig. 3) in all cases: the introduction of a new functional group into the surfactant did not result in a modification of the vesicles morphology.

3.1. Percutaneous permeation studies

In order to extend the range of drugs which can be administered across the skin and to enhance the effects of locally acting drugs, it is necessary to include penetration enhancers in formulations.

Interaction between skin and niosomes may be an important contribution for the improvement of transdermal drug delivery [31].

It has been reported that the intercellular lipid barrier in the sc would be dramatically changed to be more permeable by treatment with non-ionic surfactants in the form of niosomes that are able to act as penetration enhancers [32]. A wide range of animal models has been suggested as a suitable replacement for human skin and has been used to evaluate percutaneous permeation of molecules. These include porcine, mouse, rat, rabbit and snake models. In literature, the flux through rabbit skin was reported to be higher than that through human skin. The reason of this difference is that the thickness of the stratum corneum varies from species to species and the skin of rodent lacks the sweat glands and abounds in hair and hair follicles which is the important pathway for many drugs penetrated through skin barrier [33].

However, the ranking of prototype formulations or the evaluation of skin permeation of homologous compound achieved using in vitro methods will be a reflection of the in vivo scenario [33].

In this light, permeation properties of our niosomal systems were estimated through excised rabbit ear skin, and to investigate the non-ionic surfactant enhancer properties on Sulfadiazine per-

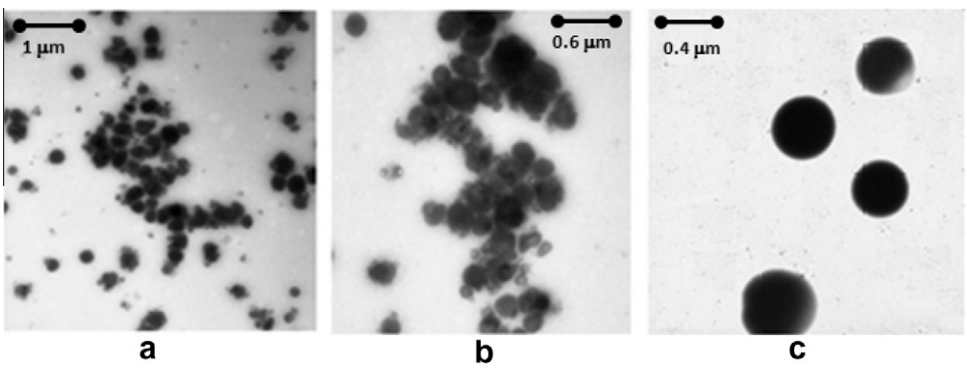

Fig. 3. Typical photomicrographs of niosomal formulations as seen by TEM for the sample: A L64, B L64ox and C Tegosoft, respectively.

Table 3
Details on the experimental percutaneous permeation procedures.

Experiment	Surfactant	Pretreatment	Treatment	Volume (mL)
A	L64 L64ox P105 P105ox Tegosoft	None	Drug-loaded niosomes	0.50 0.14 0.22 0.14 0.12
B	L64 L64ox P105 P105ox Tegosoft	None	Surfactant sub-micellar solution + drug	0.31
C	L64 L64ox P105 P105ox Tegosoft	Empty niosomes	Drug solution	0.57
D	L64 L64ox P105 P105ox Tegosoft	Surfactant sub-micellar solution	Drug solution	0.57
E		None	Drug solution	0.57

cutaneous permeation, the skin was differently pretreated with the corresponding surfactants' sub-micellar solutions or by empty niosomes for 2 h, following the administration of the opportune volume of free drug solution. Sulfadiazine was chosen as model drug since it is an antibiotic usually used for the topical cure of infected burns.

The donor compartment was always charged with an appropriate volume of sample, as reported in Table 3, so that the drug moles were constant (1.15×10^{-7} moles). This procedure was adopted to avoid dependence on the drug concentration gradient during the percutaneous permeation studies.

Details on the scientific procedures are reported below:

(a) In the first experiment, a volume of Sulfadiazine-loaded niosomes, prepared from the different surfactants, was placed in the donor compartment.
(b) In the second experiment, Sulfadiazine was solubilized in a volume of sub-micellar solution obtained from each surfactant and was placed in the donor compartment.
(c) In the third experiment, we carried out a pretreatment of the skin using a solution of empty niosomes, followed, after its removal, by the introduction of a volume of Sulfadiazine solution in the donor compartment.
(d) In the fourth experiment, we carried out a pretreatment of the skin using a sub-micellar solution of each surfactant, followed, after its removal, by the introduction of a volume of Sulfadiazine solution in the donor compartment.
(e) In the last experiment, a volume of Sulfadiazine water solution was placed in the donor compartment and used as control.

The results of our study for L64, P105, L64ox, P105ox and Tegosoft were reported in Figs. 4–8, respectively.

As shown in the Figures, the permeation of Sulfadiazine Sodium salt was not increased after pretreatment with sub-micellar solutions of surfactants, compared to the permeation obtained from the control. Also, the direct treatment with a sub-micellar solution of Pluronic L64 or P105 or Tegosoft containing the drug did not result in an enhancement of its percutaneous permeation.

Most investigators agree that direct contact between vesicles and skin is essential for efficient delivery, although surfactants apparently do not penetrate into deeper skin layers [34], but in our case, the pretreatment with empty niosomes did not enhance the percutaneous permeation of the Sulfadiazine Sodium salt. Only the direct treatment of the skin with loaded niosomes gave a relevant increase of the percutaneous permeation of the drug, confirming their role as enhancers.

Although the behavior of surfactants has been investigated in numerous studies [35], the exact mechanism by which they influence the drug percutaneous permeation is not fully understood and the results are still somewhat contradictory. In fact, in some cases, as previously reported in literature, amphiphilic molecules could act as inhibitor of penetration of a hydrophilic drug and only the co-addiction of ethanol or propylene glycol in the formulation increased the percutaneous absorption of a drug [36]. Furthermore, it is well documented that the enhancement of percutaneous penetration of drugs is due to the reversible alteration of permeability of the sc induced by phospholipids. This ambiguous behavior could be due to the chemical structure of the surfactant and to the differences in the hydrophilic/lipophilic balance. It has been reported that the presence of molecules having hydrogen bond-donating groups, due to the inter-lipid hydrogen bonding, stabilizes the bilayer and

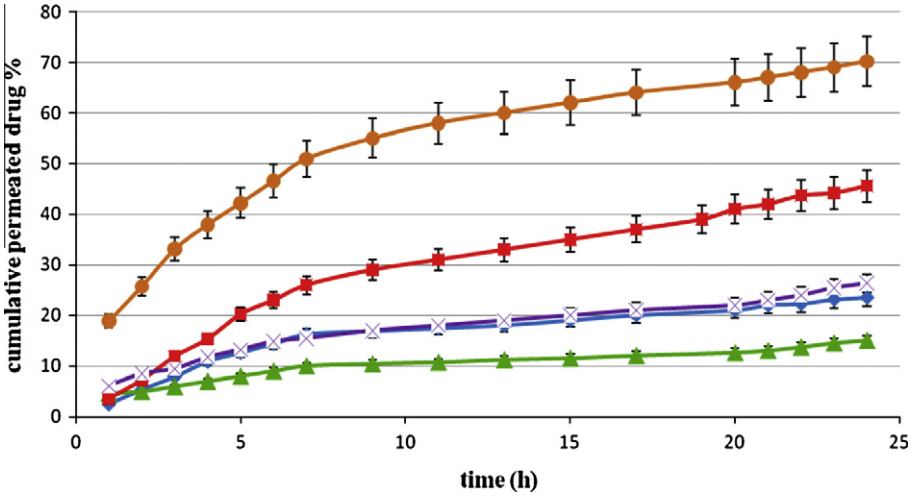

Fig. 4. Cumulative amount versus time of permeated Sulfadiazine from: (●) L64-based niosomes, (■) aqueous drug solution, (▲) L64 sub-micellar drug solution, (♦) aqueous drug solution after skin pretreatment with L64 sub-micellar solution, (×) aqueous drug solution after skin pretreatment with L64-based empty niosomes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

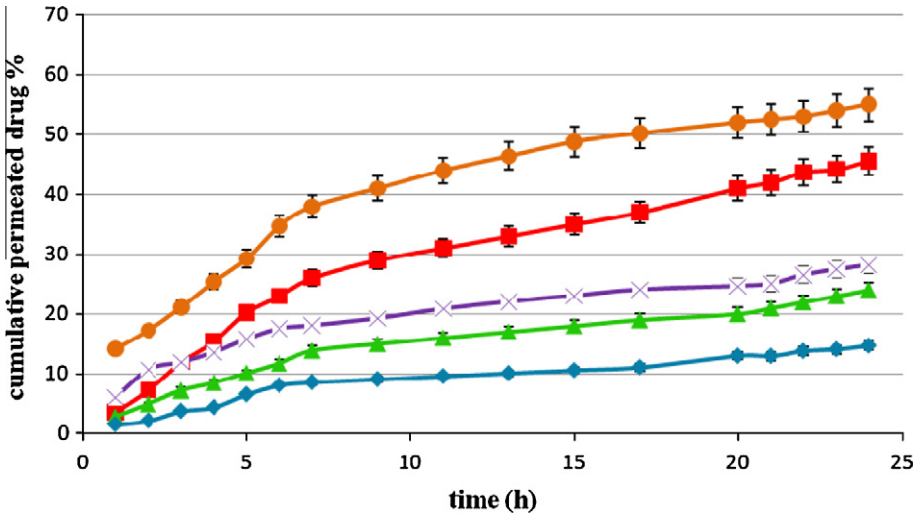

Fig. 5. Cumulative amount versus time of permeated Sulfadiazine from: (●) L64ox-based niosomes, (■) aqueous drug solution, (▲) L64ox sub-micellar drug solution, (♦) aqueous drug solution after skin pretreatment with L64ox sub-micellar solution, (×) aqueous drug solution after skin pretreatment with L64ox-based empty niosomes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

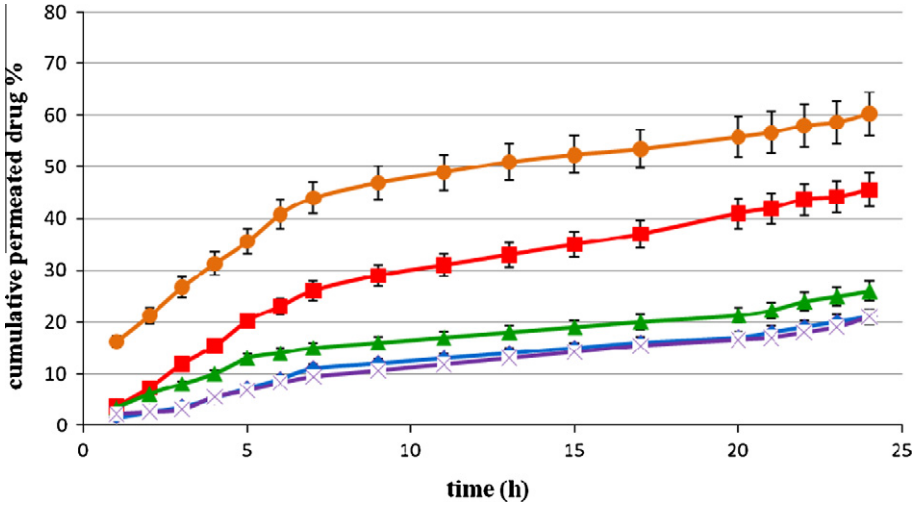

Fig. 6. Cumulative amount versus time of permeated Sulfadiazine from: (●) P105-based niosomes, (■) aqueous drug solution, (▲) P105 sub-micellar drug solution, (♦) aqueous drug solution after skin pretreatment with P105 sub-micellar solution, (×) aqueous drug solution after skin pretreatment with P105-based empty niosomes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

suppresses percutaneous permeation of the drug [37]. The surfactants used in our studies are very rich in groups that are able to create hydrogen bond: probably, this could be the reason of their low percutaneous permeation. In addition, Pluronic themselves are claimed to not enhance the permeability of hydrophilic molecules through rabbit ear skin [38].

Furthermore, in our case, the pretreatment or the direct treatment with sub-micellar solutions of surfactants did not cause any enhancing effect but decreased the skin permeation of Sulfadiazine Sodium salt, with respect to the drug solution used as control. Probably this is due to the insufficient amount of amphiphile that does not allow the arrangement in organized structures such as micelles or vesicles, claimed to cause an increase of its permeability [39]. In fact, in a sub-micellar solution, the surfactant amounts were below their critical micelle concentrations. In this study, we decided to test only sub-micellar solutions of surfactants, because Sulfadiazine sodium salt is a hydrophilic drug, and micelles are usually used to transport lipophilic drug.

The permeation of Sulfadiazine sodium salt from the aqueous solution through the skin was lower only compared to loaded niosomal samples, as shown in the figures, the cumulative percutaneous permeation achieved for each carriers was between 50% and 70%. No relevant differences in the percutaneous permeation profile were detected when L64ox and P105ox were used as surfactants, while we achieved an important difference between the oxidate and non-oxidate samples. In fact, permeation of Sulfadiazine sodium salt from non-oxidate compounds was higher than the corresponding oxidate, despite the entrapment efficiencies were lower. Probably this is due to the combined effect of better affinity of the drug for the oxidate niosomal matrices, that results in a slower release, and of repulsion between —COOH partially ionized groups and skin.

These results suggest that all surfactants tested in this work could be considered as percutaneous permeation enhancers only when they were used in the form of drug-loaded vesicular systems: no percutaneous promotion was achieved by using sub-micellar solutions of each surfactant containing the Sulfadiazine

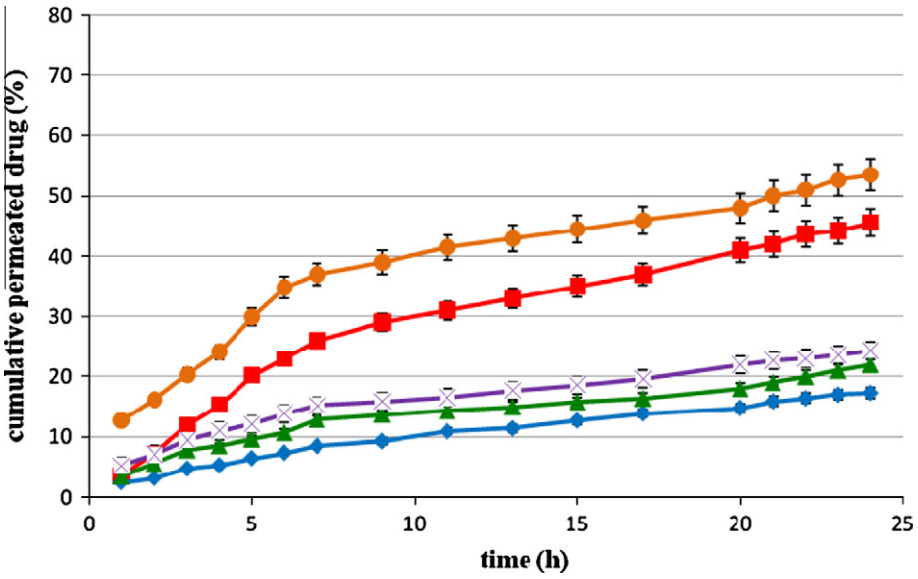

Fig. 7. Cumulative amount versus time of permeated Sulfadiazine from: (●) P105ox-based niosomes, (■) aqueous drug solution, (▲) P105ox sub-micellar drug solution, (♦) aqueous drug solution after skin pretreatment with P105ox sub-micellar solution, (×) aqueous drug solution after skin pretreatment with P105ox-based empty niosomes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

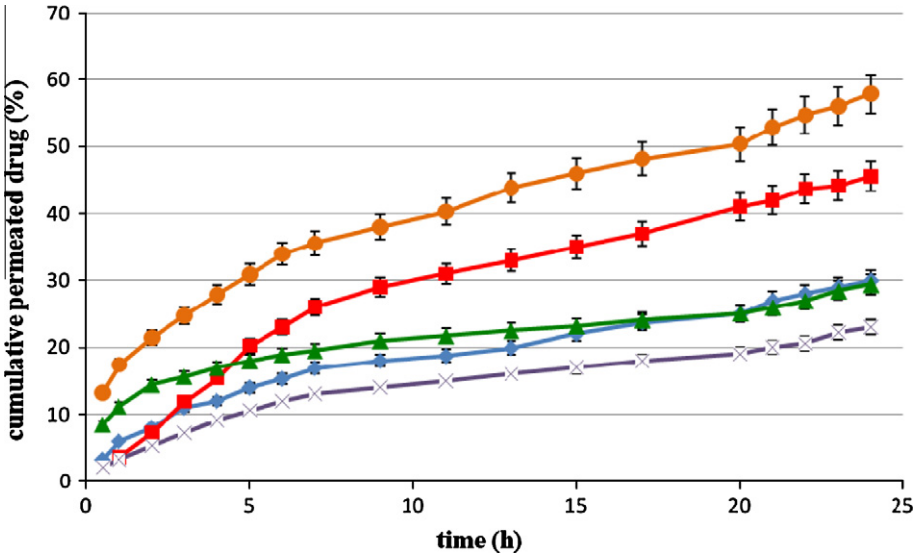

Fig. 8. Cumulative amount versus time of permeated Sulfadiazine from: (●) Tegosoft-based niosomes, (■) aqueous drug solution, (▲) Tegosoft sub-micellar drug solution, (♦) aqueous drug solution after skin pretreatment with Tegosoft sub-micellar solution, (×) aqueous drug solution after skin pretreatment with Tegosoft-based empty niosomes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

sodium salt or performing pretreatment with empty niosomes or sub-micellar solutions of these surfactants.

4. Conclusions

We investigated whether topical application of Sulfadiazine-containing niosomes could enhance drug permeation compared to drug aqueous solution. Niosomes were prepared from non-ionic surfactants, belonging to the class of Pluronic (P105 and L64) and the class of sucrose esters (TEGOSOFT LSE 65K®) for the percutaneous release of Sulfadiazine sodium salt. In addition, we changed the Pluronic surfactants' polar head increasing their hydrophilicity to assess changes in physical-chemical properties of the vesicles and the permeation of the drug.

It appeared that L64, P105 and P105ox surfactants were able to form vesicles without the addiction of membrane additives, while L64ox needed cholesterol to give niosomes. Vesicles were spherical and regular in shape and showed good Sulfadiazine sodium salt entrapment efficiency: in particular, this property was found to be more relevant when the oxidate surfactants were used for the preparation of niosomes. The enhancing effects of niosomes on the *ex vivo* percutaneous penetration of the drug were investigated and compared to those obtained by using sub-micellar solution of surfactant or achieving pretreatment of the skin with surfactants' sub-micellar solutions or empty niosomes.

The results suggested that only loaded niosomes could act as effective transdermal drug delivery systems.

In conclusion, all surfactants tested in our study could be used successfully for the transdermal delivery of Sulfadiazine sodium salt.

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References

[1] H.Y. Thong, H. Zhai, H.I. Maibach, Percutaneous penetration enhancers: an overview, Skin Pharmacol. Physiol. 20 (2007) 272–282.
[2] B.W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery, Eur. J. Pharm. Sci. 14 (2001) 101–114.
[3] H. Schreief, J. Bouwstrab, Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery, J. Control. Release 30 (1994) l–15.
[4] S.K. Rastogi, J. Singh, Effect of chemical penetration enhancer and iontophoresis on the in vitro percutaneous absorption enhancement of insulin through porcine epidermis, Pharm. Dev. Technol. 1 (2005) 97–104.
[5] O. Taro, H. Tsuyoshi, H. Masahiro, H. Takaharu, T. Tadatoshi, Effect of temperature on percutaneous absorption of terodiline, and relationship between penetration and fluidity of the stratum corneum lipids, Int. J. Pharm. 176 (1998) 63–72.
[6] E.W. Smith, H.I. Maibach, in: E. W. Smith, H.I. Maibach (Eds.), Percutaneous Penetration Enhancers, second ed., CRC Press, Taylor and Francis Group, Boca Raton, FL, 2006.
[7] V.R. Sinha, P.K. Maninder, Permeation enhancers for transdermal drug delivery, Drug Dev. Ind. Pharm. 26 (2000) 1131–1140.
[8] C. Sinico, M. Manconi, M. Peppi, F. Lai, D. Valenti, A.M. Fadda, Liposomes as carriers for dermal delivery of tretinoin: in vitro evaluation of drug permeation and vesicle-skin interaction, J. Control. Release 103 (2005) 123–136.
[9] L. Tavano, R. Muzzalupo, S. Trombino, R. Cassano, T. Ferrarelli, N. Picci, New sucrose cocoate based vesicles: preparation characterization and skin permeation studies, Colloids Surf. B: Biointer. 75 (2009) 319–322.
[10] R. Muzzalupo, L. Tavano, F.P. Nicoletta, S. Trombino, R. Cassano, N. Picci, Liquid crystalline Pluronic 105 pharmacogels as drug delivery systems: preparation, characterization and in vitro transdermal release, J. Drug Targ. 8 (2010) 404–411.
[11] F.R. Bettley, The influence of detergents and surfactants on epidermal permeability, Br. J. Dermatol. 77 (1965) 98–100.
[12] M.J. Choi, H.I. Maibach, Liposomes and niosomes, as topical drug delivery systems, Skin Pharmacol. Physiol. 18 (2005) 209–219.
[13] G.P. Kushla, J.L. Zatz, O.H. Millis, R.S. Berger, Noninvasive assessment of anesthetic activity of Lidocaine formulations, J. Pharm. Sci. 82 (1993) 118–1122.
[14] I.F. Uchegbu, S.P. Vyas, Non-ionic surfactant based vesicles (niosomes) in drug delivery, Int. J. Pharm. 172 (1998) 33–70.
[15] R. Agarwal, O.P. Katare, S.P. Vyas, Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol, Int. J. Pharm. 228 (2001) 43–52.
[16] A. Manosroi, L.L. Kongkaneramit, J. Manosroi, Characterization of amphotericin B liposome formulation, Drug Dev. Ind. Pharm. 30 (2004) 535–543.
[17] B.S. Lele, M.G. Kulkarni, Single step room temperature oxidation of poly(ethylene glycol) to poly (ethylene dicarboxylic acid), J. Appl. Pol. Sci. 70 (1998) 883–890.
[18] A.D. Bangham, M.M. Standish, J.C. Watkins, Diffusion of univalent ions across the lamellae of swollen phospholipids, J. Mol. Biol. 13 (1965) 238–252.
[19] R.R. Fenton, W.J. Easdable, H. Meng, E.S.M. Omara, M.J. Mckeage, P.J. Russel, T.W. Hambley, Preparation, DNA binding, and in vitro cytotoxicity of a pair of enantiomeric platinum(II) complexes, [(R)-and (S)-3-aminohexahydroazepine]dichloro-platinum(II). Crystal structure of the S enantiomer, J. Med. Chem. 40 (1997) 090–1098.
[20] S.W. Provencher, A constrained regularization method for inverting data represented by linear algebraic or integral equations, Comput. Phys. Commun. 27 (1982) 213–229.
[21] S.W. Provencher, CONTIN: a general purpose constrained regularization program for inverting noisy linear algebraic and integral equations, Comput. Phys. Commun. 27 (1982) 229–242.
[22] M. Trotta, E. Peira, F. Debernardi, M. Gallarate, Elastic liposomes for skin delivery of dipotassium glycyrrhizinate, Int. J. Pharm. 241 (2002) 319–327.
[23] P. Alexandridis, Poly(ethylene oxide) poly(propylene oxide) block copolymer surfactants, Curr. Opin. Colloid Int. Sci. 2 (1997) 478–489.
[24] T.M. Allen, C. Hansen, J. Rutledge, Liposomes with prolonged circulation times: factors affecting uptake by reticuloendothelial and other tissues, Biochim. Biophys. Acta 981 (1989) 27–35.
[25] M. Jamshaid, S.J. Farr, P. Kearney, I.W. Kellaway, Poloxamer sorption on liposomes: comparison with polystyrene latex and influence on solute efflux, Int. J. Pharm. 48 (1988) 125–131.
[26] L. Tavano, R. Muzzalupo, S. Trombino, R. Cassano, A. Pingitore, N. Picci, Effect of formulations variables on the in vitro percutaneous permeation of sodium diclofenac from new vesicular systems obtained from Pluronic triblock copolymers, Colloids Surf. B: Biointer. 79 (2010) 227–234.
[27] F. Ahsan, J.J. Arnold, E. Meezan, D.J. Pillion, Sucrose cocoate, a component of cosmetic preparations, enhances nasal and ocular peptide absorption, Int. J. Pharm. 251 (2003) 195–203.
[28] R. Muzzalupo, L. Tavano, S. Trombino, R. Cassano, N. Picci, C. La Mesa, Niosomes from α,ω-trioxyethylene-bis(sodium 2-dodecyloxy-propylenesulfonate): preparation and characterization, Colloids Surf. B: Biointer. 64 (2008) 200–207.
[29] M. Manconi, C. Sinico, D. Valenti, G. Loy, A.M. Fadda, Niosomes as carriers for tretinoin. I. Preparation and properties, Int. J. Pharm. 234 (2002) 237–248.
[30] G. Abdelbary, N. El-gendy, Niosome-encapsulated gentamicin for ophthalmic controlled delivery, AAPS Pharm. Sci. Technol. 9 (2008) 740–747.
[31] T. Ogiso, N. Niinaka, M. Iwaki, Mechanism for enhancement effect of lipid disperse system on percutaneous absorption, J. Pharm. Sci. 85 (1996) 57–64.
[32] L. Coderch, M. Oliva, M. Pons, A. de la Maza, A.M. Manich, J.L. Parra, Percutaneous penetration of liposomes using the tape stripping technique, Int. J. Pharm. 139 (1996) 97–203.
[33] R.L. Bronaugh, I.H. Maibach, Percutaneous Absorption: Drugs – Cosmetics – Mechanisms – Methodology, fourth ed. Informa Healthcare, Hardcover, 2005
[34] H. Schreier, J. Bouwstra, Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery, J. Control. Release 30 (1994) 1–15.
[35] M.G. Ganesan, N.D. Weiner, G.L. Flynn, N.F.H. Ho, Influence of liposomal drug entrapment on percutaneous absorption, Int. J. Pharm. 20 (1984) 139–154.
[36] R. Valjakka-Koskela, M. Kirjavainen, J. Miinkkanen, A. Urtti, J. Kiesvaara, Enhancement of percutaneous absorption of naproxen by phospholipids, Int. J. Pharm. 175 (1998) 225–230.
[37] Y. Yokomizo, H. Sagitani, Effects of phospholipids on the percutaneous penetration of indomethacin through the dorsal skin of guinea pigs in vitro, J. Control. Release 38 (1996) 67–274.
[38] M.J. Cappel, J. Kreuter, Effect of nonionic surfactants on transdermal drug delivery. II: Poloxamer and poloxamine surfactants, Int. J. Pharm. 69 (1991) 155–167.
[39] P.P. Sarpotdar, J.L. Zatz, Evaluation of penetration enhancement of lidocaine by nonionic surfactants through hairless mouse skin in vitro, J. Pharm. Sci. 75 (1986) 176–181.