

PRONIOSOMAL GEL AS A CARRIER FOR TRANSDERMAL DRUG DELIVERY OF CLOTRIMAZOLE

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ABSTRACT

Niosomes are non-ionic based surfactant vesicles that have potential application in the delivery of hydrophobic and hydrophilic drugs. Clotrimazole is an antifungal drug widely used in the treatment of fungal infections such as ring worm, tenia pedis, tenia cruriris, jock itch etc. It's oral route is associated with severe side effects in the g.i.t. Transdermal drug delivery has been recognized as an alternative route to oral delivery. Proniosomal gel offers a versatile delivery concept with the potential for drug delivery via the transdermal route. In this study different proniosomal gel bases were prepared using different non-ionic surfactants such as Span 60, Span 40, Brij 72 and Tween 80 and characterized by light microscopy, S.E.M, assessed for their rate of spontaneity, drug entrapment, and stability. The release through cellulose membrane and hairless mice skin was also carried out. The proniosomes prepared from Span 60: Cholesterol (9:1) proved to be stable with high entrapment and release efficiencies as compared to Span 40, Brij 72 and Tween 80. Proniosomal gel may be a promising carrier for the transdermal delivery of the antifungal drug Clotrimazole.

Keywords: Niosomes, Proniosomes, Clotrimazole, Transdermal drug delivery.

INTRODUCTION

For many decades treatment of an acute disease or a chronic illness has been accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, pills, creams, injectables etc. as drug carriers. This type of drug delivery system is known to provide a prompt release of drug.

Recently several technical advancements have been made. They have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity or targeting the delivery of drugs to a tissue ¹.

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task.

The liposome and niosome technologies especially have provided a spectrum of options and opportunities for designing and practicing the site specific and targeted drug therapy. The structural versatility of vesicular systems in terms of vesicle size, shape, surface morphology, composition, surface charge and bilayer fluidity and the ability to incorporate a wide range of drugs could be exploited for clinical and therapeutic benefits². Encapsulation of the drug in vesicular structures is one such system which can be expected to prolong the duration of drug in systemic circulation and to reduce the toxicity by selective up taking ³.

Niosomes can entrap both hydrophilic and lipophilic drugs either in aqueous layer or in vesicular membrane of lipid material ⁴. The advancements in the niosome lead to the evolution of proniosomal delivery systems. Proniosomes are non-ionic based surfactant vesicles which may be hydrated immediately before use to yield aqueous niosome dispersions. They can incorporate both hydrophilic as well as lipophilic drugs and due to their capability to carry a variety of drugs, these carriers are extensively used for drug delivery. Proniosomes are nowadays used to enhance drug delivery in addition to conventional niosomes. They are converted into niosomes respectively upon simple hydration or by the hydration of

skin itself after application. There size range ranges from 10-100 nm. The proniosome derived niosomes are superior to conventional niosomes in terms of storage, transportation and dosing ⁵. In this study proniosomes have been investigated as a carrier for the transdermal delivery of Clotrimazole.

Clotrimazole is an antifungal azole derivative widely used in the treatment of fungal infections. The oral use of Clotrimazole is unacceptable due to g.i.t disorder, first pass effect and short half life (2hrs). Conventional creams and gels are unacceptable as they reside for short time, show min absorption and hence less therapeutic effect. The aim of this study is to entrap the drug within the vesicles in the form of proniosomal gel and deliver it transdermally in order to obtain improved bioavailability, prolonged delivery, overcome problems of frequent dosing and increase patient compliance.

MATERIALS AND METHODS

Clotrimazole was obtained as a gift sample from National Laboratories, Baddi. Span 60 and Span 40 was purchased from C.D.H Laboratories. Brij 72 was obtained from Standard Chemicals, New Delhi. Tween 80 was purchased from Qualikems Chemicals. Cholesterol was purchased from C.D.H. Ethanol and propylene glycol was obtained from Qualikems. Potassium di-hydrogen phosphate and Di-sodium hydrogen phosphate was purchased from Qualikems.

Preparation of proniosomal gel

Proniosomal gel was prepared by the method used by Alsarra et al ⁶ with a slight modification. The compositions of different proniosomal formulations are listed in Table 1. Using a wide mouthed glass vial 100 mg of clotrimazole with surfactant, cholesterol was mixed with 2.5 ml of absolute ethanol. The open end of the vial was covered and the vial was warmed in a water bath at 70°C for 5 min. Then 3-4 drops of propylene glycol as aqueous phase was added and mixture was further warmed in a water bath for about 2 min so that a clear solution was obtained. The mixture was allowed to cool to room temperature until the dispersion was converted into proniosomal gel.

Table 1: Formulation of Proniosomal gel of Clotrimazole

Formulation Code	Clotrimazole (mg)	Span 60 (mg)	Span 40 (mg)	Brij 72 (mg)	Tween 80(mg)	Cholesterol (mg)
S 60	100	1800	-----	-----	-----	200
S 40	100	-----	1800	-----	-----	200
B 72	100	-----	-----	1800	-----	200
T 80	100	-----	-----	-----	1800	200

Microscopical examination

Optical Microscopy

In a glass tube 0.2 g of proniosome gel of different surfactants was diluted with 10 ml of distilled water containing 2-3 drops of propylene glycol. It was vortexed for about 2 min. A few drops the formed niosomal dispersion were spread on a glass slide and examined. Photomicrographs were taken using a Canon digital camera ^{6,7}.

Scanning Electron Microscopy

The shape, surface characteristics of the prepared proniosome derived niosome vesicles was examined using a S.E.M. Model No. SEM 505 PHILIPS (Made in Holland). 0.2 g of gel was diluted with 10 ml of distilled water. Sample was applied on aluminium stub using a double sided carbon tape. Then the gold-palladium coating was done. Photographs were observed using SEM equipped with a digital camera at 20 kV accelerating voltage ⁶.

Clotrimazole Encapsulation Efficiency

To 0.2 g of proniosomal gel, weighed in an open glass tube was added 10 ml of phosphate buffer pH 7.4. The CT- containing niosomes were separated from unentrapped drug by centrifugation by (Remi compifuge) at programme 1; 20,000 rpm at 20°C for 30 min. The supernatant was recovered and assayed by U-V method for CT content. The percentage of drug encapsulation (EP(%)) was calculated by the following equation

$$EP (\%) = [(C_t - C_r) / C_t] \times 100\%$$

Where C_t is the concentration of total CT and C_r is the concentration of free CT ⁶.

In vitro release study

The in vitro release studies were performed by the method used by Alsarra et al with a slight modification. 1 gm of proniosomal gel of different surfactants was weighed. It was spread on the cellophane dialyzing membrane which was securely mounted on the bottom end of an open ended glass tube using cellotape. The assembly was immersed in a 250 ml beaker containing phosphate buffer pH 7.4 which was placed over a magnetic stirrer. The whole assembly was clamped using a burette stand. Sink condition was maintained and samples were withdrawn at time interval of 1 hr, 2 hr, 3hr, 4hr, 5hr, 6hr. Drug release was studied using Shimadzu U-V Spectrophotometer at 262 nm ⁶.

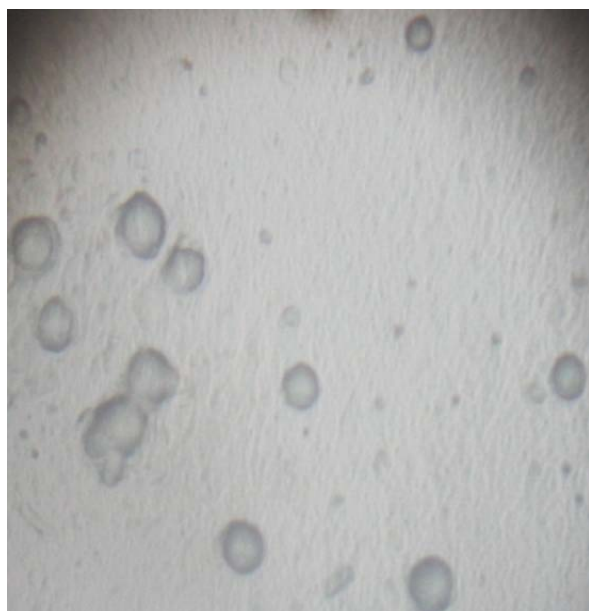


Fig. 1.1: S 60 niosomes

In vitro skin permeation

The same procedure as in the in vitro release study was carried out replacing the cellulose membrane with natural rat skin. The hair on the back of the rat was removed using the shaver. The hairless skin specimen of rat was tied to the open ended cylinder using thread with the epidermis side facing the donor compartment. The percent cumulative amount of drug permeated through the skin was plotted as a function of time (hr) for each formulation.

Rate of Spontaneity (Hydration)

Approximately 10 or 20 mg of proniosomal formulation was transferred to the bottom of a clean stoppered glass bottle and spread uniformly around the wall of the glass bottle with the help of a glass rod. At room temperature 2ml of phosphate saline (0.154 M NaCl) was added along the wall of the glass bottle and left in a test tube stand. After 20 min a drop of this saline solution was withdrawn and placed on Neubauers Chamber to count the number of vesicles. The number of niosomes eluted from proniosomes was counted ⁸.

Stability studies

The ability of the vesicles to retain the drug was assessed by keeping the proniosomal gel at different temperature conditions i.e. Refrigeration temperature (4-8°C), Room temperature (25°C), and oven (45°C). Throughout the study, proniosomal formulation were stored in aluminium foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of one month and drug leakage from the formulations was analysed for drug content spectrophotometrically at 262 nm ⁸.

RESULTS AND DISCUSSION

Microscopic Examination

Optical Microscopy

The Photomicrographs and S.E.M micrographs of different formulations S 60, S40, B72 and T 80 are illustrated in fig 1 and 2 respectively. It was observed that the vesicles of the niosomes formed by hydration of proniosomal gels are almost spherical in shape as also reported by Aboelwafa et al ⁹. It was noticed that the vesicles produced from formula S60 have larger size than S40. B72 did not form uniform vesicles. The vesicles of T80 were ruptured due to its high H.L.B value.

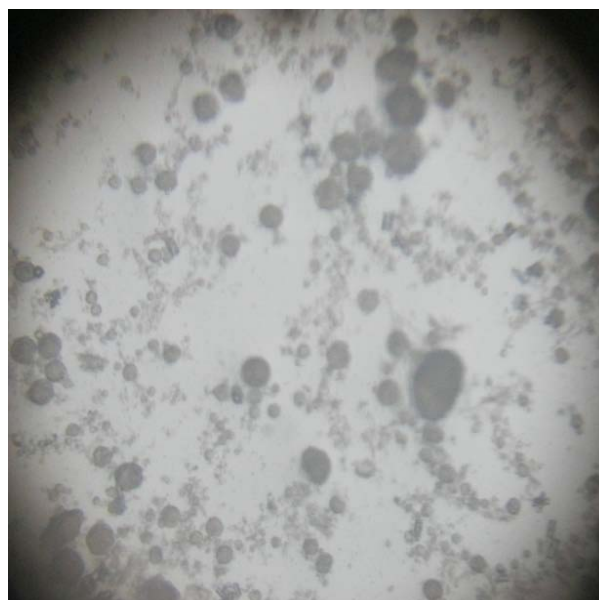


Fig.1.2: S 40 niosomes

Electron Microscopy

S.E.M photographs as illustrated in fig 2 showed that the niosomes formed were spherical in shape and the vesicles of Span 60 were comparatively larger in comparison to the other surfactants. Brij 72 and Tween 80 did not form uniform vesicles.

Rate of Spontaneity

The rate of spontaneity was highest with Span 60 as compared to Span 40, Brij 72 and Tween 80. Tween 80 exhibited lowest rate of spontaneity of 2.1 ± 0.57 . This may be because Tween 80 is liquid at room temperature.

Encapsulation Efficiency

The % Encapsulation of different surfactants followed the order Span 60 > Span 40 > Brij 72 > Tween 80. Span 60 showed higher entrapment efficiency which might be due to the fact that Span 60 has highest phase transition temperature⁹. This could also be attributed to the structure, orientation, and packing behavior of the surfactants. Span 60 has longest saturated chain length and shows highest entrapment.

In-vitro release study

The invitro release studies as carried out showed that the % release of span 60 was highest in comparison to other surfactants used in the study. Span 60 showed 57.68% release, span 40 showed 33.41% release, Tween 80 showed 14.87% release and Brij 72 showed 21.5% release within a time interval of 6 hr. This may be because

Span 60 formed largest and most uniform vesicles in comparison to other surfactants. Brij 72 and Tween 80 did not form uniform vesicles. The vesicles formed using Tween 80 were ruptured may be due to the high H.L.B value of the surfactant.

In vitro permeation study

By comparing the results obtained from permeation of Clotrimazole through hairless rat skin, it was found that the release of Clotrimazole proniosomal gel through rat skin was significantly higher than its permeation across skin indicating that the skin is a truly barrier controlling the permeation of the drug.

The developed proniosome gels exhibited high skin permeation compared to the control solutions containing equivalent amount of Clotrimazole. This could be attributed to the lower ability of drug to penetrate skin layers and the permeation enhancing effect of the non-ionic surfactants and cholesterol used in the preparation of the proniosome gels.

Stability Studies

The results of the study showed that the proniosomal gel formulation was quite stable at refrigeration and room temperature as not much leakage of drug was found at these temperatures. Percent drug retained at 45°C might have decreased due to melting of the surfactant and cholesterol at this temperature. Therefore proniosomal gel formulations can be stored at either room temperature or refrigeration. This is in accordance with the results of Ankur et al⁸.

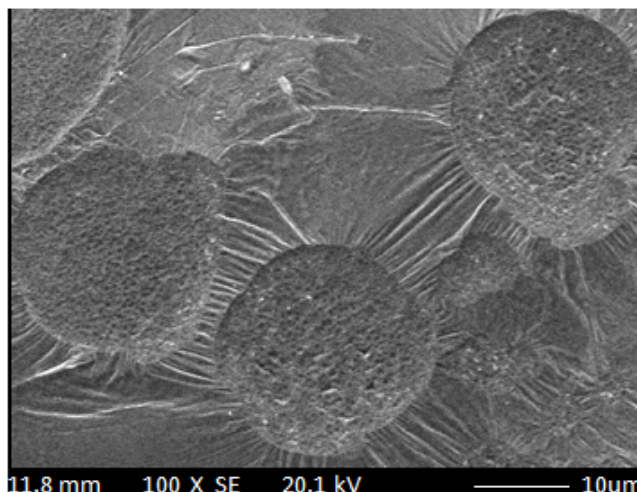


Fig. 2.1: S.E.M of Span 60 niosomes

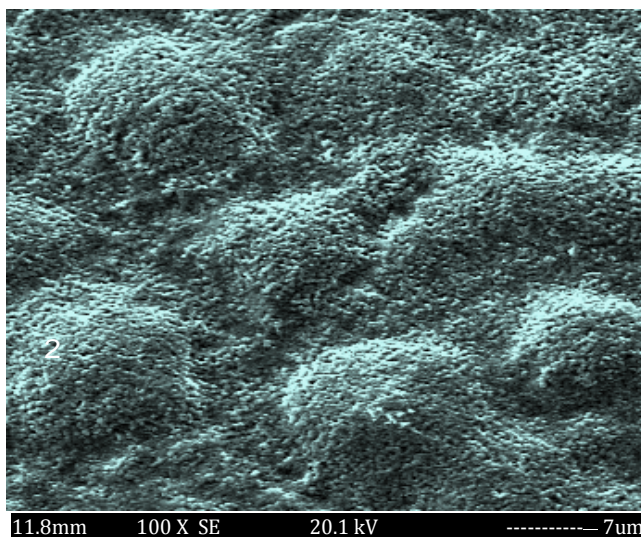


Fig. 2.2: S.E.M of Span 40 niosomes

Table 2: Rate of spontaneity of different formulations

S 60	10.6 ± 0.57
S 40	8.6 ± 1.52
B 72	3.6 ± 1.15
T 80	2.1 ± 0.57

Table 3: Encapsulation efficiency of different formulations

S 60	75.18%
S 40	53.75%
B 72	46.25%
T 80	16.5%

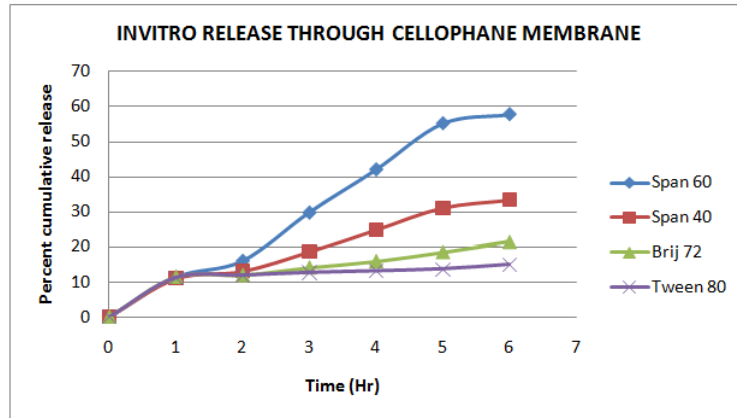


Fig. 3: Plot of percent cumulative release of different formulations

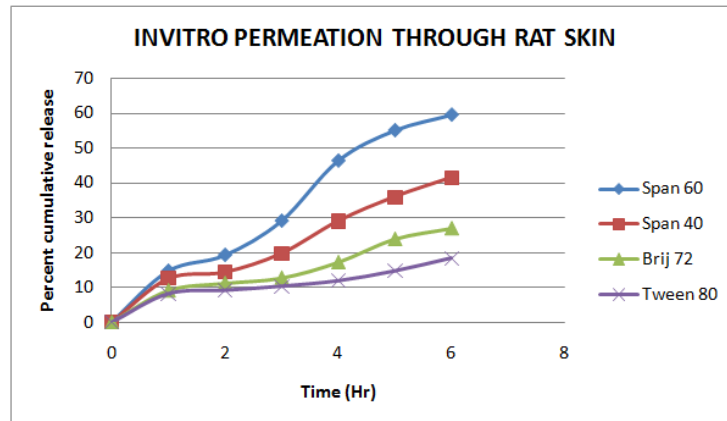


Fig. 4: Plot of percent cumulative drug permeated through hairless rat skin

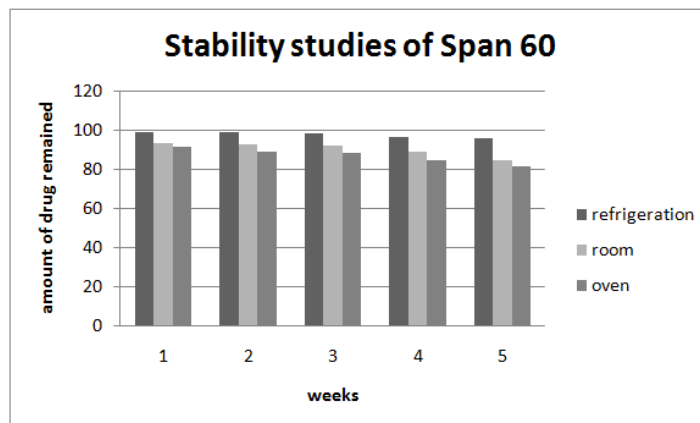


Fig. 5: Graph of stability study of span 60

Span 60 proniosomes were the most stable in comparison to other surfactants.

CONCLUSION

Clotrimazole was successfully entrapped within the non-ionic surfactant vesicles. The above mentioned results suggest that Span 60 and Span 40 can be successfully used for entrapping the drug. The results also suggest that the type of surfactant, its H.L.B, transition temperature affect the capability of proniosomal gel formation and the niosome forming ability from proniosomes. The inclusion of an optimal ratio of surfactant in the vesicles contributes to the enhancement of drug permeation from proniosomal gel formation. The in vitro permeation studies suggest that proniosomal gel formulations enhance the rate of permeation of the drug across skin. This penetration enhancement effect may be attributed to both the presence of non-ionic surfactants and cholesterol. Thus proniosomes may be a promising carrier for Clotrimazole due to their simple and cost effective production.

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