

Development and *in vitro* permeation studies of proniosomal based transdermal delivery system of Atenolol

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Abstract: Proniosomes refer to a flexible vesicular carrier with the potential for drug administration through the transdermal route. A proniosome gel type transdermal delivery system of Atenolol was prepared and extensively studied both *in vitro* drug release and *ex vivo* permeation studies. The prepared formulations were evaluated for vesicle size, entrapment efficiency, *in vitro* drug loading, and drug release studies. The release of drug had shown considerable improvement in controlled manner from the prepared gel formulation. It was observed that Span 40 & 60 (A 8) based formulations shows vesicles of minimum size and higher entrapment efficiency compared to the other formulations. Proniosomal transdermal therapeutic system (A 8) was found to be the optimized formulation as it posses good drug release and shows permeation in a steady-state manner over a desired period of time. Also the drug diffusion across snake sheded skin, guinea pig abdomen skin, albino rat, porcine ear correlates better with *in vitro* drug release studies. The formulation was found to be stable when stored at room temperature and at refrigeration temperature ($4 \pm 2^\circ\text{C}$) for 90 days.

Keywords: Atenolol, proniosomes, guinea pig skin, snake sheded skin, porcein ear skin.

INTRODUCTION

Atenolol is a β_1 -adrenergic receptor blocking agent which has been widely prescribed for cardiovascular diseases such as myocardial infarction, angina pectoris and hypertension devoid of membrane stabilizing or intrinsic sympathomimetic activity (Ozge Inal *et al.*, 2008). Due to incomplete absorption from the lower gastrointestinal tract it has an oral bioavailability of about 50-60 %, while the remaining is excreted unchanged in faeces (Jun-Shik Cho *et al.*, 2003). Though the oral route for Atenolol was widely accepted, they are associated with side effects such as mesenteric arterial thrombosis, nausea, and ischemic colitis. Reduction of the drug concentration on the receptor side and fluctuation in plasma drug levels have also been reported (Sastry *et al.*, 1997; Brown *et al.*, 1976). Thus, formulating an appropriate drug delivery for antihypertensives which maintain a proper blood level for a long period of time with reduced adverse effects associated with decreasing frequency in dosage administration is very important (Jug *et al.*, 2009).

For specific drug action, functional molecules have to be transported by a carrier(s) (Liposomes or niosomes) to the target site and released for drug action (Ankur Gupta *et al.*, 2007). Niosomes proved to be an alternate to liposomes because they pose less chemical stability problems and low cost, but they are linked with problems in physical stability, such as aggregation, leakage on storage, fusion and sedimentation (Ijeoma Uchegbu *et al.*, 1998). The provesicular method can be put forward to the

niosomes which show signs of better stability when related to liposomes. The proniosomes which exhibits more stable characteristics during sterilization and storage, reduces these problems by using dry, free-flowing particles that form niosomal dispersion immediately when in contact with water (Hanan *et al.*, 2011; Hu *et al.*, 1999).

The approach for the development of new formulation not only circumvents the limitations of the conventional dosage forms but can also act as a new trend by modifying widely reported and established formulations. Nowadays, researchers were paying their attention on the way to controlled manner of drugs delivery through the skin (Pandey *et al.*, 2000). A challenge to transdermal drug delivery (TDD), was lipid environment of the skin stratum corneum, causes the rate limiting barrier of drug permeation. Vesicular delivery was considered very hopeful to prevail over this permeation barrier of skin (Md Khalid Anwer *et al.*, 2009). Proniosomes offers a versatile delivery for drugs via transdermal route (Fang *et al.*, 2001).

The present study was aimed to develop and characterize proniosomal carrier systems of Atenolol using non-ionic surfactants span in various grades such as 20, 40, 60, and 80 with cholesterol containing formulation for transdermal delivery.

MATERIALS AND METHODS

Soya lecithin was a gift sample obtained from Lipoid, Nattermannalle, Switzerland. Span 20, Span 40, Span 80

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and cholesterol were procured from SD Fine Chemicals (Mumbai, India). Atenolol was a gift sample obtained from Sun Pharma Ltd, Mumbai, India. Ethanol-AR, sodium hydroxide, orthophosphoric acid, and potassium dihydrogen orthophosphate were purchased from E-Merck (Mumbai, India).

Preparation of Proniosomes

Proniosomes were prepared by the coacervation-phase separation technique. Accurately weighed amounts of the surface-active agent, cholesterol and drug were transferred to a glass vials and mixed with absolute alcohol (about 0.5/ml) and warmed in water bath (55-60°C) for 5 min with agitation until complete dissolution of cholesterol. Then phosphate buffer pH 7.4 (1.6 ml) was added and the mixture was then warmed for about 2 min to get a clear solution. The mixtures were allowed to cool down at room temperature and noticed for the development of homogenous solution, two phases liquid, which was translucent, transparent or white creamy proniosomal gel. The mixture was allowed to cool to room temperature until the dispersion was transformed to proniosomal gel. Proniosomal gel was then added with 1 % Carbopol gel in 1:1 ratio. The gel formed was stored in dark until used for further characterization (Ibrahim Alsarra *et al.*, 2005).

In order to optimize and evaluate various Atenolol proniosomal formulations were prepared using different grades of non-ionic surfactants in various combinations of Span (sorbitan esters) as Span 20, Span 40 and Span 80 with addition of cholesterol (table 1).

Table 1: Composition of various proniosomal formulations (mg)

Formulation code	Span 20	Span 40	Span 60	Span 80	Lecithin	Cholesterol
A 1	900	-	-	-	900	200
A 2	-	900	-	-	900	200
A 3	-	-	900	-	900	200
A 4	-	-	-	900	900	200
A 5	450	450	-	-	900	200
A 6	450	-	450	-	900	200
A 7	450	-	-	450	900	200
A 8	-	450	450	-	900	200
A 9	-	450	-	450	900	200
A 10	-	-	450	450	900	200

Vesicle physical analysis

Microscopic examination

A thin layer of formed proniosomes were spread on a slide and the nature of vesicle observed and the presence of drug crystals which are insoluble were focused under light microscope at various magnification powers (10× and 40×). Photomicrographs were taken using Fujifilm Finepix F 40 fd (8.3 MP) digital camera with 3 × optical zoom (Mahmoud Mokhtar *et al.*, 2008).

Scanning electron microscope

SEM was carried out to identify the shape, surface characteristics, and size of the niosomes. The proniosomal gel was diluted with 10 ml of phosphate buffer pH 7.4. Obtained niosomal system was placed superficially on an aluminum stub using dual-sided adhesive carbon tape. Then the sputter-coated with gold palladium (Au/Pd) specimen were examined using SEM.

Transmission electron microscopy (TEM)

The morphology of the hydrated niosomal dispersions obtained from proniosomes was also determined by transmission electron microscopy. Dilution of a drop of niosomal dispersion was made using 10 folds of deionized water, from which a drop was applied to a carbon-coated 300 mesh copper grid and left for 1 min to adhere to the proniosomes with the carbon substrate. The residual dispersion was taken away by absorbing the drop with the corner of a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 1 s. The sample was air-dried and examined under the transmission electron microscope (JEOL 100 CX transmission electron microscope at 80 KV) (Hu *et al.*, 1999).

Drug encapsulation efficiency

To the proniosomal formulations, was added 10 ml of phosphate buffer (pH 7.4) and the aqueous solution was sonicated for 10 min. The drug containing niosomes were separated from the untrapped drug at 25,000 rpm for 30 min at 20°C by centrifugation. The supernatant was removed and diluted with phosphate buffer. The resulting solution was assayed by the UV spectrophotometer. The drug encapsulation in percentage was calculated by using the following equation (Ibrahim Alsarra *et al.*, 2005; Md Khalid Anwer *et al.*, 2009). $EP (\%) = [(Ct-Cr)/Ct] \times 100$, where EP is the encapsulation percentage, Ct-concentration of total drug, and Cr-concentration of free drug.

In vitro drug release study

In vitro release of prepared proniosomal gels were carried using modified Franz-diffusion cell. The efficient diffusion area of the cell was 1.41cm² and had a receptor volume of 15 ml. The dialysis cellophane membrane was mounted between the donor and receptor compartment. The receptor medium was phosphate saline buffer pH 7.4. The prepared proniosomal gel was placed on one side of the dialysis membrane. The receptor compartment was bounded by a water jacket to retain the temperature at 37±1°C. The receptor fluid was stirred by a Teflon-coated magnetic bead operated to a magnetic stirrer (Jug *et al.*, 2009).

At predetermined time intervals, samples in the acceptor chamber was removed and replaced with an equal volume of receptor media for drug content determination. Drug concentration was determined UV spectrophotometrically (Shimadzu-1700) at 275 nm.

In vitro skin permeation study

The *in vitro* permeation of Atenolol was determined using different animal skins such as shed snake skin, albino porcine ear, male albino rat, male guinea pig in order to observe the absorption of drug. Shed snake skin of “Naja Naja” offers considerable advantages over human material, as it was relatively abundant. The permeability co-efficient of lipophilic drugs was in the same range as those through the human skin. The shed skin was soaked in phosphate buffer (pH 7.4) for 30 min before use.

Albino porcine ear was obtained from a local slaughter house. The whole skin was soaked in water at 60°C for 45 s and the epidermis was removed by heat separation technique. The epidermis was washed with pH 7.4 phosphate buffer and then used.

The skin of the male albino rat, which was taken from flank of the animal, was cleaned by removing hairs using scissors. The prepared skin was washed with pH 7.4 phosphate buffer and then used (Jain S *et al.*, 2003).

The skin of the male guinea pig was taken from the abdominal region and cleaned by removing the hairs using scissors. The prepared skin was washed with pH 7.4 Phosphate buffer and then used (Ramesh panchagunla, 1997). The studies were carried out based on the approval obtained from the Institutional animal ethical committee (IAEC).

The *in-vitro* skin permeation studies were also performed by using modified Franz diffusion cell. The temperature of receptor phase was maintained at $37 \pm 1^\circ\text{C}$ throughout the experiment. At predetermined time intervals, samples from the receptor compartment were taken to determine the amount of drug permeated through the above mentioned various animal skins. Samples withdrawn were analyzed spectrophotometrically (Shimadzu-1700) at 275 nm.

Stability studies

The drug retention pattern was assessed by storing the proniosomal gel at different temperature conditions, i.e., refrigeration temperature ($4 \pm 2^\circ\text{C}$) and room temperature ($37 \pm 2^\circ\text{C}$). Proniosomal gel formulations were stored in aluminum foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of 90 days and leakage of drug from the formulations was analyzed for drug content spectrophotometrically (Ankur Gupta *et al.*, 2007).

RESULTS

The preparation method of proniosomal formulation includes the mixture of alcohol, surfactant, and aqueous phase were used to form the concerted proniosomal gel, which can be transformed to stable niosomal dispersion by dilution with excess aqueous phase spontaneously.

Vesicle physical analysis

Vesicles with lesser diameter are believed to have enhanced permeation through the skin as smaller vesicles tend to fuse readily (Chandra A and Sharma PK, 2008). Formulation A8 were smaller in size, shows higher entrapment efficiency and uniform surface area. The SEM and TEM images show that the proniosomal formulation prepared with Span 40 & 60 (A8) was well-identified spherical nano vesicular and homogenous.

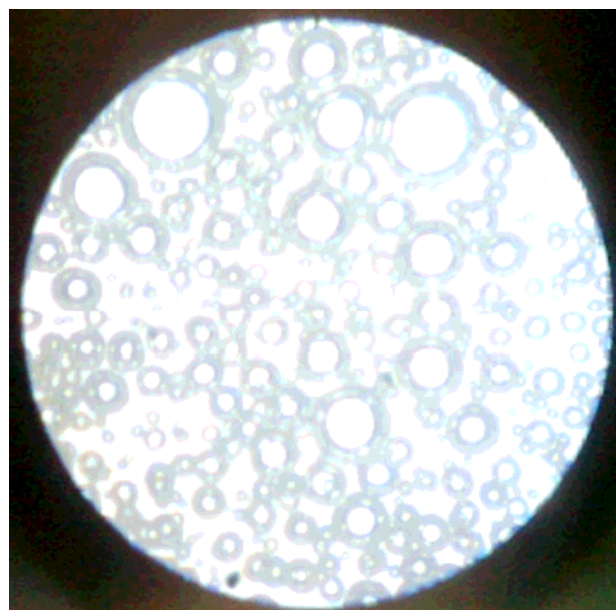


Fig. 1: Optical microscopy of Formulation A 8.

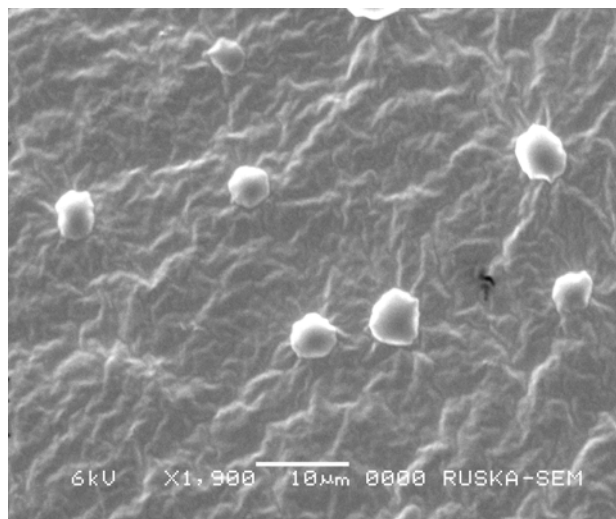


Fig. 2: SEM image of formulation A 8.

Drug encapsulation efficiency

The entrapment was calculated as a percentage of the total amount of Atenolol used in preparation of the proniosomes. All the formulations evaluated were found to have a high value of % entrapment (>90%, table 2).

Good encapsulation of Atenolol was found in the proniosomal gel prepared using Spans 40 and 60 (A8).

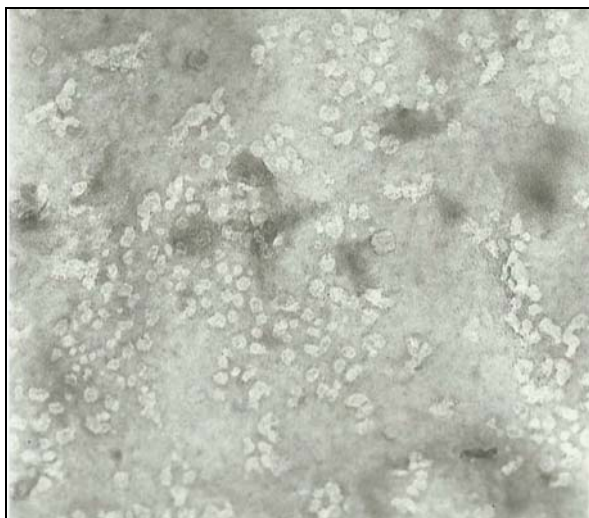


Fig. 3: TEM image of formulation A 8.

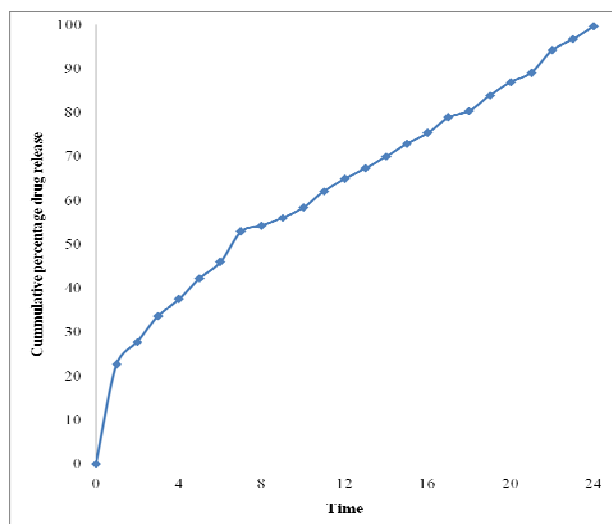


Fig. 4: *In vitro* drug release of formulation A 8

***In vitro* drug release study**

In vitro drug release for all formulations of the proniosomal gels was observed. *In vitro* release studies are often performed to predict how a delivery system might work in an ideal situation as well as give some indications of its *in vivo* performance since drug release dictates the amount of drug available for absorption. All the formulation exhibited zero order release. The drug permeation was maximum (99.68%) from formulation containing Span 40 & 60 (A8) among all tested formulations.

***In vitro* skin permeation study**

Although animal skin has different (generally higher drug permeability) compared to human skin, skin of rodents

(mice, rats, rabbits and guinea pigs) was used in *in vitro* and *in vivo* percutaneous permeation studies due to its availability (Mahmoud Mokhtar *et al.*, 2008; Biana Godin and Elka Touitou, 2007). Fig. 5 shows the cumulative amounts of Atenolol through various animal skins (phosphate buffer pH 7.4).

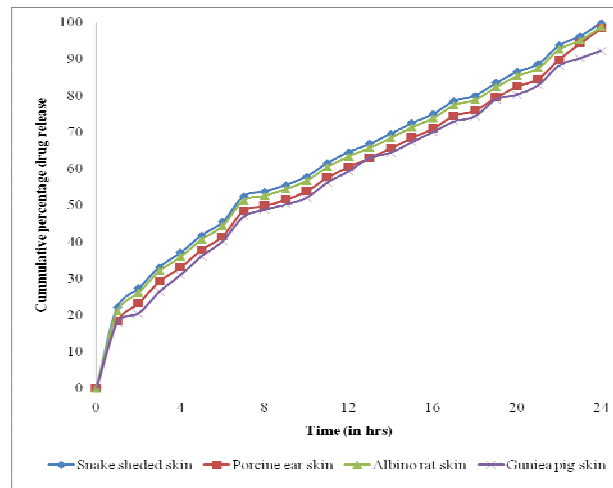


Fig. 5: *In vitro* drug permeation studies of A 8.

Stability studies

There was no considerable reduction in the drug content value of the transdermal formulation before and after three months of storage (table 3). The proniosomal gel was also examined microscopically which showed round shaped vesicles.

DISCUSSION

Vesicle physical analysis

Circular vesicle bodies were observed with uniform and very small size microscopically, which was shown in fig. 1, and the SEM and TEM image was shown in fig. 2 & 3 respectively.

Drug encapsulation efficiency

Formulation containing Span 40 and Span 60 exhibited very high entrapment (Kandasamy Ruckmani and Veinramuthu Sankar, 2010; Pratap S Jadon *et al.*, 2009). This might be due to the fact that Spans 40 and 60 are solid at room temperature and exhibits a higher phase transition temperatures [Tc] (Ankur Gupta *et al.*, 2007; Ahmed A Aboelwafa *et al.*, 2010). It was found that in the proniosomal gel formulation, the entire drug will be intercalated into the bilayers as opposed to the aqueous spaces within the gel.

***In vitro* drug release study**

The impact in the drug release varies according to the change in composition of the non-ionic surfactant. The formulation containing Spans 40 and 60 form the vesicle

of a larger size which shows greater entrapment of drug (Peeyush Vasistha and Alpana Ram, 2013). Also, the drug leakage from the vesicles formed of Span 40 & 60 was very low due to its high phase transition temperature and low permeability (Manconi M *et al.*, 2002). Lack of lag phase was observed because of the low sampling time of 1/h and the permeation of drug. Hence formulation A 8 had been selected as optimum formulation for *in vitro* skin permeation studies and stability testing studies.

Table 2: Entrapment efficiency of all the formulations

S. No	Formulation	Percentage encapsulation efficiency $\pm$ SD
1	A 1	97.25 $\pm$ 1.1
2	A 2	99.10 $\pm$ 0.9
3	A 3	98.77 $\pm$ 0.3
4	A 4	97.86 $\pm$ 0.4
5	A 5	98.89 $\pm$ 0.5
6	A 6	99.10 $\pm$ 0.4
7	A 7	98.32 $\pm$ 0.7
8	A 8	99.21 $\pm$ 0.3
9	A 9	98.67 $\pm$ 0.3
10	A 10	98.99 $\pm$ 0.5

***In vitro* skin permeation study**

The presence of unsaturated double bond in the oleate side chain of span 40 & 60 was responsible for the significant enhancement of Atenolol permeation (Mahmoud Mokhtar *et al.*, 2008). The arrangement of unsaturated fatty acids changes the stratum corneum lipid structure upon binding to the keratin filament; hence increase drug permeability across skin (Matthias Forster *et al.*, 2009).

Stability studies

No any significant difference in the size of the vesicles has been noted before and after storage. Hence, the proniosomal gel can be stored at either refrigeration or room temperature.

Table 3: Stability studies of formulation A 8

S. No.	Temperature	Initial	After 3 months
1	At refrigerator	99.21 $\pm$ 0.3	98.73 $\pm$ 0.2
2	At Room temperature	99.21 $\pm$ 0.3	98.31 $\pm$ 0.5

CONCLUSION

Antihypertensive treatment demands prolonged and controlled release of Atenolol which can be achieved through proniosomal gel as a drug delivery system. All the proniosomal gel formulations were evaluated for the encapsulation efficiency, vesicle shape and size, *in vitro* drug release and stability studies and the results were found in the acceptable range. On the basis of higher drug

permeation, low vesicle size, maximum encapsulation efficiency, and higher bioavailability, formulation (A8) has been selected as an optimized therapeutic system of Atenolol. Further, the *in vitro* permeation across the different skins reveals the potential of proniosomal formulation for improved absorption of Atenolol across gastrointestinal membrane. In conclusion, the proniosomes proved to be efficient carriers for the delivery of Atenolol across skin. However, further *in vivo* studies need to be conducted to check the feasibility of proniosomal carriers for enhanced bioavailability of Atenolol.

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