

Original Research

In vitro transdermal delivery of propranolol hydrochloride through rat skin from various niosomal formulations

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Abstract

Objective(s): The purpose of the present study was to prepare and to evaluate a novel niosome as transdermal drug delivery system for propranolol hydrochloride and to compare the in vitro efficiency of niosome by either thin film hydration or hand shaking method.

Materials and Methods: Niosomes were prepared by Thin Film Hydration (TFH) or Hand Shaking (HS) method. Propranolol niosomes were prepared using different surfactants (span20, 80) ratios and a constant cholesterol concentration. In vitro characterization of niosomes included microscopical observation, size distribution, laser light scattering evaluation, stability of propranolol niosomes and permeability of formulations in phosphate buffer (pH=7) through rat abdominal skin.

Results: The percentage of entrapment efficiency (%EE) increased with increase in surfactant concentration in all formulations. Among them, F3 formulation (containing span80:cholesterol ratio of 3:1) showed the highest entrapment efficiency (86.74±2.01%), Jss (6.33µg/cm².h) and permeability coefficient (7.02 × 10⁻³cm/h). By increasing the percentage of entrapment efficiency (resulting in increase in surfactant concentration), the drug released time is not prolonged. Among all the formulations, F4 needed more time for maximum drug release. Among these formulations, F4 was also found to have the maximum vesicle size as compared to other formulations. It was observed that niosomal suspension prepared from span 80 was more stable than span 20.

Conclusion: This study demonstrates that niosomal formulations may offer a promise transdermal delivery of propranolol which improves drug efficiency and can be used for controlled delivery of propranolol.

Keywords: Niosomes, Propranolol hydrochloride, Transdermal delivery, in vitro

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Introduction

Transdermal delivery has many advantages over conventional methods of drug administration, because it avoids hepatic first-pass metabolism, potentially decreases side effects and improves patient compliance. Propranolol, a beta-adrenergic blocking agent used in the treatment of hypertension, is reportedly subjected to an extensive and highly variable hepatic first-pass metabolism following oral administration (1,3). Controlled administration of propranolol via transdermal delivery system could improve its systemic bioavailability and therapeutic efficacy by avoiding first-pass effect, as well as decreasing the dosing frequency required for treatment. This study investigates the *in vitro* skin permeation of propranolol delivery from niosomal preparation.

Niosomal drug delivery has been studied using various methods of administration (3) including intramuscular (4), intravenous (5), oral and transdermal (6,7). In addition, as drug delivery systems, niosomes have shown to enhance absorption of some drugs across cell membranes (8), localize drugs in targeted organs (9) and tissues and elude the reticuloendothelial system. Niosomes have been used to encapsulate colchicines (10), estradiol (11), tretinoin (12,13), dithranol (14,15) enoxacin (16) and for application such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine (4,5,8,17-22). Niosomes are preferred over other vesicular systems as they offer some advantages (23, 24) as following: it provides water-based vehicle suspension. offering better patient compliance in comparison with oily dosage forms. They possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties which can accommodate drug molecules with a wide range of solubilities. The characteristics of the vesicle formulation are variable and

controllable. Altering vesicle composition, size, lamellarity, trapped volume, surface charge and concentration can control the vesicle characteristics. The vesicles may act as a depot, releasing the drug in a controlled manner. Other advantage of niosomes includes their osmotically active and stable structures as well as their tendency to increase the stability of entrapped drug. Handling and storage of surfactants requires no special conditions. They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs. They can be made to reach the site of action by oral, parenteral as well as topical routes. The surfactants are biodegradable, biocompatible and non-immunogenic. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells. Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.

Materials and Methods

Materials

Propranolol was purchased from Daropaksh Pharmaceuticals Company, Iran; Spans (20, 80), cholesterol, diethyl ether and methanol were obtained from Merck Chemical Industries, Germany. All chemicals and solvents were of analytical grade.

Methods

Preparation of Niosomes

Niosomes were prepared by thin film hydration method using different grades of surfactants (span 20 & 80) and cholesterol in constant ratio as the composition shown in Table 1. The surfactants and cholesterol were dissolved in diethyl ether and solvent was then evaporated under reduced pressure

Table 1. percentage drug Entrapment Efficiency (%EE) of selected niosomal formulations (mean \pm S.D, n=3).

Formulation	(%EE)
F1	77.05 \pm 1.22
F2	81.19 \pm 1.09
F3	86.74 \pm 2.01
F4	62.01 \pm 1.85
F5	66.85 \pm 1.66
F6	70.99 \pm 0.97

using rotary flash evaporator (super fit rotary vacuum) at 150 rpm for 5-10 min. This intermittent vortexing at 50°C results in deposition of thin layer of solid mixture on the sides of the flask. Then it was hydrated with aqueous phase containing the drug (1 mg/ml) in 40 ml of distilled water, vortexed and heated at 60-70°C for 1 hour. The resulting multilamellar vesicles were cooled in an ice bath and sonicated by using probe type Ultrasonicator (Elma, Germany) for 3 min at 150V for preparing of unilamellar vesicles of niosomes. These niosomal vesicles are stored at 4°C in a refrigerator. Plain niosomes, as control for each formulation, were prepared without the drug using the same procedure (25).

Entrapment Efficiency Percentage (%EE)

Percentage of entrapment efficiency was determined by centrifuge method. A niosomal suspension (15 ml) was centrifuged at 2000 rpm for 30 min at 4°C.

The supernatant liquid was diluted with phosphate buffer (pH=7) and was assayed by UV spectrophotometry at 289 nm (26). The percentage of drug encapsulation was calculated by the following equation:

$$EE (\%) = [(C_t - C_f)/C_t] \times 100$$

Where C_t is the concentration of total drug and C_f is the concentration of untrapped drug.

Stability of propranolol niosomes

The samples were stored at 4°C and 25°C for 8 weeks and stability and drug content per gram of all samples were determined after 8 week (27).

Animal experiments

Male adult Albino Wistar rats (weighing 150-200 g and aged 10-12 weeks) were obtained from Animals Laboratory, Ahvaz Jundishapur University of Medical Sciences. The hair on the abdominal skin was removed with an electric clipper, taking care not to damage the skin. The rats were anaesthetized with ether prior to sacrificing them. Abdominal full-thickness skin was removed and any extraneous subcutaneous fats cleaned from the dorsal side using cooled acetone solution. Whole skin thickness was measured using a digital micrometer (AAOC, France).

Vesicle size determination

Mean size of niosomal formulations were measured at 25°C by photon correlation spectroscopy, Scatterscop particle size analyzer (Malvern-Korea). Light scattering was monitored at 25°C at a scattering angle of 90°.

Scanning Electron Microscopy(SEM)

The prepared niosomes size and shape was studied using SEM. The niosomes prepared by thin film hydration method were mounted on an aluminum stub with double-sided adhesive carbon tape. The vesicles were then sputter-coated with silver using a vacuum evaporator and examined with the scanning electron microscope (LEO, VP 1455- Germany) (Figure1).

In vitro skin permeation studies

In vitro skin permeation of propranolol niosomes were studied using modified vertical Franz-diffusion cells with an effective diffusion area of approximately 3.46 cm².

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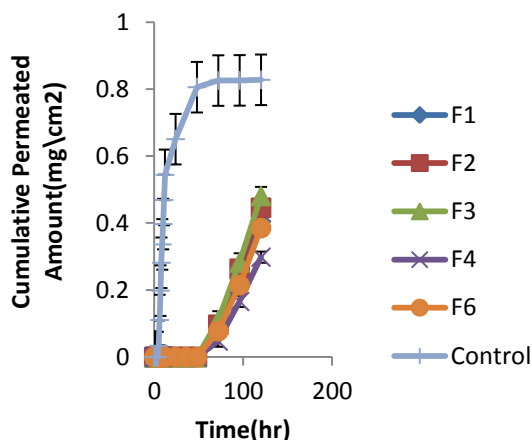


Figure 1. Scanning Electron Microscopy (SEM) photographs of F4 formulation.

Full thickness Albino Wistar rat skin was placed securely between donor and receptor compartment with the epidermis site facing the donor compartment. The receptor compartment was filled with 20 ml phosphate buffer (pH=7) solution which was continuously stirred with a small magnetic bead at 300 rpm and thermo stated at $37\text{C}^{\circ} \pm 1\text{C}^{\circ}$ throughout the experiment. After 16 hr equilibrium, 3 g of propranolol niosomal were placed on to the skin surface. At predetermined time intervals (0.5, 1, 2, 3,.....,120 h) a 2 ml of sample was withdrawn and replaced with an equal volume of fresh phosphate buffer(pH=7) solution to ensure sink conditions.

Statistical Analysis

All data are expressed as mean \pm SD. Statistical comparison was made using one-way ANOVA and $p < 0.05$ was considered statistically significant.

Results

Entrapment Efficiency Percentage (%EE)

The percentage of entrapment efficiency of all formulations was found to be in decreasing order of $F_3 > F_2 > F_1 > F_6 > F_5 > F_4$. EE (%) of different formulations are shown in (Table1). In niosomal formulations prepared

using sorbitan monoesters, span 80 showed the maximum entrapment efficiency at 3:1 (surfactant:cholesterol) molar ratio.

Vesicle size determination

The vesicle size was found in the range of 3.1 μm to 35.35 μm as shown in Table 2. The vesicle size of propranolol niosomal formulation was found to be in decreasing order of $F_4 > F_5 > F_6 > F_1 > F_2 > F_3$. Among these formulations, F_4 was found to have maximum vesicle size and F_3 have minimum vesicle size as compared to other formulations.

Stability Study

Stability studies were performed on F_1 and F_4 niosomal formulations for a period of 8 weeks by subjecting them to aging at 4°C . Indirect relationship between the entrapment efficiency of the drug in the vesicles and aging was observed. As the storage period increased, the degree of entrapment efficiency decreased. It was observed that formulations prepared by span 80 were more stable than niosomes prepared by span 20. Also, niosomal suspensions were more stable in 4°C than 25°C . The results are shown in Table 3.

In vitro permeation studies

The results of in vitro skin permeation are shown in Table 4. Permeation profiles of propranolol hydrochloride through the excised rat skin from the niosomal formulations and control is shown Figure 2.

The results showed in Figure (2) and tabulated in Table (3) indicate that the propranolol permeation through rat skin in successfully controlled. The results indicate that all niosome formulations decreased the permeability across rat skin compared with control.

While propranolol is released from the water drug solution (control sample) and permeated completely in less than 3 hrs, niosomal

Table 2. Compositions of selected niosome formulations and particle size (mean±SD, n=3).

Formulations	Surfactant	Drug	surfactant	Cholesterol	Particle Size(µm)	Poly dispersity Index (PDI)
F ₁	Surfactant 80	1mg/ml	3 g	3 g	16.51 ± 1.06	0.375±0.14
F ₂		1mg/ml	6 g	3 g	6.93 ± 2.15	0.389±0.05
F ₃		1mg/ml	9 g	3 g	3.10 ± 1.08	0.379±0.11
F ₄	Surfactant 20	1mg/ml	3 g	3 g	35.96 ± 2.91	0.395±0.08
F ₅		1mg/ml	6 g	3 g	30 ± 3.38	0.371±0.07
F ₆		1mg/ml	9 g	3 g	22.96 ± 2.59	0.383±0.021

formulations were able to delay the process up to 62.38 h. Formulations F₄ and F₃ showed the minimum and maximum J_{SS}, respectively, with a range of 3-6.33 µg/cm²/h for all of the formulations. The J_{SS} values of all of the formulations were significantly less than that of control (p<0.05). The results of permeability coefficient (P) showed the maximum value of 7.02×10^{-3} cm/h and a minimum of 5.03×10^{-3} cm/h for F₃ and F₄ samples, respectively. There was a significant decrease in permeability coefficient (P) for all of the formulations when compared with control (p<0.05), indicating their ability to control drug permeation. There was also a significant increase in T_{Lag} for different formulations if compared with control (p<0.05), confirming their retarding properties.

Discussion

Amongst many reported methods for the preparation of niosomes, thin film hydration method was selected since this method was able to encapsulate hydrophobic drug with higher entrapment efficiency and smaller particle size. The niosomal formulations with Tweens display poor entrapment with lipophilic or amphiphilic drugs whereas Spans give higher entrapment with high stability. This is due to the fact that hydrophilic surfactants (such as Tweens) owing to high aqueous solubility do not form proper

vesicular structure in aqueous medium, whereas due to more lipophilic nature, Spans form vesicles and entrap the lipophilic drug or amphiphilic drugs. Niosomes are composed of non-ionic surfactants which are biocompatible and relatively non-toxic and themselves serve as an excellent permeation enhancer (15).

In this study in order to assess the influence of the drug carrier on the diffusion of drug through skin, in vitro permeation studies (Figure 2), using stripped Albino Wistar rat skin and vertical Franz diffusion cell was utilized. In the present study transdermal controlled permeability of propranolol hydrochloride molecule (water-soluble and low molecular weight (295.8 Da) drug was studied.

One of the mechanism by which niosomes may contribute to transdermal drug delivery may be described to the fusion of vesicles on the surface of the skin and hence enhanced skin permeation (28, 29). Moreover, it has been proven that niosomes enhance penetration and retention of topically applied drug (30).

Niosomal propranolol formulations were designed to control drug transdermal permeation. The higher T_{Lag} of the formulations, it is expected that they may cause structural changes in skin layers and hence affect the drug distribution in different layers. The results also show that Span 80, a surfactant with HLB of 4.3,

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Table 3. Physical stability of niosomal formulations at room (25C⁰) temperature and refrigerate (4C⁰) (Mean±SD, n=3).

Formulations	Storage Temperature	% Entrapment Efficiency		
		1st Day	3 weeks	8 weeks
F ₁	Room (25C ⁰)	61.04± 0.057	57.35 ± 0.066	51.42 ± 0.017
F ₁	Refrigerator (4C ⁰)	62.51± 0.023	61.49 ± 0.071	59.24 ± 0.013
F ₄	Room (25C ⁰)	59.73±0.099	50.07 ± 0.072	46.86 ± 0.028
F ₄	Refrigerator (4C ⁰)	60.27 ± 0.087	57.83 ± 0.068	54.98 ± 0.023

Table 4. *In Vitro* permeability parameters for propranolol hydrochloride and control (Mean±SD, n=3).

Formulations	J _{SS} (µg/cm ² .h)	T _{Lag} (hr)	D(cm ² .h ⁻¹)	P(cm/h)
F ₁	4.34 ± 0.015	58.33 ± 0.1	1.4 × 10 ⁻⁵ ± 3.06 × 10 ⁻⁷	5.94 × 10 ⁻³ ± 0.007
F ₂	5.3 ± 0.011	56.16 ± 3.92		6.72 × 10 ⁻³ ± 0.003
F ₃	6.33 ± 0.012	51.26 ± 2.02		7.02 × 10 ⁻³ ± 0.009
F ₄	3 ± 0.001	62.38 ± 5.6	1.31 × 10 ⁻⁵ ± 1.21 × 10 ⁻⁶	5.03 × 10 ⁻³ ± 0.001
F ₅	3.660 ± 0.017	57.68 ± 9.6	1.44 × 10 ⁻⁵ ± 2.35 × 10 ⁻⁶	5.9 × 10 ⁻³ ± 0.004
F ₆	4.66 ± 0.01	51.14 ± 3.4	1.6 × 10 ⁻⁵ ± 1.14 × 10 ⁻⁶	6.58 × 10 ⁻³ ± 0.005
Control	6.066 ± 0.009	3.01 ± 1.08	2.91 × 10 ⁻⁴ ± 8.69 × 10 ⁻⁵	0.02 ± 0.0036

has increased loading efficiency in comparison to Span 20 (HLB=8.6). The reason may be the ability of more lipophilic surfactant (Span 80) which results in enhanced solubility of hydrophilic drug (propranolol) in lipid phase. Practically, increase of surfactant-lipid ratio has enhanced permeability rate of niosomal formulations. The average particle size of Span 80 formulations is also less than that of Span 20 containing samples. The minimum and maximum mean particle size were obtained for F₃ and F₄ formula, respectively, which is in accordance with their release and permeation results. It can be suggested that along with decrease in particle size, the surface area of particles increase and subsequently release and permeation increase. Figure 1 shows the SEM images of F₄ niosomal formulation. According to the results, niosomal formulations caused a reservoir effect for

drug that lead to the drug entrapment in niosomal composition and also skin layers and therefore showed a retardation effect. In other words, niosomes have lowered diffusion coefficient (D) and hence their P and J_{SS} indices. Ruckmani et al have also previously reported a retardation in cytarabine hydrochloride release for niosomal formulations containing sorbitan ester/cholesterol or polyoxyethylene sorbitan esters/cholesterol (20). Comparing their results with our findings, it could be suggested that high molar ratio of cholesterol can significantly lower the release rate of propranolol HCl. Our findings are in accordance with Bisby et al study that reported the effect of cholesterol concentration on release of calcein from niosomal formulations. Their results showed that increasing the cholesterol molar control content to more than 5% was considerably decreased the drug release

(31). It is generally accepted that higher surfactant ratio increases hydrophilic drug solubility in lipid phase, cholesterol, the drug affinity to the vehicle, and therefore enhances their entrapment efficacy. Another suggested mechanism is lowering the size average of particles, increasing the surface area and hence enhancing their loading and permeability properties (33). The results of permeation study are evidences for such mechanisms. There have been the most rapid release and permeation for F₃ which had the minimum particle size and the maximum loading index. On the contrary, F₄ that had the largest particle size and minimum loading efficacy, showed a considerable delay in drug release and permeation.

Also, due to relatively higher lipophilicity of external skin layers, i.e. stratum corneum, more lipophilic vehicle prepared from Span 80 have more readily penetrated into the skin, interacted with skin constituents followed by the release of their drug content, a phenomenon that may not be considered for Span 20 containing formulations. Therefore, it may be concluded that niosomal formulations of propranolol HCl are able to reduce P and J_{SS} coefficients by decreasing diffusion coefficient. The proposed mechanisms are the reservoir effect and retention capacity of niosomes. The effect is a concentration-dependent phenomenon. In other words, the more molar ratio of surfactant, the higher diffusion coefficient followed by P and J_{SS} enhancement. The effect of surfactants is due to disruption of lipid bilayer in the stratum corneum (34).

Conclusions

Thin film hydration method used for the preparation of propranolol niosomes was found to be a proper technique to encapsulate hydrophobic drug in non-ionic surfactants. The non-ionic surfactant

prepared showed reasonable drug entrapment, suitable size and good controlled drug permeability.

In this work, niosomes were prepared by variable surfactant and constant cholesterol concentrations. The impact of surfactant and cholesterol in the entrapment efficiency and release rate was significant.

From this work, it is concluded that by increasing surfactant concentration entrapment efficiency increases. Among all the formulations, F₃ formulation (with span 80 & cholesterol ratio 3:1) showed highest entrapment efficiency of 86.74±2.01%. It was observed that niosomal formulation prepared from span 80 was more stable than that of span 20.

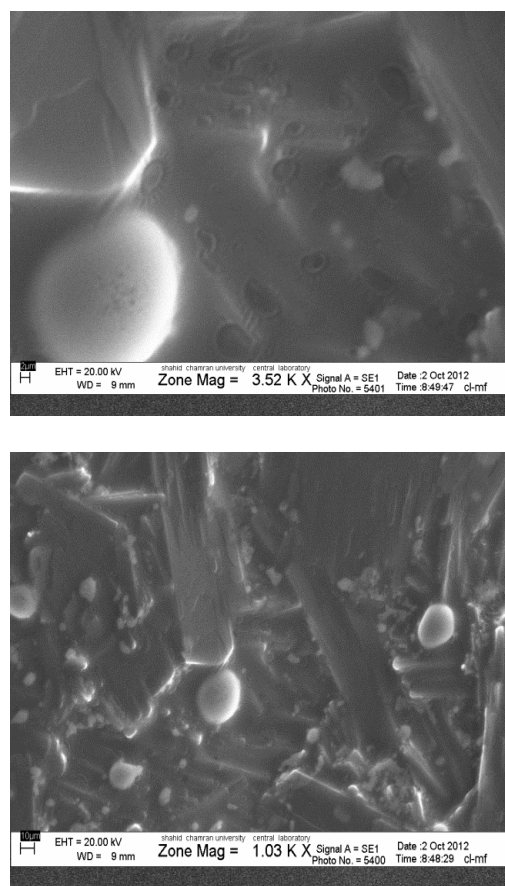


Figure 2. Permeation profiles of propranolol hydrochloride through the excised rat skin from the niosomal formulations and control.

Also, all of the niosomal formulations were more stable in refrigerator temperature than room temperature.

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