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Enhanced Transdermal Delivery of Salbutamol Sulfate via Ethosomes

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ABSTRACT

The main objective of the present work was to compare the transdermal delivery of salbutamol sulfate (SS), a hydrophilic drug used as a bronchodilator, from ethosomes and classic liposomes containing different cholesterol and dicetylphosphate concentrations. All the systems were characterized for shape, particle size, and entrapment efficiency percentage, by image analysis optical microscopy or transmission electron microscopy, laser diffraction, and ultracentrifugation, respectively. In vitro drug permeation via a synthetic semipermeable membrane or skin from newborn mice was studied in Franz diffusion cells. The selected systems were incorporated into Pluronic F 127 gels and evaluated for both drug permeation and mice skin deposition. In all systems, the presence of spherical-shaped vesicles was predominant. The vesicle size was significantly decreased ($P < .05$) by decreasing cholesterol concentration and increasing dicetylphosphate and ethanol concentrations. The entrapment efficiency percentage was significantly increased ($P < .05$) by increasing cholesterol, dicetylphosphate, and ethanol concentrations. In vitro permeation studies of the prepared gels containing the selected vesicles showed that ethosomal systems were much more efficient at delivering SS into mice skin (in terms of quantity and depth) than were liposomes or aqueous or hydroalcoholic solutions.

KEYWORDS: Transdermal, salbutamol sulfate, liposomes, ethosomes.

INTRODUCTION

In the past decade, topical delivery of drugs by liposomal formulations has evoked considerable interest. Recently, it has become evident that traditional liposomes are of little or no value as carriers for transdermal drug delivery, because they do not deeply penetrate skin but remain confined to upper layers of the stratum corneum.[1] Confocal microscopy studies have shown that intact liposomes are not able to penetrate into the granular layers of the epidermis.[2]

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Recent approaches to modulating drug delivery through skin have resulted in the design of 2 novel vesicular carriers, deformable liposomes and ethosomes.[3]

Deformable liposomes, transfersomes, were the first generation of elastic vesicles introduced by Cevc and Blume and were reported to penetrate intact skin while carrying therapeutic concentrations of drugs when applied under nonoccluded conditions.[4] Transfersomes consist of phospholipids and an edge activator, which destabilizes lipid bilayers of the vesicles and increases their deformability.[5] Several studies[6-9] have reported that deformable liposomes were able to improve both in vitro and in vivo skin delivery of various drugs.

Ethosomes were developed by Touitou et al as additional novel lipid carriers composed of ethanol, phospholipid, and water.[1] Ethosomes were reported to improve the skin delivery of various drugs.[10,11]

Ethanol is known as an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum.[12] Its inclusion in liposomes to form ethosomes has already been investigated.[13-15]

Salbutamol sulfate (SS) is a drug widely used for the treatment of bronchial asthma, chronic bronchitis, and emphysema.[16] The drug is readily absorbed from the gastrointestinal tract. However, it is subject to first-pass metabolism in the liver and possibly the gut wall.[17] Therefore, this drug is now rarely delivered via the oral route. It is usually given by inhalation or slow intravenous injections, in the management of severe asthmatic attacks. Actually, most (or all, in several countries) products containing this drug are administered by inhalation. The plasma half-life of the drug has been estimated to range from 4 to 6 hours, so the recommended dose in adults and children is usually given every 4 to 6 hours.[18]

The aim of the present work was to study the possibility of transdermal controlled delivery of SS. Transdermal drug delivery systems offer many advantages over their corresponding classical oral, injectable, and inhaler systems, including (1) improving the systemic bioavailability of drugs because the first-pass metabolism by the liver and digestive system is avoided, and (2) achieving a controlled constant drug delivery profile, which is especially important for those suffering from nocturnal attacks and need a longer duration of therapeutic action from a single application.[19]

Achieving controlled transdermal delivery of SS is challenging, as it is a hydrophilic drug with a pK_a of 9.2 and a log P value of 0.11.[20] Therefore, this study was focused on

optimizing the factors influencing the preparation of an efficient ethosomal system that could be incorporated into a suitable gel to be easily applied by patients. We wanted to create a system that could control the rate of drug release for 24 hours, with an input rate of ~100 μg/h.[20]

MATERIALS

SS was a gift from Egyptian International Pharmaceutical Industries Co (10th of Ramadan, Egypt). Phosphatidylcholine from soybean lecithin and dicetylphosphate were obtained from Sigma Chemicals (St Louis, MO). Cholesterol was from Dolder Ltd (Basel, Switzerland). Diethyl ether, chloroform, formaldehyde, ethanol, disodium hydrogen phosphate, and potassium dihydrogen phosphate were from E Merck (Darmstadt, Germany). Uranyl acetate was purchased from Riedel-de Häen (Seelze, Germany). Semipermeable membrane "Spectra por molecular porous membrane tubing" with a molecular weight cutoff of 12 000 to 14 000 was obtained from Spectrum Laboratories (Laguna, CA). Pluronic F 127 was from BASF (Mount Olive, NJ). All other reagents were of analytical grade.

METHODS

Preparation of Liposomes

Liposomes were prepared by the solvent evaporation method.[21] Exactly 60 mg of phosphatidylcholine from soybean lecithin, 10 to 30 mg cholesterol, and 0 to 7.5 mg dicetylphosphate were dissolved in a small volume of diethyl ether:chloroform (1:1) mixture in a round-bottom flask. The aqueous phase containing SS (10 mg) was added to the organic phase such that the organic-to-aqueous-phase ratio was 5:1. The mixture was then sonicated (Sonic water bath, Decon Laboratories, Hove, UK) for 10 minutes. A stable white emulsion was produced from which the water and the organic solvent mixture were slowly evaporated at 55ºC using a rotary vacuum evaporator (Rotavapor, Type R110, Büchi, Flawil, Switzerland) until a thin film was formed on the wall of the flask. The resulting film was kept under vacuum to eliminate the traces of organic solvent. This film was then hydrated with an appropriate amount of an aqueous solution and left at 55ºC in a thermostatic controlled water bath (W10, Medingen, Sitz Freital, Germany) for 1 hour. The resulting liposomal suspension was left to stand at room temperature for 1 hour and then sonicated for 20 minutes.

Preparation of Ethosomes

Ethosomes were prepared following the previously described procedure, but the resulting film was hydrated with a hydroalcoholic solution (20%-40% [vol/vol] ethanol).[13]Table 1 shows the composition of the different batches corresponding to all formulas.

Vesicle Characterization

Vesicle Morphology

Visualization by image analysis optical microscopy: Samples of the prepared vesicles were suitably diluted with saline and examined using an optical microscope. The images of the vesicles were transferred to an IBM-compatible computer through a video camera (JVC, Victor Co, Yokohama, Japan). At least 300 vesicles were measured for each sample, and the shape was analyzed automatically using image analysis software (Leica Imaging Systems, Cambridge, UK).

Visualization by transmission electron microscopy (TEM): A drop of the sample solution was placed on a copper grid, and the material excess was removed with a filter paper. A 2% uranyl acetate solution was dropped onto the grid. The excess of staining solution was removed. Finally, the grid was examined under a transmission electron microscope (Jeol Jem 1230, Tokyo, Japan) at 80 kV.

Table 1. Composition of Various Liposomes (1-6), Ethosomes (7-9), and 2 Control Solutions*

Batches	Phosphatidylcholine (mg)	Cholesterol (mg)	Dicetylphosphate (mg)	Ethanol (% vol/vol)	SS (mg)
Control 1	—	—		—	10
Control 2	—	—		30	10
Formula 1	60	10		—	10
Formula 2	60	20		—	10
Formula 3	60	30		—	10
Formula 4	60	20	2.5	—	10
Formula 5	60	20	5	—	10
Formula 6	60	20	7.5	—	10
Formula 7	60	20	5	20	10
Formula 8	60	20	5	30	10
Formula 9	60	20	5	40	10

*SS indicates salbutamol sulfate.

Vesicle Size Distribution

The vesicle size of each liposomal suspension (formulas 1-6) and each ethosomal suspension (formulas 7-9) was determined, in triplicate, by laser diffraction at 25°C (Malvern Mastersizer-S, Malvern Instruments, Manchester, UK). For size measurements, the preparation was appropriately diluted with ethanol-water (30% vol/vol, ethanol) solution for ethosomes and purified water for liposomes. The polydispersity index (PI) was determined as a measure of homogeneity. Small values of PI (<0.1) indicate a homogeneous population, while PI values > 0.3 indicate high heterogeneity.[22] Data were fed into the SPSS statistics program (SPSS Inc, Release 14.0 for Windows, Chicago, IL) applying a 1-way analysis of variance (ANOVA) test with least squared difference (LSD) multiple comparisons.

Determination of Entrapment Efficiency Percentage

Vesicle preparations were kept overnight at 4°C and ultracentrifuged (Megafuge 1.0 R, Heraeus, Hanau, Germany) for 2 hours at 15 000 rpm. The free (unentrapped) SS concentration was determined in the supernatant spectrophotometrically (Shimadzu UV–1601 PC Double Beam, Kyoto, Japan) at λ_{max} 276 nm. The SS entrapment percentage was calculated from the following formula:

$$EE = [\langle Qt - Qs \rangle \div Qt] \times 100 \qquad (1)$$

where EE is the entrapment efficiency, Q_t is the theoretical amount of SS that was added, and Q_s is the amount of SS detected only in the supernatant.

In Vitro Permeation and Skin Deposition Studies

Experiments were run in Franz diffusion cells having an effective permeation area of 4.90 cm^2. The temperature was maintained at 37°C ± 0.5°C. The receptor compartment contained 20 mL of phosphate-buffered saline (pH 7.4) and was stirred constantly by a magnetic stirrer at 50 rpm. The design of the in vitro experiments was conducted in a way similar to that described by El Sayed et al.[3] The experiments were performed in 2 stages. The first stage examined drug permeation through the skin. The receptor medium was an isotonic phosphate buffer (pH 7.4) containing 0.11% (wt/vol) formaldehyde as a preservative.[23] The permeation studies were performed using synthetic semipermeable membranes or the skin of newborn mice (age 6 days or younger) (Cairo University Labs, Cairo, Egypt). The aim of the preliminary investigations, performed through a synthetic barrier, was to demonstrate the effect of various factors like cholesterol and dicetylphosphate concentration. The formula that exhibited the optimum drug permeation through the synthetic barrier was chosen for further permeation studies via the biological barrier. All animals were treated according to the principles of laboratory animal care (National Institutes of Health publication #86–32).[24] Mice skin was obtained after sacrificing the animals, by peeling the skin from the underlying cartilage.[25] A preliminary wash of the skin was done with normal saline, followed by drying between 2 filter papers. The skin was used directly in the study without storage. Synthetic semipermeable membranes or mice skin membranes were mounted, with the stratum corneum side up and the donor compartment dry and open to the air, and floated on receiver solution for 24 hours for equilibration and prehydration. As described by El Maghraby et al,[26] this approach was suggested to maintain a transepidermal hydration gradient.[27] This proceedure was expected to be the driving force for skin penetration of deformable liposomes.[9] The receiver content was then replaced by fresh medium. Free SS was removed by ultracentrifugation for 2 hours at 15 000 rpm. Purified sediment was then diluted using purified water in the case of liposomes or hydroalcoholic solutions in the case of ethosomes. The volume of each formulation was adjusted to 2 mL, containing 10 mg of SS, for the in vitro permeation study. Test formulations were applied to the skin surface, which had an available diffusion area of 4.90 cm^2. Aqueous SS solution and SS hydroalcoholic solution (30% vol/vol, ethanol) were used as controls (n = 3). One-milliliter samples of the receptor medium were removed at appropriate intervals for determination of SS concentration spectrophotometrically, after precipitation of proteins using acetonitrile, and immediately replaced with fresh medium. At the end of this stage (24 hours), the donor compartment and the skin surface were washed 3 times with warm (45°C) receptor medium. Parallel experiments were run at the same time intervals using blank solutions free from the drug. The assay was validated. The accuracy, repeatability, specificity, and reliability were ensured. The recovery was 98.71%. The cumulative amount of the drug, Q ($\mu g/cm^2$), that permeated the mice skin per unit surface area was determined, and the flux, J ($\mu g/cm^2/h$), of the formulas was calculated.

The second stage was employed to determine the amount of drug deposited on the skin. The receptor content was completely removed and replaced by 50% (vol/vol) ethanol in distilled water and kept for a further 12 hours; then the absorbance was measured spectrophotometrically. This receiver solution diffused through the skin, disrupting any liposome structure and extracting deposited drug from the skin, thus giving a measure of skin deposition.[3,26] Each vesicular system was investigated in 3 cells. The significance of results was checked by the SPSS statistical program applying a 1-way ANOVA test and a significance level of $P < .05$.

Dispersion of Ethosomes in Pluronic F 127 Gel

The best achieved ethosomal dispersion, formula 8, was incorporated into 20% and 30% (wt/wt) Pluronic F 127 gels

(formulas 10 and 11, respectively). The specified amount of Pluronic F 127 powder was slowly added to cold physiological saline (5°C-10°C). The resulting dispersion was stirred gently with a magnetic stirrer bar, to ensure complete dissolution, and left overnight at 4°C. Eventually a viscous solution was obtained. For the gel to be formed, this solution was kept at 30°C for not less than 3 hours. Appropriate amounts of formula 8 containing SS (10 mg) were then incorporated into 5 g of the prepared gels with continuous stirring until homogeneous ethosomal gels were achieved. The in vitro permeation and mice skin deposition of SS from these gels was performed as described previously. The significance of results was compared with that achieved from formula 8.

Table 2. Physical Characteristics of the Vesicles: Diameter, Polydispersity Index, and Entrapment Efficiency Percentage (Mean ± SD, n = 3)

Batches	Vesicle Diameter (nm)	PI	Entrapment Efficiency (%)
Formula 1	470.2 ± 19.2	0.23 ± 0.07	23.3 ± 2.1
Formula 2	518.6 ± 23.4	0.18 ± 0.04	27.5 ± 1.9
Formula 3	548.2 ± 30.6	0.24 ± 0.07	30.0 ± 2.3
Formula 4	462.6 ± 21.6	0.21 ± 0.03	35.9 ± 3.1
Formula 5	411.2 ± 22.5	0.17 ± 0.03	39.7 ± 3.1
Formula 6	387.3 ± 24.4	0.32 ± 0.05	40.1 ± 3.0
Formula 7	331.8 ± 27.2	0.13 ± 0.06	44.9 ± 4.1
Formula 8	271.2 ± 25.3	0.11 ± 0.03	49.8 ± 3.2
Formula 9	267.8 ± 26.3	0.10 ± 0.04	50.6 ± 3.7

*PI indicates polydispersity index.

Results and Discussion

Vesicle Morphology

The examination of the prepared formulations by Leica optical microscope and TEM revealed the predominance of spherical-shaped vesicles. As shown in Figure 1, the vesicles are uniform in size and appear to be multilayered.

Vesicle Size Distribution

Laser diffraction studies showed significant differences in size between liposomes and ethosomes ($P < .05$). As illustrated in Table 2, the diameters of various liposomes ranged from 387.27 to 548.21 nm, while for ethosomal systems, the diameters were smaller and extended from 267.82 to 331.81 nm. The small PI values of most formulations could indicate their homogeneity. The smallest values (0.11 and 0.10) were observed with ethosomal formulations 8 and 9, respectively. On the other hand, the PI value of formula 6 (0.32) could indicate that it is a heterogeneous dispersion.

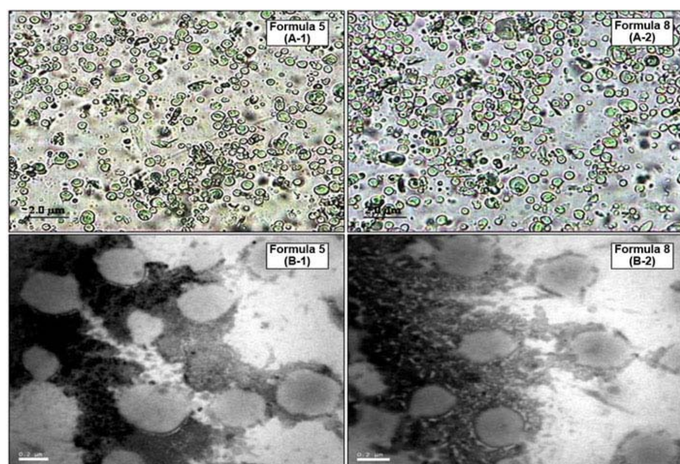

Figure 1. Optical micrographs of formula 5 (A-1) and formula 8 (A-2) and transmission electron micrographs of formula 5 (B-1) and formula 8 (B-2).

It is clear that increasing cholesterol concentration resulted in a marked increase in the mean vesicle size. For example, formula 3, which contained 30 mg of cholesterol, had a 16.58% bigger mean vesicle size than did formula 1, which contained 10 mg of cholesterol. Similar results were obtained by Lopez-Pinto et al,[13] who found that the mean liposome diameter of minoxidil increased from 412 to 520 nm upon increasing cholesterol concentration from 0 to 40 mg.

The effect of the negative charge inducer dicetylphosphate, commonly added to prevent aggregation of the prepared vesicles, was studied. The results showed that there was a significant decrease ($P < .05$) in the mean vesicle size when the amount of dicetylphosphate was increased. For example, the mean vesicle size of formula 6, which contained 7.5 mg of dicetylphosphate, was 33.91% lower than that of formula 2, which contained no dicetylphosphate. Incorporation of dicetylphosphate into vesicle bilayer membranes made the surfaces have negative charges. This resulted in marked repulsions and, hence, an expected increase in the distance between adjacent bilayers.[28] However, the electrostatic attraction between the positively charged drug and the negatively charged dicetylphosphate overcame the expected repulsion of the like charges. This resulted finally in a slight reduction in the mean vesicle size.

A further reduction in the mean vesicle size was achieved in ethosomal formulations. It was found that this reduction in mean vesicle diameter was directly proportional to the ethanol content of the vesicles. For example, the mean vesicle diameter of formula 9, which contained 40% alcohol, was 44.60% smaller than that of formula 5, which had no alcohol.

These results are in accordance with the findings of other authors,[10-13] who concluded that this generalized reduction in the mean vesicle diameters is due to the presence of ethanol in the vesicles. Lasic et al[28] suggested that ethanol probably causes a modification of the net charge of the system

and confers it some degree of steric stabilization that may finally lead to a decrease in the mean vesicle size.

Entrapment Efficiency

As shown in Table 2, the maximum entrapment percentage of 50.63% was reached at 40% (vol/vol) ethanol (formula 9). It was observed that liposomes were more stable at higher ethanol concentrations. Like dicetylphosphate, ethanol may exert a stabilizing effect in the formulation, preventing or at least delaying the formation of vesicle aggregates, because of the electrostatic repulsions. There are 2 factors governing the stability and hence the entrapment efficiency of the vesicles. The first is the ethanol concentration; the vesicles containing high ethanol concentrations have thinner membranes, corresponding to the formation of a phase with interpenetrating hydrocarbon chains.[29] The second factor is the presence of cholesterol, which contributes to vesicle stability as it provides a greater rigidity to the lipid layers and confers to the system a higher stability, a reduced likelihood of vesicle fusion, and a greater resistance to the high rotational energy exerted by ultracentrifugation.[30]

In Vitro Drug Permeation and Mice Skin Deposition Studies

The release of SS from liposomal vesicles decreased as cholesterol amount increased, as shown in Figure 2. The release data showed that the percentage of SS permeated through the semipermeable membrane after 24 hours was 99.57%, 97.07%, and 70.56% for cholesterol amounts of 10 mg, 20 mg, and 30 mg, respectively. Initially, the permeation rate was higher (up to 8 hours), because of the release of the surface-absorbed drug followed by a nearly zero-order release (up to 24 hours). The permeation of SS from its aqueous control solution was almost complete after 8 hours because of the effect of the synthetic membrane itself, as it controls the release of the drug through it.

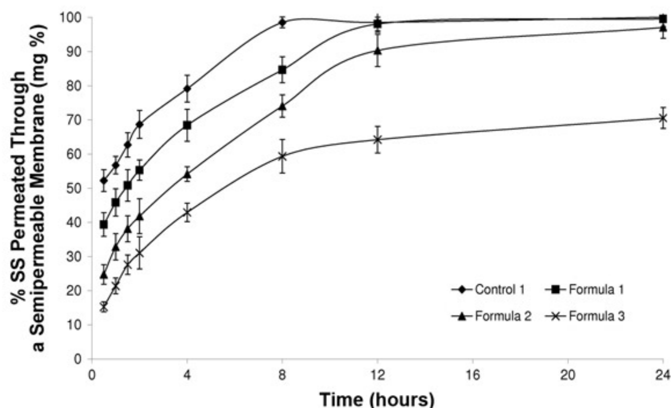

Figure 2. Effect of increasing cholesterol concentration on in vitro drug permeation from liposomes at pH 7.4 through a semipermeable membrane in Franz diffusion cells at 37ºC ± 0.5ºC. SS indicates salbutamol sulfate.

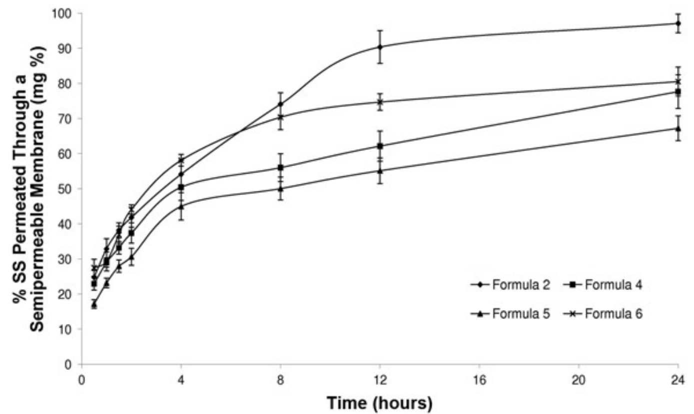

Figure 3. Effect of increasing dicetylphosphate concentration on in vitro drug permeation from liposomes at pH 7.4 through a semipermeable membrane in Franz diffusion cells at 37ºC ± 0.5ºC. SS indicates salbutamol sulfate.

Liposome system formula 2 was selected for further optimization studies, as it offered the best compromise in characteristics: mean vesicle size, drug entrapment efficiency percentage, and drug release rate.

From the drug release profiles shown in Figure 3, it was concluded that there is a marked retardation in the drug release rate in formulas containing dicetylphosphate as compared with formula 2. There is an inversely proportional relationship between the amount of dicetylphosphate and the drug release rate. This relationship held true until a dicetylphosphate concentration of 5 mg—that is, formula 5. The percentage of the drug that permeated the semipermeable membrane from formulas 4 and 5 was 77.63% and 67.20%, respectively, after 24 hours, compared with 97.07% from formula 2, which contained no charge inducer. Interestingly, upon increasing the dicetylphosphate concentration to 7.5 mg in formula 6, the percentage of drug permeated rose again, reaching 80.49%. However, it was still lower than that obtained with formula 2. This could be attributed to layer instability upon addition of large amounts of a charge inducer, causing repulsion between charged layers.[31,32] The high PI value of these vesicles (0.32) could support this suggestion.

Based on the previous results, liposomal formula 5 was selected for further in vitro drug permeation and mice skin deposition studies. It was compared with ethosomal formulations containing 20%, 30%, and 40% ethanol (formulas 7, 8, and 9, respectively) and 2 control solutions.

The permeation of SS from the previously mentioned formulas via skin of newborn mice is shown in Figure 4. It is clear that the cumulative SS percentage that permeated the

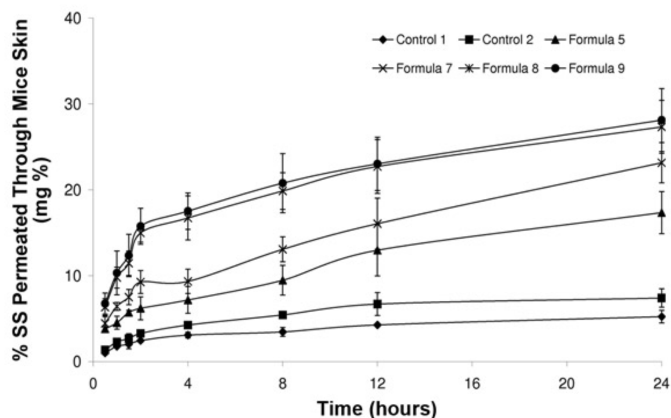

Figure 4. In vitro drug permeation from the selected liposomes and ethosomes with increasing ethanol concentrations, at pH 7.4, through mice skin in Franz diffusion cells at 37ºC ± 0.5ºC. SS indicates salbutamol sulfate.

mice skin after 24 hours for both control 1 and control 2 was significantly lower (5.23% and 7.40%, $P < .05$) than that of other liposomal or ethosomal formulations, indicating that vesicular systems can improve skin delivery, especially for water-soluble drugs like SS. The percentage SS permeated from liposomal formula 5 after 24 hours was 17.34%. This is significantly lower than that released from the ethosomal formulas ($P < .05$), where the percentage permeated was 23.15%, 27.32%, and 28.12% for formulas 7, 8 and 9, respectively.

Similar conclusions could be obtained upon analysis of the drug flux of the same formulas to the receiver compartment, shown in Table 3. The drug flux values of ethosomal formulas 7, 8, and 9 were significantly higher ($P < .01$) than that of liposomal formula 5. This could indicate that ethanol enhances drug penetration across the skin layers. Of the prepared ethosomal formulations, the highest drug flux (14.42 ± 0.83 μg/cm^2/h) was achieved with formula 8. Taking into account the drug flux values (4.95 ± 0.23 and 5.59 ± 0.16) of the control 1 and control 2 solutions, respectively, it is possible to appreciate the enhancer effect of ethanol. The mechanism of release kinetics was evaluated by fitting the permeation data to the zero-order, first-order, and Higuchi diffusion models. All permeation profiles fit the Higuchi diffusion model, and a linear relationship was found between the amount of drug released and the square root of time. It could be concluded that the vesicles acted as reservoir systems for continuous delivery of the encapsulated drug.

Several studies[4,32,33] have investigated possible mechanisms by which vesicles could improve skin delivery of drugs. First, vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin. Second, vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and modify the intercellular lipid lamellae. This facilitates penetration of free drug molecules into and across the stratum corneum. It was also claimed that intact deformable liposomes penetrated through the stratum corneum, under influence of the naturally occurring in vivo transcutaneous hydration gradient, and through the underlying viable skin into blood circulation. Recently, an in vivo electron microscopic study demonstrated a fast (within 1 hour of application) partitioning of intact surfactant-based elastic vesicles into human stratum corneum, but almost no vesicles could be found in the deepest layers of the stratum corneum. Therefore, it was expected that, as a result of the osmotic force, vesicles would not penetrate beyond the level of the lowest layers in the stratum corneum. Results of studies showing that deformable liposomes improved skin deposition of only some drugs could support this proposal. The encapsulated drug can penetrate deeper transdermally, thus, drugs have to be released from vesicles in order to reach the systemic circulation. Several studies also suggested a possible penetration-enhancing mechanism (mechanism 2) for surfactant-based elastic vesicles and for deformable liposomes.[33,34]

On the other hand, ethosomes were reported to be effective at delivering molecules to and through the skin to the systemic circulation. Ethanol is a well-known permeation enhancer. However, previous studies comparing permeation enhancement of drugs from ethosomal systems and hydroethanolic solutions showed that permeation enhancement from ethosomes was much greater than would be expected from ethanol alone. The stratum corneum lipid multilayers are densely packed and highly conformationally ordered at physiological temperature. Ethanol interacts with lipid molecules in the polar head group region, resulting in a reduction in the phase transition temperature (Tm) of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effects of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier. Ethanol may also provide the vesicles with a softness and flexibility

Table 3. Drug Flux Values of the Different Formulas Into the Receiver Solution via Mice Skin (Mean ± SD, n = 3)

Batches	Drug Flux (μg/cm^2/h)
Control 1	4.95 ± 0.23
Control 2	5.59 ± 0.16
Formula 5	8.40 ± 0.34
Formula 7	11.92 ± 0.45
Formula 8	14.42 ± 0.83
Formula 9	14.08 ± 0.56
Formula 10	5.25 ± 0.29
Formula 11	4.43 ± 0.13

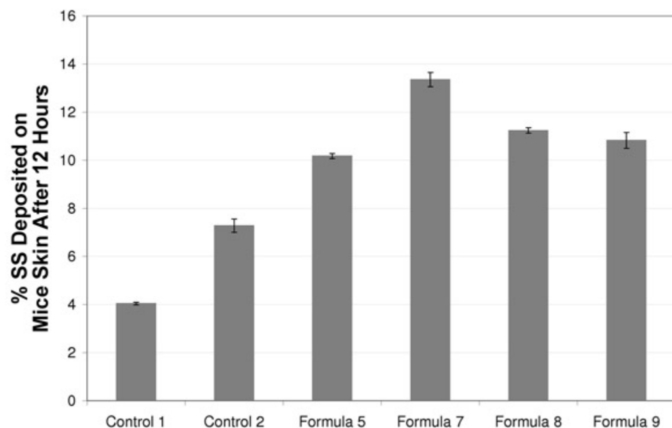

Figure 5. Percentage of SS deposited on mice skin in vitro after 12 hours of extracting different control, liposomal, and ethosomal formulas with 50% hydroalcoholic solution at 37ºC ± 0.5ºC. SS indicates salbutamol sulfate.

that allows them to more easily penetrate into deeper layers of the skin. The ethosome vesicles can forge paths in the disordered stratum corneum. The release of drug in the deep layers of the skin and its transdermal absorption could then be the result of fusion of ethosomes with skin lipids and drug release at various points along the penetration pathway.[33]

These findings may explain the results of in vitro skin deposition from different formulas, as shown in Figure 5. The percentage of SS deposited in skin from liposomal formula 5 and from ethosomal formulas 7, 8, and 9 is higher than the percentage deposited from both control 1 and 2. Transport of the drug by liposomes or ethosomes into the stratum corneum bypasses the main barrier to drug permeation, which considerably improves skin delivery. Drug encapsulation may greatly improve skin deposition. However, several factors may affect the extent to which this role improves transdermal flux, such as drug release from the vesicles in the stratum corneum. The rate and amount of released drug is a balance between 2 factors: (1) drug affinity to vesicles, and (2) drug solubility in lipids of the stratum corneum.

Statistical analysis of the previous results (ie, the percentage of SS permeated through mice skin after 24 hours and the percentage of SS deposited in the skin) revealed that formulas 8 and 9 are more efficient than formula 7 in delivering SS transdermally. Indeed, there was no significant difference ($P > .05$) between the results of formulas 8 and 9; therefore, formula 8, having a lower ethanol content (30% vol/vol), was chosen for further studies.

As the ethanol content is increased (30% and 40% vol/vol) in formulas 8 and 9, respectively, the rate of drug permeated is increased and the percentage drug deposited (11.42% and 10.83%, respectively) is decreased, compared with 13.37% of the drug deposited with formula 7. This may be explained with respect to the role of ethanol in ethosome formulation, which favors or enhances the permeation of the hydrophilic drug SS through the stratum corneum and demonstrates that, to permeate skin, the drug must be released first. So the percentage SS deposited in skin was higher with formula 7, which contains 20% ethanol, indicating that it needs more time to be permeated through the skin layers; the fact that formula 7 has the lowest permeation rate among the ethosomal formulations clarifies this. For hydrophilic drugs like SS, the penetration-enhancing effect seems to play a more important role in the enhanced skin delivery than it does for lipophilic drugs, since permeation of hydrophilic molecules tends to be relatively slower and hence more enhanceable. Results of the current study support the existence of the penetration-enhancing effect. The intact vesicle permeation mechanism will also have an important role, especially in improving skin deposition. However, drug release from vesicles in the stratum corneum is a critical step that affects transdermal flux.[35]

The permeation of SS from various Pluronic F 127 gels is presented in Figure 6. It is clear that the drug permeation from formula 10 (20% wt/wt Pluronic gel) was higher than that of formula 11 (30% wt/wt Pluronic gel). As for the original ethosomal suspensions, the permeation profiles of these formulas were fitted to the Higuchi diffusion model.

Conclusively, formula 10 was considered to be the most suitable system for the delivery of SS for 2 reasons. The first is its higher drug flux than that of formula 11, as shown in Table 3. The second reason is related to the cumulative percentage of the drug permeated through mice skin over 24 hours, ~22% (≈2200 µg). This value is close to the target system that delivers SS at a rate of 100 µg/h over the same period. The surface active properties of Pluronic gels would add further advantages to this system that is capable of delivering SS at a controlled release rate.

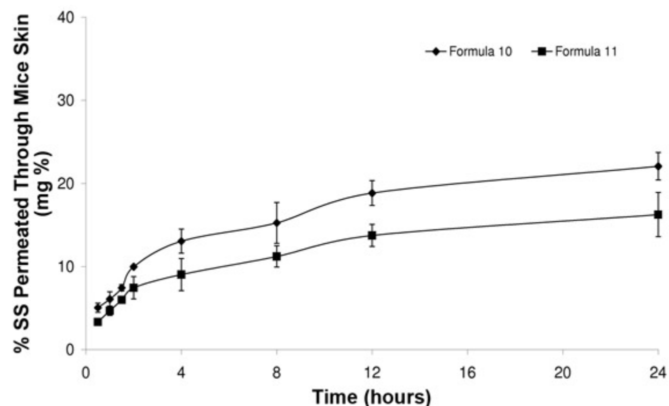

Figure 6. In vitro drug permeation from Pluronic F 127 gels containing the selected ethosomes at pH 7.4 through mice skin in Franz diffusion cells at 37ºC ± 0.5ºC. SS indicates salbutamol sulfate.

CONCLUSION

The presence of ethanol in the aqueous compartment of the ethosomal vesicles favored the encapsulation of SS and enhanced its permeation via the skin of newborn mice because of the synergistic effect of ethanol, vesicles, and skin lipids. Ethosomal systems are capable of delivering higher amounts of SS at a controlled release rate through mice skin than classic liposomes are. Further studies on the delivery of hydrophilic drugs through human skin via ethosomes should be performed in the future.

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