

Proniosomes as Nano-Carrier for Transdermal Delivery of Atenolol Niosomal Gel

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Received: 3rd Sept, 17; Revised 14th Oct, 17, Accepted: 14th Nov, 17; Available Online: 25th Dec, 2017

ABSTRACT

Objective of the study is to prepare Proniosomes that refers to a flexible vesicular carrier with the potential for drug administration through the transdermal route. Method: Proniosomes were prepared by the coacervation-phase separation technique. The prepared formulations were evaluated for vesicle size, entrapment efficiency. The optimal proniosomes formula (A8) was prepared with different aqueous phase, incorporated in a gel base and studied for pH, viscosity, spreadability, stability, *in vitro* drug release and *ex vivo* permeation. Results: Niosomes formulations prepared with Span 40 and 60 have spherical and smaller Nano size. 25 mg atenolol loading has resulted 190.9 ± 15.033 nm sizes. EE% of the optimum formula prepared with distilled water was 62.11 to 92.38. Rheological behavior showed combined shear thinning and thixotropic and gel was spreadable. Tested formulations were stable on cooling (4-8 °C). *In vitro* drug release followed zero order kinetic, and gave sustained release. Release rate was significantly higher across cellulose membrane compared with rate skin. Amount of drug obtained after skin extraction was 92.6 ± 0.5% indicate enhanced permeation rate. Conclusion: All the proniosomal gel formulations were found through the acceptable range of vesicular size and entrapment efficiency. Formulation A8 has been selected as an optimized therapeutic system of atenolol.

Keywords : Atenolol, Proniosomes, Nano carrier, Transdermal, *in vitro* release.

INTRODUCTION

Atenolol is a β-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 activity¹. 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 feces². Though the oral route for Atenolol was widely accepted, they are associated with side effects such as mesenteric arterial thrombosis, nausea, and ischemic colitis. Reductions of the drug concentration on the receptor side and fluctuation in plasma drug levels have also been reported³. Thus, formulating an appropriate drug delivery for antihypertensive 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 important⁴. Recently, there has been a continuous interest towards trans-dermal drug delivery (TDD), as it would avoid problems associated with the other routes of administration. A timely warning challenge to TDD formulators was issued by Hadgraft and Guy⁵; represented in the lipid matrix of the skin stratum corneum, which presents the rate limiting barrier of drug permeation⁶. Thus, several technological advances have been made in the recent decades to overcome skin barrier properties and enhance percutaneous drug penetration⁷.

Drug delivery systems using colloidal particulate carriers such as liposomes⁸, niosomes⁹, or proniosomes proved to have distinct advantages over conventional dosage with an increasingly important role in drug delivery; as particles can act as drug containing reservoir, and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site¹⁰.

Proniosomes are liquid crystalline-compact niosomal hybrid which could be converted into niosomes upon hydration with water offering a versatile vesicle delivery concept with potential for drug delivery via transdermal route¹¹. Upon skin application proniosomes get hydrated with water from skin under occlusion¹². Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the skin. Previous experimental results and supportive theoretical analysis suggested several mechanisms to explain the ability of niosomes to modulate drug transfer across skin; it is thought that structure modification of stratum corneum is one of the most probable reasons for enhancing the permeability of drugs: the intercellular lipid barrier in stratum corneum would be dramatically changed to be more loose with liposomes and niosomes¹³. Both phospholipids and non-ionic surfactants in proniosomes can act as penetration enhancers, since it was found that some phospholipids are able to fluidize the stratum corneum lipid bilayers and diffuse through them¹⁴.

Proniosomes; provide additional convenience of transportation, distribution, storage and dosing. They are known to avoid many of the problems associated with either the aqueous niosome dispersion, as problems of physical stability (aggregation, fusion, leaking), or liposomes, as degradation by hydrolysis¹⁵, or oxidation¹⁶, as well as sedimentation, aggregation or fusion during storage¹⁷, in addition to its high cost, difficulties in sterilization, variable purity problems of phospholipids¹⁸ and in large scale production¹⁵ of a product with adequate physical and chemical stability. Proniosomes not only do they offer a promising means of drug delivery, but also could enhance the recovery rate of the skin barrier¹⁹. All this make proniosomes; “dry niosomes”, a promising industrial product¹⁰.

Since both hydrophilic and hydrophobic substances can be embedded in niosomes vesicles²⁰, thus it is known that sparingly soluble drugs can be entrapped in vesicles²¹. Consequently, proniosomes are expected to offer a special advantage for atenolol which is lipophilic with relative hydrophilicity. Thereby, the present study aims at designing a new transdermal formulation for atenolol characterized by safety and high therapeutic efficacy, through designing an optimum proniosome gel formulation so as to reduce the daily administered dose of atenolol with a subsequent improvement in patient compliance and drug safety.

MATERIALS AND METHODS

Materials

Atenolol was kindly donated by Epico (Egypt). Soya lecithin was a gift sample obtained from Cargill (Germany). Cholesterol from lanolin, Span 20, Span 40, Span 60 and Span 80 were purchased from Fluka (Switzerland). Ethyl alcohol absolute was obtained from Adwic (Egypt).

Glycerin 99.5% was obtained from El-shark al-Awsat for chemical trade company (Egypt). Cellulose membrane was purchased from Sigma–Aldrich (USA). Carbomer 934P, Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, potassium chloride and Triethanolamine were supplied from El Nasr Pharmaceutical Chemicals (Egypt), all of analytical grade.

Methods

Preparation of proniosomes

Proniosomes were prepared by the coacervation-phase separation technique. Accurately weighed amounts of the surface-active agents, lecithin, 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. The aqueous phase was added and the mixture warmed with sonication for about 15 min to get a clear solution. The mixtures were allowed to cool down at room temperature and noticed for the development of yellowish creamy proniosomes dispersion. After cooling dispersions were transformed to niosomal gel which added with 1% Carbopol 934 gel in 1:1 ratio. The gel formed was stored in dark until used for further characterization²². In order to

optimize and evaluate various atenolol proniosomes-derived niosomes formulations, different grades of non-ionic surfactants were used as Span (sorbitan esters) 20, Span 40, Span 60 and Span 80 with addition of lecithin and cholesterol. Data are collected in Table 1. Three different aqueous phases (phosphate buffer pH 7.4, distilled water or 0.1% aqueous glycerol solution) were also used.

Drug loading

The optimum proniosomal formula was loaded with four different atenolol concentrations i.e. (25, 50, 75 and 100 mg). Each concentration was prepared thrice with different aqueous phases i.e. (phosphate buffer pH 7.4, distilled water or 0.1% aqueous glycerol solution).

Characterizations of prepared proniosomes

Light microscopy

A thin layer of formed proniosomes dispersion were spread on a slide, diluted with a small drop of deionized water then dried. 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 zoom²³.

Transmission electron microscopy (TEM)

The morphology of the prepared proniosome formulations was determined by TEM (JEOL 100 CX transmission electron microscope at 80 KV): a drop of the dispersion was diluted 10-fold using deionized water, then a drop of the diluted dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min to allow some of the proniosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. After twice rinsing the grid (deionized water for 3–5 s) a drop of 2% aqueous solution of uranyl acetate was applied for 1 s. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried¹⁰.

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 niosomes containing drug were separated from the untrapped drug at 25,000 rpm for 45 min at 4°C by centrifugation. The supernatant was recovered and assayed by the UV spectrophotometer at 275 nm. The drug encapsulation in percentage was calculated by using the following equation (24), EP (%) = $[(Ct-Cr)/Ct] \times 100$, where EP is the encapsulation percentage, Ct-concentration of total drug, and Cr-concentration of free drug.

Particle size analysis and Zeta potential

The particle size and polydispersity index of formulated proniosomes either drug loaded or unloaded was determined by using Zeta sizer by dynamic light scattering (Nano ZS, Malvern, and Worcester-shire, UK). Before measurement samples were dispersed in distilled water. Three replicates were measured and values were presented as mean ± standard deviation (SD).

pH measurement

The pH values of the optimum prepared proniosomes-derived niosomes gel formulations were measured using a pre-standardized digital pH meter (Jenway 3510, Staffordshire, UK) at 25 °C. One gram of each formulation was dispersed in 10 ml deionized water and the electrode was dipped into the dispersion until constant reading was obtained. The pH measurements were average of three replicates²⁵.

Rheological measurements

Rheology's of proniosomes loaded drug prepared with three different aqueous phases were performed with a cone and plate rheometer (Anton Paar®GmbH, Ostfildern, Germany). Up and down portions of the flow curves were determined using parallel plate geometry (50 mm diameter), where, the gap between the two plates was 1 mm. About 0.5 g of the tested formulation was applied to the plate and left until the temperature of the plate reached $25 \pm 1^\circ\text{C}$. The measurements were made over range from 10, 15, 20, 25, 50, 75 up to 250 rpm with 20 s between each two successive speeds. The rheological behavior of each formulation was evaluated by plotting the shear stress versus the obtained shear rate values. The flow behavior was studied according to Farrow's equation²⁶.

$$\text{Log } D = N \text{ Log } S - \text{Log } \dot{\eta}$$

Where, D is the shear rate (sec^{-1}), S is the shear stress (Pa), N is Farrow's constant and $\dot{\eta}$ is the viscosity (Pa.s). N is the slope of the plot of $\text{log } D$ against $\text{log } S$, which indicates the deviation from Newtonian flow. When N is less than one, it indicates dilatant flow (shear rate thickening). If N is greater than one, it indicates pseudo plastic or plastic flow (shear rate thinning). When the system showed thixotropic behavior, the hysteresis area (H.A.) between the upward and a downward curve was measured adopting the trapezoidal rule.

Spreadability test

The evaluation of spreadability (S) was performed at room temperature using the parallel plate method²⁷. Half gram of each formulation was applied on a drawn circle of 1 cm in diameter. Samples were pressed between two glass plates (each of them weight 200 g), then gradually more plates were added to increase the mass on the sample, with interval of 1 min between each added plate. The diameter of the spreading area reached by samples between each addition of a glass plate was measured in millimeters along two perpendicular axes. Results were expressed in terms of the spreading area as a function of the applied mass. The spreading area was calculated according to the following equation:

$$S_i = (d^2 \Pi)/4$$

Where, S_i is the spreading area (mm^2) after the application of certain mass (g) and d is the mean diameter (mm) reached by each sample²⁸.

The spreadability factor (S_f) was also calculated to represent the spreading of a formulation on a smooth horizontal surface when a gram of weight is placed on top of it. The following equation was used to calculate the S_f . $S_f = A/W$

Where, S_f ($\text{mm}^2 \cdot \text{g}^{-1}$) is the spreadability factor resulting from the ratio between the maximum spreading area (A ;

mm^2) after the addition of the sequence of weights used in the experiment and the total weight reached (W ; g)^{27,28}.

Assessment of physical stability for proniosomes

Aggregation or fusion of the vesicles as a function of temperature was determined as the change in entrapment efficiency after storage and measuring Nano size, PDI and Zeta potential. The vesicles of the optimal formula were stored in glass vials at room temperature or kept in refrigerator ($4-8^\circ\text{C}$) for 3 months. The retention of entrapped drug was measured 72 h after preparation and then after 1, 2 and 3 months of storage in selected formulations²⁹. Stability for each formulation was defined in terms of retaining its initial entrapment efficiency for three months duration. Stable formulations were defined as those showing high entrapment efficiency ($>60\%$) and high atenolol retention value ($>90\%$) at each time interval, also non-significant change in size, PDI and Zeta potential. The optimum formula A8 prepared by three different aqueous phase was proceed through the three month stability investigation.

Atenolol retained in proniosomes =

$$\frac{\text{Entrapped atenolol after storage}}{\text{Entrapped atenolol before storage}} \times 100$$

Entrapped atenolol before storage

In vitro release study

An automated, temperature-controlled continuous flow through Franz diffusion cells (six cells) with a diffusional area of 3.14 cm^2 was used to evaluate the amount of drug released from the developed formulations. The diffusion cells were thermoregulated with a water jacket at 37°C . Cellulose nitrate membrane filters were soaked with phosphate buffer of pH 7.4 and mounted to diffusion cells. The receptor phase contained 7.1 mL of phosphate buffer and passed through the receptor chamber at a controlled rate using a multichannel peristaltic pump. 200 mg of each sample of (noisome loaded atenolol gel formulated with different aqueous phase) was applied to the donor compartment using a calibrated pipette, and the cells were covered to prevent evaporation of vehicle and ensure integrity of the formulations throughout the respective study period. Accurate samples (2 mL) were withdrawn at time intervals 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 18, 20, 22 and 24 hr. and analyzed by spectrophotometer at 275 nm.

Skin irritancy test

Irritancy test was carried out to determine possible localized reaction of the selected formula on the skin since skin safety is of prior consideration for transdermal delivery systems. A single dose of 200 mg of the selected medicated formulation i.e. A8 was applied to the left side of the shaved back of male albino rat ($250 \pm 0.5 \text{ gm}$) and the right side was considered as control. The control area was further divided into two sub areas, one receiving the selected formulation unloaded with the drug (positive control) and the other receiving no treatment (negative control). The development of erythema was monitored daily for 6 days. Extents of development of erythema were indicated on the basis of the following³⁰.

0: No erythema development; 2: barely visible few blood vessels and light erythema development; 4: main blood

Table 1: Composition of various proniosomes formulations (mg).

Formulation code	span 20	span 40	span 60	span 80	lecithin	cholesterol
A1	150	-	-	-	150	150
A2	-	150	-	-	150	150
A3	-	-	150	-	150	150
A4	-	-	-	150	150	150
A5	75	75	-	-	75	75
A6	75	-	75	-	75	75
A7	75	-	-	75	75	75
A8	-	75	75	-	75	75
A9	-	75	-	75	75	75
A10	-	-	75	75	75	75

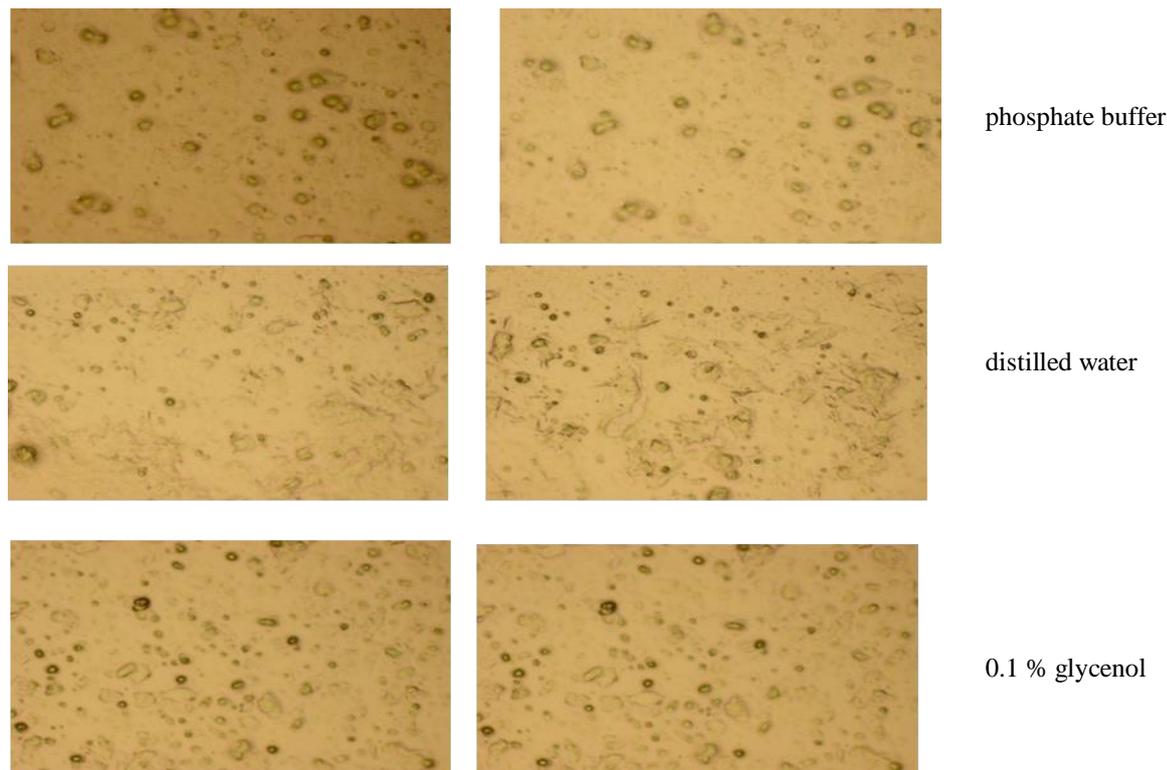


Figure 1: Light Microscopy of some proniosomes formulations.

vessels visible and slight erythema development; 6: main blood vessels more obvious and slight erythema development. Irritation potential was calculated using the following equation:

Resultant index =

$$\frac{A \cdot B}{\text{Number of observation days}}$$

Where A and B are representing erythema value and corresponding day, respectively. This method was repeated three times each with proniosomes gel prepared with certain aqueous phase (phosphate buffer 7.4, distilled water, 0.1% glycerol).

Ex- VIVO STUDY
Preparation of full thickness skin
 Sprague–Dawley male rats (8–10 weeks old and weighing 250–300 g) were obtained from the Central Animal Facility. The research adhered to the “Principles of laboratory Animal Care” (NIH publication No. 8023,

revised 1978). Methodology was approved by faculty of pharmaceutical sciences and pharmaceutical industries ethics Committee, Future University (REC-FPSPI-8/50). Hairs from the dorsal surface were removed using an animal hair clipper (Sterling 2, Wahl, UK). Animals were euthanized by the use of overdose of sodium thiopental following which full thickness skin was harvested. The subcutaneous tissue was removed surgically, and the dermis side was wiped with isopropyl alcohol to remove adhered fat. The skin was washed with phosphate-buffered saline (pH 7.4)³¹. All measurements were carried out in a single ventilated room (temperature, ~28–30°C; relative humidity, ~38–40%).

Permeation experiments

Full thickness skin was mounted between the two compartments of Franz diffusion cells with the stratum corneum side up and with effective area of (3.14 cm²). The receptor compartment (capacity 7.1 mL) was then filled

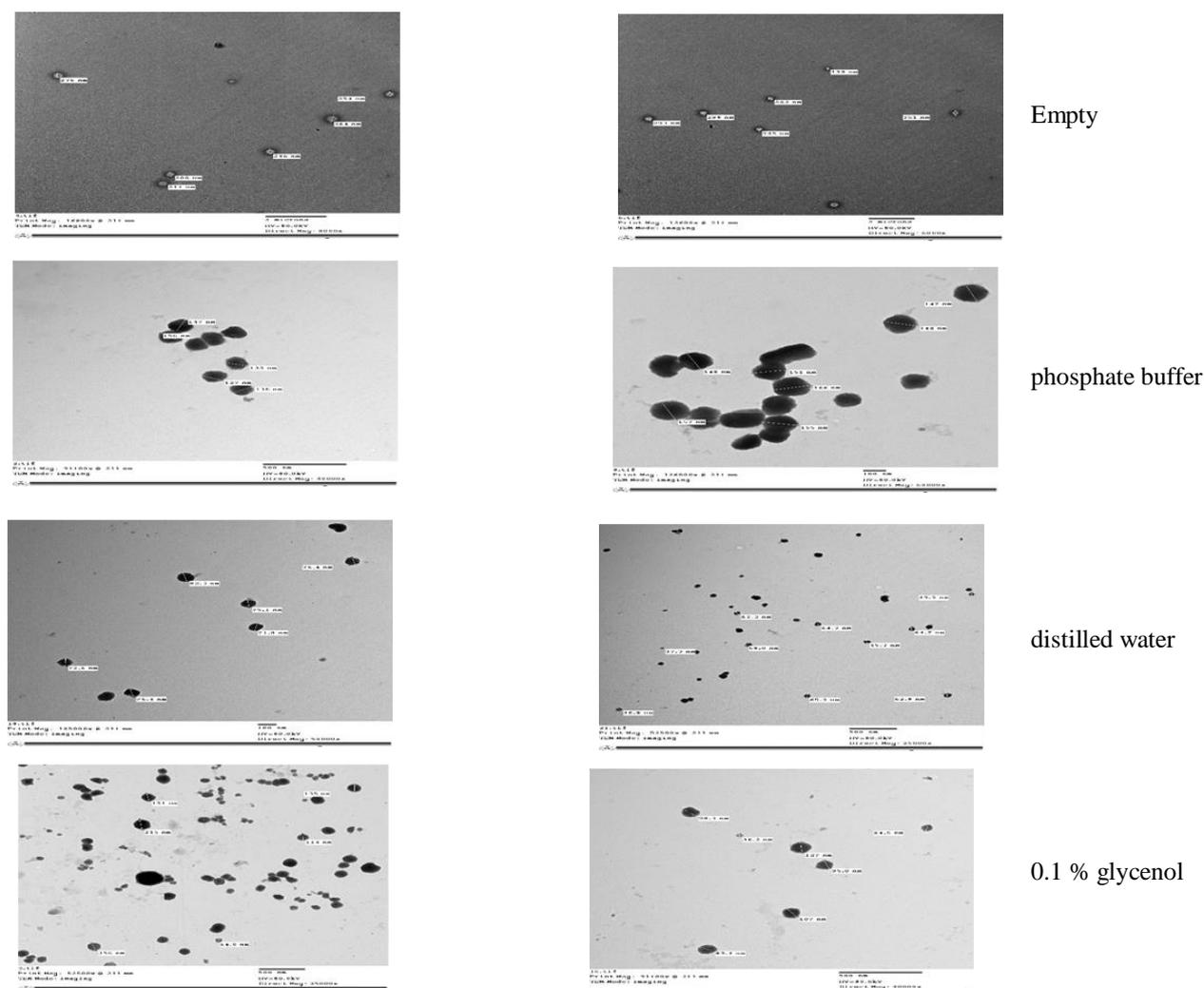


Figure 2 : TEM of some proniosomes formulations showing different sizes of spherical Nano vesicles.

with phosphate-buffered saline (pH 7.4) and maintained at $37\pm 1^\circ\text{C}$ with a constant stirring speed at 300 rpm using a magnetic stirrer. The dermal side of the skin which was placed in contact with the receptor compartment fluid was equilibrated for 30 min³². Then proniosome prepared with different aqueous phase gel in a dose equivalent to 25 mg atenolol were evenly spread on SC side of the skin in donor compartment and covered with Para film (American Can, USA). Aliquots (2 ml) were withdrawn from the receptor compartment with immediate replacement with fresh medium to maintain the sink conditions constantly and a constant volume as well. The concentrations of the drug in the withdrawn samples were determined using spectrophotometer at wave length 275 nm. Data were analyzed by plotting mean percentage cumulative amount of drug permeated versus time to investigate the best fit to distinct kinetic model (zero, first or Higuchi order) to elucidate the drug permeation mechanism. The excess formulation was carefully removed from the skin surface after 24 h and skin was washed three times with phosphate-buffered saline (pH 7.4) before drying gently with the cotton swab. The skin was then stored at -20°C until further analysis.

Quantification of drug in the skin

After completion of the permeation experiment, the excess formulation was removed from the skin surface. The skin was washed three times with phosphate-buffered saline (pH 7.4) and dried. SC was then separated from dermal tissues (epidermis and dermis (E+D)) using serial tape stripping. The procedure was standardized to avoid damage to the skin. These tape strips and the remaining skin portions (E+D) were collected in separated Eppendorf. Drug extraction from the various skin layers including tape strips was accomplished by adding 1 mL of 0.1 N NaOH to the Eppendorf, followed by high-speed vortexing for 30 s and then incubation at 70°C water bath for 30 min. To this alkaline extract, methanol was added to precipitate the proteins and then samples were centrifuged at $2,300\times g$ for 10 min. The supernatants were then analyzed for atenolol content in each skin layer (SC and dermal layer (E+D) and reported as percentage of applied dose/cm² area) using spectrophotometer, by measuring the absorbance at λ_{max} 275nm using phosphate buffer at pH 7.4³³.

RESULTS

Table 2: Effect of atenolol loading concentrations (mg) on particle size, PDI, Zeta potential and EE% (n=3).

Drug loaded (mg)	Proniosomes prepared using phosphate buffer PH7.4				Proniosomes prepared using distilled water				Proniosomes prepared using 0.1 % glycerol			
	Size d.nm	PDI	Zeta-potential	EE%	Size d.nm	PDI	Zeta-potential	EE%	Size d.nm	PDI	Zeta-potential	EE%
25	498.8 ± 24.259	0.473 ± 0.026	-44.3 ± 2.69	81.2	190.9 ± 15.031	0.368 ± 0.262	-30.0 ± 3.04	90.5	273.8 ± 43.696	0.610 ±0.033	-36.1 ± 4.74	87.5
50	625.6 ± 17.668	0.622 ± 0.014	-55.1 ± 3.04	82.4	293.4 ±5.237	0.437 ±0.014	-40.0 ± 1.84	85.6	186.7 ± 13.450	0.290 ±0.05	-41.4 ±1.27	75.2
75	891.3 ± 29.068	0.71 ± 0.03	-64.8 ± 3.75	77.8	302.0 ± 16.371	0.414 ±0.016	-51.0 ± 1.41	80.5	187.2 ± 30.363	0.317 ± 0.032	-61.1 ±2.97	73.6
100	958.0 ± 30.016	0.763 ± 0.010	-77.1 ± 4.95	73.5	379.5 ± 8.237	0.430 ±0.020	-60.0 ±0.707	79.6	253.7 ± 18.352	0.375 ± 0.015	-64.1 ± 2.19	70.2

Table 3: EE% of proniosomes derived-niosomes formulations.

Formula	EE%		
	using phosphate buffer	using distilled water	Using 0.1% glycerol
A1	44.99	63.33	52.15
A2	58.21	71.25	60.98
A3	65.83	73.52	72.06
A4	60.46	62.11	62.14
A5	64.11	69.83	67.23
A6	70.45	75.02	69.72
A7	66.14	65.18	61.34
A8	74.59	92.38	76.91
A9	75.41	87.47	79.39
A10	76.61	85.61	69.11

The preparation method of proniosomal formulation includes the mixture of alcohol; surfactants, cholesterol and lecithin were used to form the concerted proniosomal gel, which can be transformed to stable niosomal dispersion by dilution with excess aqueous phase spontaneously. Three different aqueous phases were used. Characterization of proniosomes formulation

Vesicle physical analysis

Vesicles with lesser diameter are believed to have enhanced permeation through the skin as smaller vesicles tend to fuse readily³⁴. The prepared proniosome gel formulations revealed niosomes vesicular structure with entrapped atenolol. Examination by light microscope shows formulation A8 both loading with 25 mg drug and unloaded were smaller in size and uniform surface area Figure 1.

Transmission electron microscope (TEM) images of (A8) formulations prepared with three different aqueous phase show that the niosome formulations prepared with Span 40 & 60 were well-identified spherical, smooth surface,

homogenous and smaller dimensions Nano vesicular. Figure 2 showed both unloaded and drug loaded niosomes prepared with three different aqueous phase.

Drug content

Table 2 summarized the size, PDI and Zeta potential ± S.D of four different drug concentrations loaded proniosomes (25, 50, 75, and 100 mg). Each concentration was prepared with three different aqueous phases. From the previous table it is clear that as drug concentration increased, both size dimensions and PDI are increased with changing values of negatively charged Zeta potential. Niosomal dispersion prepared using distilled water and loaded with 25 mg drug have smaller size, PDI and suitable potential; 190.9 ± 15.031 nm, 0.368 ± 0.262 , -30.0 ± 3.04 mV respectively. For 50 mg drug loaded niosomes, size range from 186.7 ± 13.45 to 625.6 ± 17.66 nm, size of 75 mg drug loaded was from 187.2 ± 30.36 to 891.3 ± 29.06 nm, while for 100 mg drug the size range was 253.7 ± 18.35 to 958.0 ± 30.01 nm. PDI and Zeta potential indicted proniosomes stability loaded with 25 mg drug, they are higher in other drug concentrations ranged from 0.473 ± 0.026 to 0.763 ± 0.01 and -44.3 ± 2.69 to -77.1 ± 4.95 respectively for proniosomes prepared using phosphate buffer. 0.290 ± 0.05 to 0.610 ± 0.03 and -36.1 ± 4.74 to -64.1 ± 2.19 respectively for proniosomes prepared using 0.1% glycerol. EE% was highest in proniosomes prepared with distilled water and loaded with 25 mg drug i.e. 90.5% followed by proniosomes loaded with 75 mg, then 50 mg and finally loaded with 100 mg. drug concentration had nonsignificant difference on EE% between niosomal dispersion prepared using phosphate buffer 7.4 and 0.1% glycerol.

Entrapment efficiency

The entrapment was expressed as a percentage of the total amount of atenolol used in preparation of the proniosomes are summarized in Table 3 and shown in Figures 3, 4, 5 and 6. Dispersion prepared using phosphate buffer (pH 7.4) exhibited EE% ranged from 44.99 to 76.61. That prepared

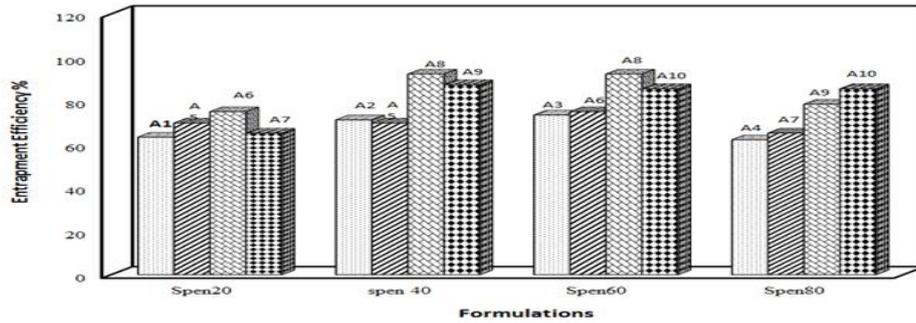


Figure 3 : Entrapment efficiency of proniosomes prepared using phosphate buffer (PH7.4).

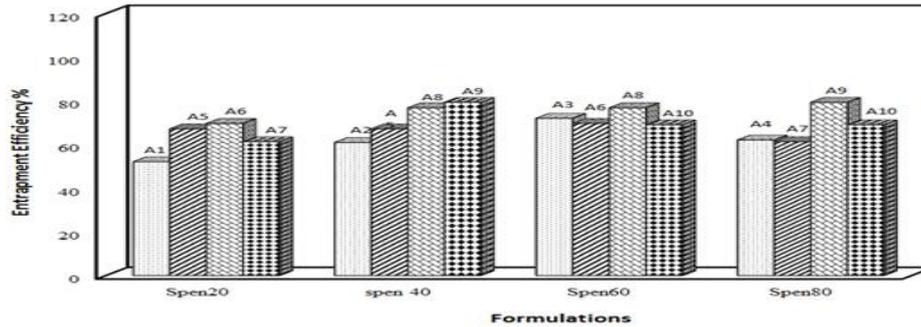


Figure 4 : Entrapment efficiency of proniosomes prepared using distilled water.

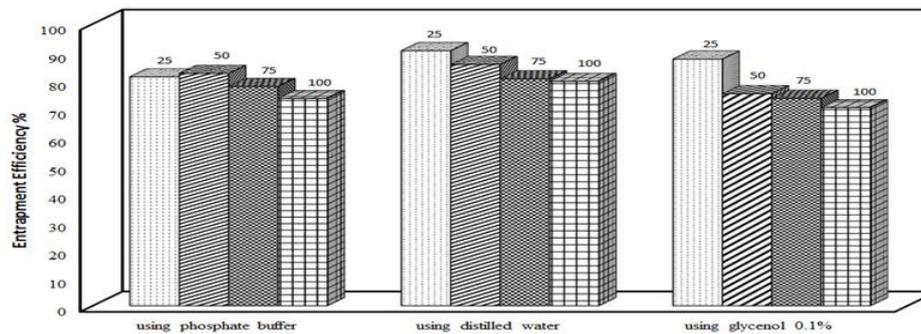


Figure 5 : Entrapment efficiency of proniosomes prepared using 0.1% glycerol.

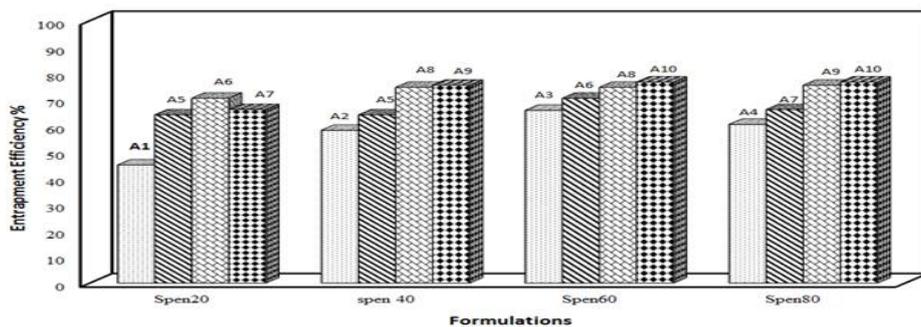


Figure 6 : Entrapment efficiency of proniosomes prepared using different loaded drug concentration and prepared with different aqueous phase.

using distilled water had EE% from 61.83 to 92.38. While niosomes prepared using 0.1% glycerol had EE% between 52.15 and 79.39. Niosomes formed from Span 40 and 60 exhibited high entrapment. Formula A8 had the highest EE% in different preparations especially that prepared using distilled water. Entrapment efficiency percentage

was measured for different loaded drug concentrations; it was higher in proniosomes loaded with 25 mg drug prepared with distilled water 90.5% than proniosomes prepared with phosphate buffer 81.2% followed by proniosomes diluted with 0.1% glycerol solution 87.5%. EE% for formula loaded with 50 mg drug was 85.6

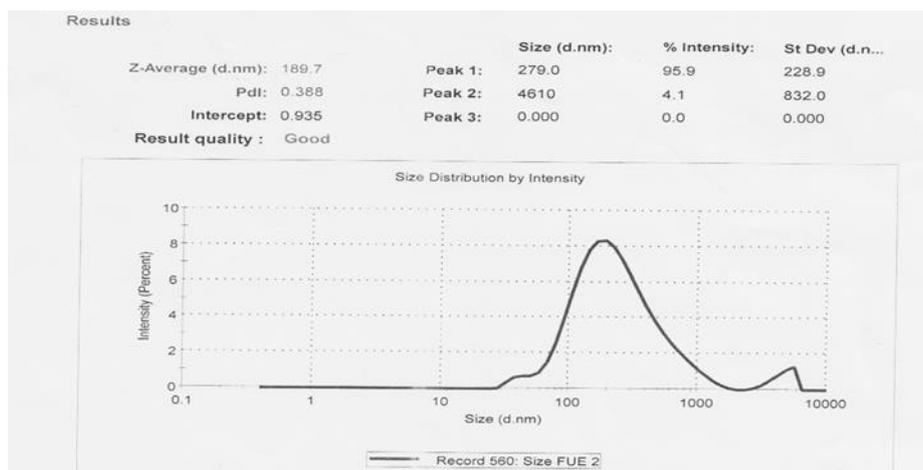


Figure 7 : proniosomes size of A8 loaded with 25 mg drug and prepared with distilled water.

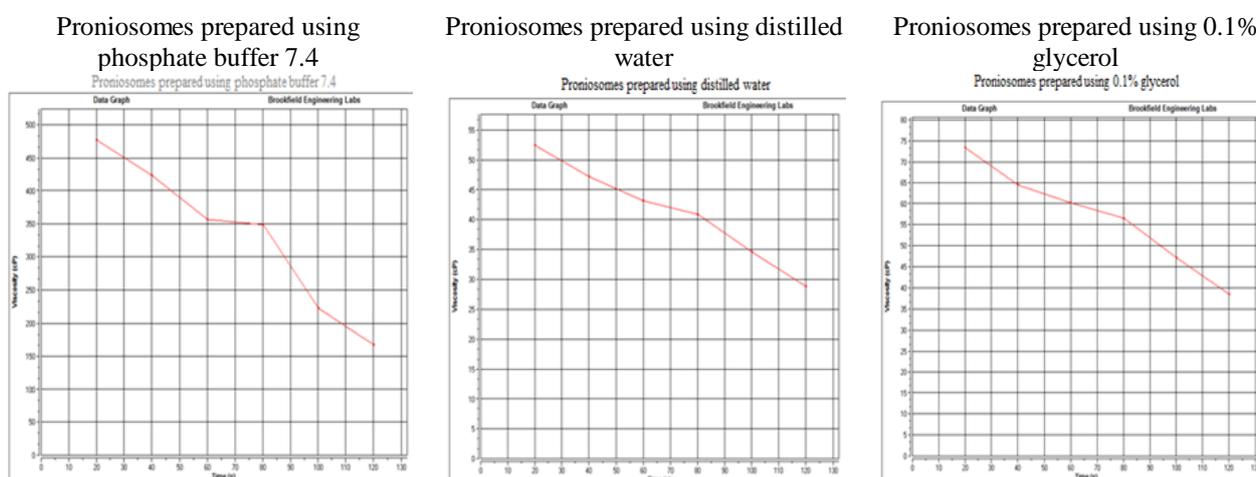


Figure 8: Rheological behavior of proniosomes gel loaded with 25 mg drug and prepared with different aqueous phase.

prepared with distilled water, 82.4 prepared with phosphate buffer and 75.2 for that prepared with 0.1% glycerol solution. Proniosomes loaded with 75 mg atenolol had EE% 80.5 prepared with distilled water, 77.8 prepared with phosphate buffer and 73.6 prepared with 0.1% glycerol solution. Proniosomes loaded with 100 mg drug had EE% 79.6 prepared using distilled water, 73.5 prepared with phosphate buffer and 70.2 prepared with 0.1% glycerol solution.

Particle size analysis

Table 4 clarifies proniosomes-derived niosomes both unloaded and loaded with atenolol of ten formulas for each. Loaded vesicles were formulated with three different aqueous phases. Nano-diameters size, PDI and Zeta potential \pm S.D measurements indicated that unloaded vesicles had parameters of 259.2 ± 21.31 to 867.0 ± 40.71 nm sizes, 0.370 ± 0.021 to 0.796 ± 0.031 PDI and -34.1 ± 0.990 to -49.5 ± 1.63 mV Zeta potential. Loaded vesicles prepared with phosphate buffer 7.4 assumed sizes 406.4 ± 21.634 to 918 ± 12.07 nm, PDI 0.578 ± 0.031 to 0.901 ± 0.01 and Zeta potential started with -43.4 ± 0.849 up to -68.6 ± 3.46 mV. Distilled water prepared niosomes had sizes ranged from 195 ± 8.31 to 462.2 ± 19.55 nm, PDI

0.362 ± 0.025 to 0.745 ± 0.013 and potential -31.6 ± 1.89 to -65.4 ± 0.636 mV. Vesicles prepared with 0.1% glycerol solution had sizes 278 ± 23.05 to 744.5 ± 8.62 nm, PDI 0.456 ± 0.01 to 0.9 and potential -45.7 ± 0.424 to -69.3 ± 0.354 mV. Vesicles were prepared with distilled water was smaller in size than those prepared with buffer or glycerol. Average particle size of niosomes measured by Zeta sizer was found to be higher than measuring with TEM. For buffer prepared niosomes sizes measured by TEM ranged from 125 to 216 nm, those with distilled water had sizes 33.9 to 127 nm and 0.1% glycerol prepared niosomes had sizes 54.7 to 205 nm. Fig. 7 showed example of these measurements. *Measurement*

The pH measurements of niosomal dispersion loaded with atenolol and prepared with three different aqueous phases formulations were measured. The pH values were in the range from 6.99 ± 0.132 to 7.116 ± 0.201 indicating that the pH values of the prepared formulations lies within the acceptable range for topical preparations. This is explained by the fact that the pH value of the SC and the upper viable epidermis has been recorded to be 4.0–4.5 and 5.0–7.0, respectively, and the local pH value of the SC extracellular lipid matrix is 6³⁵.

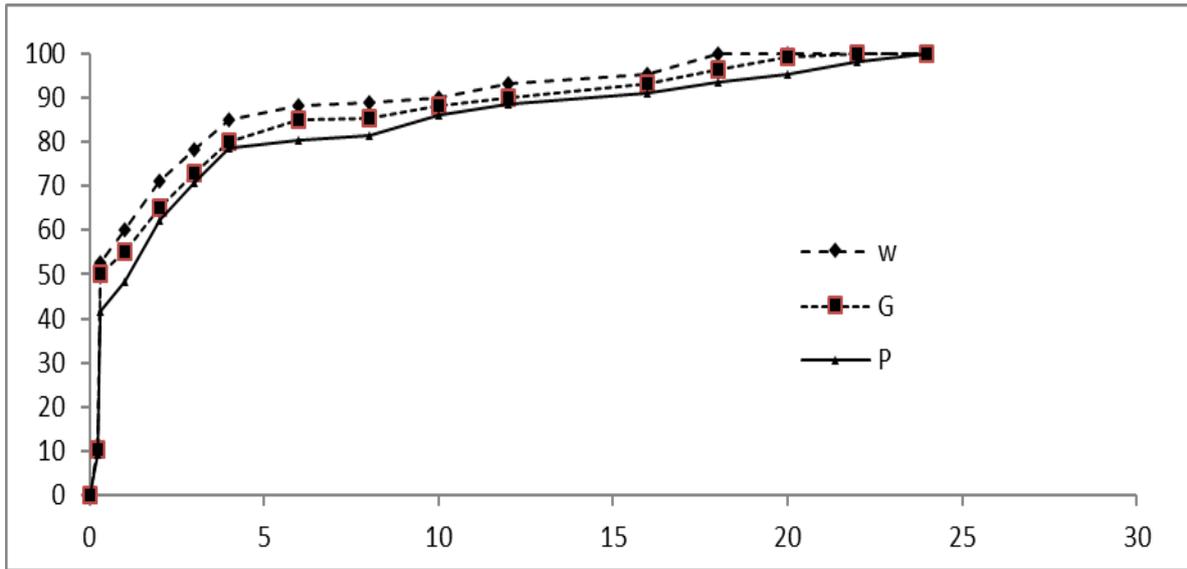


Figure 9: Release profile of atenolol from optimum proniosomes formulation prepared using phosphate buffer (PH 7.4), distilled water and 0.1% glycerol.

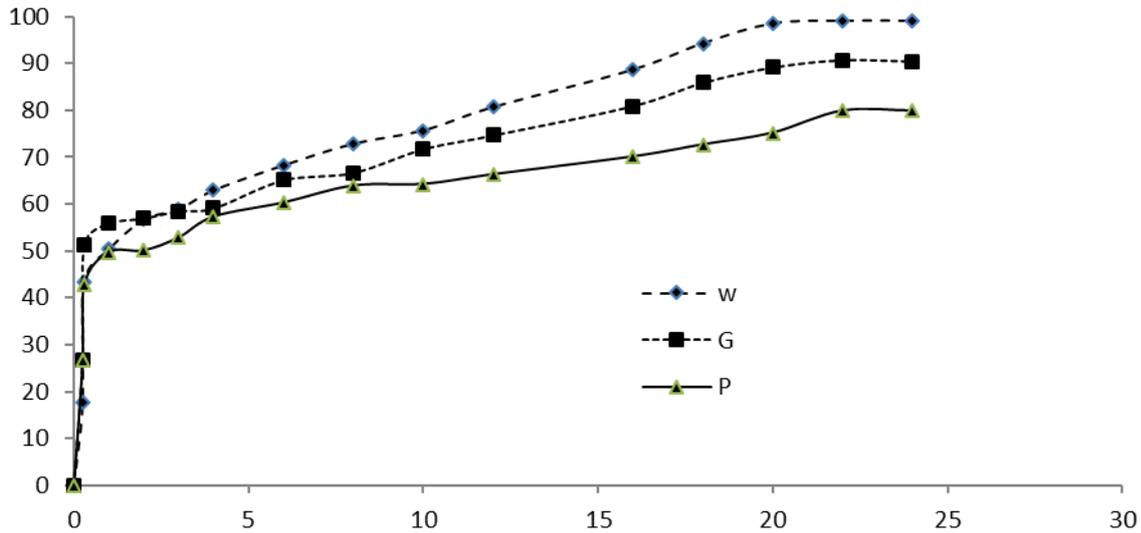


Figure 10: Release profile of atenolol from optimum proniosomes formulation prepared using phosphate buffer (PH 7.4), distilled water and 0.1% glycerol on rate skin.

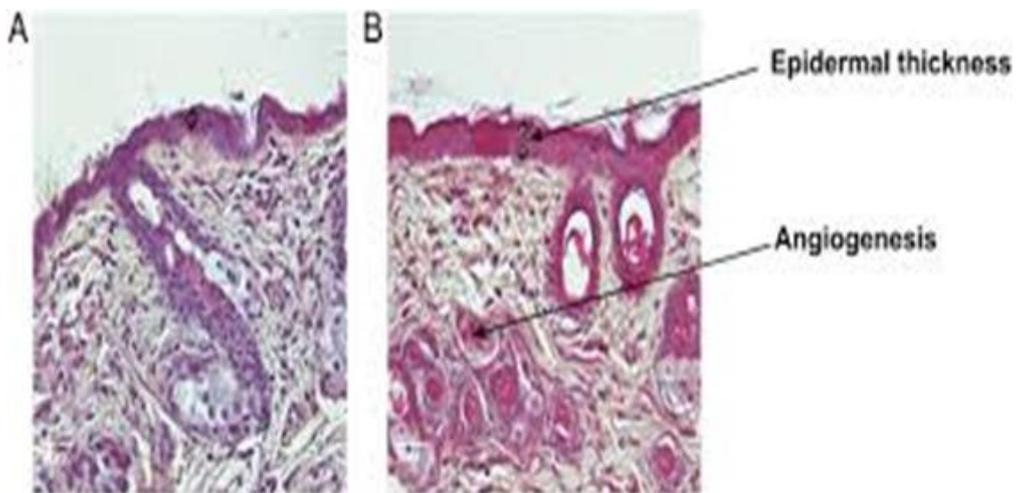


Figure 11: Histographical images of normal skin and atenolol proniosomes-driven noisomes gel in rate tissue.

Rheological study

The rheological behavior of the prepared proniosomes formulations were studied to determine the appropriate gel formula and concentration that can form semisolid matrices in a single-step process. The rheological data namely Farrow's constant (N) and the hysteresis area (H.A.) for the selected formulations reveal that the gel formulations exhibited Farrow's constant (n) values larger than one, which indicates shear thinning characteristics with variable thixotropic (data are not shown). The yield values for the investigated proniosomes gel formulations were greater than 20 Pa. Products with yield points below this value will flow readily by themselves. Semisolids with yield value above 20 Pa will flow more slowly. The combined shear thinning behavior and thixotropic are desirable characteristics for topical formulations, as they facilitate processing during manufacture and the flow from the container, and improve spreading on the skin. In addition, the applied film can gain viscosity instantaneously and thus resist running³⁶. Figure 8 showed rheological behavior of different proniosomes gel.

Spreadability test

Spreadability is another important characteristic of dermatological medicines to be evaluated during developmental, because it is responsible for the correct dosage transfer to the target site and easy way of application on the substrate²⁸. The values of the S_f of the formulations as a function of the added weight are summarized in Table 5. None significant difference was observed in S_f for proniosomes derived niosomes gel formulations. Although there was slight increase in S_f for formulations prepared with deionized water than that prepared with phosphate buffer 7.4 and 0.1% glycerol solution.

Assessment of physical stability for proniosomes

At refrigeration temperature (4-8 °C)

After three months of stability monitoring the formulations prepared using phosphate buffer (pH 7.4), distilled water and 0.1% glycerol were stable. All the previously mentioned formulations showed, both, high entrapment efficiency (>60%) and high atenolol retention value (>90%), by the end of each month. The studied formulations also gave the optimum Nano size, PDI and Zeta potential Table 6.

Storage at room temperature

Studying the stability of different proniosomes formulations prepared with different aqueous phases for three months at room temperature revealed that the formulations prepared using distilled water are the most stable, since they were the only showing both, high entrapment efficiency (>60%) and high atenolol retention value (>90%), throughout the three months. However, those prepared using phosphate buffer (pH 7.4) and 0.1% glycerol, showed certain stability till either the first or second month of storage but none was stable by the end of the third month. Comparing stability of different proniosomes formulations stored at 4 °C after 3 months the following observed: (1) proniosomes are generally more stable at low temperature, (2) distilled water, not only

did it provide the optimal entrapment conditions and retention value for atenolol, but also, yield the most stable proniosomes, followed by buffered preparations and finally, those prepared using 0.1% glycerol.

In vitro release studies

Drug release for optimum formulations of the proniosomal gels was observed as demonstrated in Figure 9. *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. Atenolol was released from the proniosomes with an initial fast release phase followed by a sustained release pattern. The release of atenolol from the investigated stable formulations followed zero order kinetics with an immediate release of drug (no lag time). The drug permeation was maximum (100%) from formulations containing Span 40 & 60 (A8) prepared with different aqueous phase especially that prepared with distilled water. Non-significant difference ($p < 0.05$) in *in vitro* release of A8 between formulations of different aqueous phase. Also all formulations reached to plateau and gave sustained release over 24 h.

Skin irritancy test

The selected proniosomes formulation (A8) with different aqueous phase showed an irritation potential of 0.37 ± 0.1 , 0.39 ± 0.0 , 0.35 ± 0.1 thus proving to be non-irritant as it was mentioned by Van-Abbé et al (31), that a value between 0 and 9 in an irritancy test indicates that the applied formulation is generally non-irritant to human skin. No obvious erythema, oedema or inflammation was observed on rats' skin after one week of application of the selected formulations.

Ex-vivo permeation studies

Skin of rats was used in *ex vivo* percutaneous permeation studies due to its availability²³. Figure 10 showed the cumulative percent of atenolol was released from niosomal gel prepared with three different aqueous phases (phosphate buffer pH 7.4, distilled water and 0.1% glycerol). Release rate was significant higher across cellulose membrane compared to rate skin. (A8) formula effectively enhanced atenolol release also slow permeation rate. After 24 h of the study period, 99.08% of atenolol proniosomal formulation prepared with distilled water was permeated thorough the rat skin. 90.68% drug released from formulation diluted with 0.1% glycerol and 79.84% atenolol was released from proniosomal formulation prepared with phosphate buffer 7.4. It became clear that proniosomes possess higher skin penetration ability and use of different aqueous phase may affect rate of release.

Drug in rat skin

Histopathological images of normal skin and skin after application of niosomal gel loaded with atenolol have been presented in Figure 11 .

As depicted by the images, no major morphological changes were observed either on the skin surface or at the hair follicular openings. However, occasional depositions could be seen at certain places especially accumulation of proniosomal gel on the ridges present on skin surface and

Table 4 : Particle size analysis of proniosomes -derived niosomes formulations (n=3).

Formulations	unloaded using distilled water			Drug loaded using phosphate buffer 7.4			Drug loaded using distilled water			Drug loaded using glycerol 0.1%		
	size d.nm	PDI	Zeta potential \pm S.D	size d.nm	PDI	Zeta potential \pm S.D	size d.nm	PDI	Zeta potential \pm S.D	size d.nm	PDI	Zeta potential \pm S.D
A1	530 \pm 25.55	0.796 \pm 0.031	-48.3 \pm 0.495	880.0 \pm 33.12	0.802 \pm 0.012	-64.7 \pm 0.0707	408.0 \pm 12.27	0.51 \pm 0.012	-64.3 \pm 1.77	550.9 \pm 15.8	0.595 \pm 0.031	-45.7 \pm 0.424
A2	547.1 \pm 15.151	0.493 \pm 0.052	-46.7 \pm 2.05	893.0 \pm 15.632	0.868 \pm 0.023	-67.3 \pm 0.354	398.0 \pm 11.73	0.494 \pm 0.013	-60.0 \pm 0.495	552.0 \pm 14.11	0.531 \pm 0.012	-51.8 \pm 1.63
A3	769.3 \pm 37.301	0.645 \pm 0.047	-48.0 \pm 1.34	918.0 \pm 2.07	0.900 \pm 0.030	-68.6 \pm 3.46	462.2 \pm 19.55	0.529 \pm 0.023	-62.6 \pm 2.76	744.5 \pm 8.62	0.583 \pm 0.031	-60.3 \pm 0.429
A4	259.2 \pm 21.31	0.370 \pm 0.021	-49.5 \pm 1.63	801.0 \pm 20.16	0.901 \pm 0.010	-68.6 \pm 5.87	343.8 \pm 16.71	0.333 \pm 0.010	-60.8 \pm 0.212	367.9 \pm 22.12	0.443 \pm 0.15	-55.9 \pm 0.424
A5	538.3 \pm 352.11	0.536 \pm 0.011	-44.2 \pm 0.778	885.0 \pm 30.751	0.900 \pm 0.024	-67.8 \pm 0.212	337.7 \pm 14.20	0.735 \pm 0.013	-60.5 \pm 1.70	387.7 \pm 19.73	0.900 \pm 0.10	-60.8 \pm 2.33
A6	867.0 \pm 40.71	0.657 \pm 0.023	-45.3 \pm 0.707	902.0 \pm 10.613	0.800 \pm 0.035	-67.5 \pm 0.566	241.2 \pm 16.62	0.580 \pm 0.021	-60.2 \pm 0.141	419.8 \pm 12.23	0.442 \pm 0.025	-69.3 \pm 0.354
A7	422.6 \pm 22.62	0.478 \pm 0.015	-44.8 \pm 0.354	516.0 \pm 25.273	0.721 \pm 0.026	-63.1 \pm 2.11	365.5 \pm 17.51	0.559 \pm 0.014	-55.2 \pm 1.77	355.4 \pm 18.61	0.552 \pm 0.013	-63.5 \pm 5.16
A8	453.8 \pm 9.812	0.522 \pm 0.014	-34.1 \pm 0.990	496.0 \pm 12.812	0.631 \pm 0.012	-43.4 \pm 0.849	195.0 \pm 9.31	0.497 \pm 0.021	-31.6 \pm 1.89	428.5 \pm 10.41	0.512 \pm 0.010	-57.3 \pm 0.073
A9	487.1 \pm 30.615	0.530 \pm 0.026	-46.5 \pm 0.707	505.0 \pm 18.901	0.900 \pm 0.010	-61.2 \pm 2.83	339.5 \pm 20.52	0.530 \pm 0.034	-66.1 \pm 2.26	380.7 \pm 13.61	0.530 \pm 0.021	-49.8 \pm 1.91
A10	583.5 \pm 39.340	0.531 \pm 0.031	-47.0 \pm 3.75	406.9 \pm 21.634	0.578 \pm 0.031	-60.2 \pm 0.566	196.0 \pm 11.64	0.362 \pm 0.025	-65.4 \pm 0.636	278.0 \pm 23.05	0.456 \pm 0.011	-65.0 \pm 1.56

at the hair follicular openings in case of formula treated skin. The extraction efficiency was measured by comparing the amount of drug added and that obtained after extraction, it was found to be $92.6 \pm 0.5\%$. The amounts (% applied dose/ cm²) of atenolol gel in SC, dermal layer (E+D) and receptor compartment (RC) in rat skin were found to be 0.09 ± 0.004 , 0.33 ± 0.027 and 0.5 ± 0.013 , respectively.

DISCUSSION

The proniosomes are promising drug carriers, because they possess greater chemical stability and lack of many disadvantages associated with liposomes. It has additional merits with niosomes are low toxicity due to non-ionic nature, nor equipment of special precautions and conditions for formulation and preparation. One of the most useful aspects of proniosomes is their ability to target drugs to particular area. Proniosomes can be used to target drugs to the reticuloendothelial system (RES). The RES preferentially takes up proniosomes vesicle³⁷. The uptake of proniosomes is controlled by circulating serum factors called opsonins. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of the drugs can also be used for treating parasitic infections of the liver. Proniosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to proniosomes (as immunoglobulin bind readily to the lipid surface of the niosome) to target them to specific organ. Proniosomes are used as carriers in transdermal delivery of anti-hypertensive drugs as captopril in the treatment of hypertension. Proniosomal system causes extended release of drug in the body. The drug is encapsulated with sorbitol esters, lecithin and cholesterol.

Investigation of niosomes by optical and TEM microscopy reveal the presence of spherical niosomes showing gradual increase in transparency from the center to the periphery but some other vesicles appeared as dark spherical spots which exist in disperse and aggregate

Table 5: Spreadability factor and pH value of (A8) gel loaded with 25 mg atenolol prepared with different aqueous phase (n=3).

Formula (A8)	Spreadability factor S_f (nm^2/g) \pm S.D	pH value \pm S.D
prepared using phosphate buffer 7.4	1.821 ± 0.052	6.90 ± 0.03
prepared using distilled water	1.978 ± 0.0453	7.00 ± 0.20
prepared using 0.1% glycerol	1.554 ± 0.00	6.50 ± 0.02

collections including the presence of pores and minor surface roughness. The

particle size of niosomes is increasing according formulation with different ratios of non-ionic surfactants as niosomes containing Span 40 and 60 form vesicles of suitable Nano diameter. Unloaded vesicles had the largest Nano diameter that may due to porous surface allow swallowing.

Studying of drug concentrations as a parameter of formula optimization revealed that 25 mg drug loaded is an optimum drug content. The vesicles were smaller in size with both PDI and Zeta potential indicating stability and higher EE% than other concentrations.

Formulation containing Span 40 and Span 60 exhibited very high entrapment³⁸. 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]³⁹. 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. The encapsulation efficiency of tween is relatively low as compared to span. The geometry of vesicle to be formed from surfactants is affected by its structure, which is related to critical packing parameters. On the basis of critical packing parameters of surfactants can predicate geometry of vesicle to be formed. Critical packing parameters can be defined using following equation.

$$CPP = \frac{v}{lc \times a_0}$$

CPP = 0.5 micelles form

CPP = 0.5 – 1 spherical vesicles form

CPP = 1 = inverted vesicles form

V– Hydrophobic group volume lc = the critical hydrophobic group length, a_0 = the area of hydrophilic head group. Span60 is the good surfactant because it has CPP value between 0.5 and 1⁴⁰.

Ratio and type of nonionic surfactants are affecting niosomes size. The formulation containing Span 40 and 60 form the vesicles of larger size. Selection of surfactant was done on the basis of their HLB value. As hydrophilic lipophilic balance (HLB) is a good indicator of vesicle forming ability of any surfactant, HLB number in between 4 and 8 was found to be compatible with vesicle formation. It is also reported that the hydrophilic surfactant owing to high aqueous solubility on hydration do not reach a state of concentrated systems in order to allow free hydrated units to exist aggregates and coalesced to form lamellar structure. The water soluble detergent polysorbate 20 forms niosomes in the presence of cholesterol. This is despite the fact that the HLB number of this compound is 16.7 and the degree of entrapment is affected by the HLB of a

surfactant. Transition temperature of surfactants also affects the entrapment of drug in vesicles. Spans with highest phase transition temperature provide the highest entrapment for the drug and vice versa. Span 40 and Span 60 produces vesicles of larger size with higher entrapment of drug. The drug leaching from the vesicles is reduced due to high phase transition temperature and low permeability. Higher HLB value of Span 40 and Span 60 results reduction in surface free energy which allows forming vesicles of larger size. The length of alkyl chain of surfactant has a prominent effect on permeability of prepared niosomes as length of surfactant increases entrapment efficiency also increases. Hence, long chain surfactant results in high entrapment. Thus, span 60 has a longer saturated alkyl chain (C16) compared to span 20 (C10), so it produces niosomes with higher entrapment efficiency⁴¹. Generally, no significant difference is noticed in the entrapment efficiency between proniosomes formulations with changing nonionic surfactants Span of different grad while fixing amounts of Lecithin and cholesterol. This might be attributed to a previous report stating that a fixed amount of lipid in the formula produces constant number of niosomes and has a definite encapsulating capacity; increasing this amount has no beneficial effect. Also each definite formula had own EE%, it is clear that it is highly influenced by the type of non-ionic surfactant and the aqueous medium used, since variable results were obtained in each case. The pH of prepared atenolol proniosomes delivered niosomes dispersion are ranged from 6.5 ± 0.02 to 7 ± 0.20 gave proper permeability and stability. The actual pH of the hydration medium depends on the solubility of the drug being encapsulated.

Thixotropic properties that is desirable for topical formulations⁴². This phenomenon indicates the breakdown of the three-dimensional structure of the system that is of particular interest in the area of semisolid technology, since it makes the system more fluid when submitted to external pressure, and therefore, it spreads more easily in the region to which the pressure is applied⁴³. This thixotropic behavior would facilitate preparation, pouring, spreading, handling, and application to the skin and also would rapidly produce a coherent film over the skin surface.

The efficacy of topical therapy depends on the patient spreading the formulation in an even layer to deliver a standard dose. The optimum consistency of such a formulation helps ensure that a suitable dose is applied or delivered to the target site. This is particularly important with formulations of potent drugs. A reduced dose would not deliver the desired effect, and an excessive dose may

Table 6: Stability studies after three months of optimum proniosomes prepared formula (A8) using different aqueous phase at 4°C.

optimum formula A8	prepared using phosphate buffer 7.4	prepared using distilled water	prepared using distilled water	prepared using glycerol	0.1%
Size	299.5	144.1	144.1	243.4	
PDI	0.337	0.384	0.384	0.442	
Zeta potential \pm S.D	-42.1 ± 3.25	-39.3 ± 0.424	-39.3 ± 0.424	-53.6 ± 1.13	
EE%	91.35	95.49	95.49	90.57	
Retention value %	93.8	95.6	95.6	91.2	

lead to undesirable side effects. The delivery of the correct dose of the drug depends highly on the spreadability of the formulation⁴⁴. Gel is easily spreadable by small amount of shear. The spreadability of the gels was optimum for all formulations; three dimensions of hydrated gel are easily to breakdown with shear.

Percentage drug retained in proniosomes derived niosomes were selected as a parameters for the stability, since the stability of the formulation would reflect in drug leakage and a decrease in the percentage drug retained. Size, PDI and Zeta potential analysis was done for determining the colloidal properties of the prepared formulations. The impact of drug release slightly varies according to the composition of aqueous phase, the rate of release was more fast using distilled water may related to hydrophilic character of the drug with fixed amount and type of nonionic surfactants and lipids. The release of atenolol from niosomal gels is a combination of the release of drug from niosomes and the diffusion through the polymer network channel structures of the gel. The initial fast release was due to desorption of the drug molecules that were present on the surface of formed niosomes. In later phase, the atenolol release was regulated by diffusion through the inflated bilayers and exhibited sustained release pattern⁴⁵. The entrapped atenolol molecules could leak out gradually from the vesicles into the surrounding gel. Previous studies reported that the cholesterol present in the monolayer would limit 1, 2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE) mobility and favor an upright orientation of the DPPE hydrocarbon chains thus enhancing Van der Waals interactions. Consequently, it would contribute to a spontaneous mixing between the two membrane lipids and to an increased stability of the monolayer⁴⁶. Also, inclusion of cholesterol within the niosomal structure could markedly reduce the flux of drug during the release phase, which is in accordance with the membrane stabilizing activity of this lipid⁴⁷. High cholesterol content facilitated the formation of compact niosomal bilayers with reduced permeability and thus hindered the release of drug entrapped⁴⁸. Moreover, drug release is mostly reflective of the viscosity of gel formulations. Viscosity of the gel formulation is a very important factor that affects the release of drug since it may reduce diffusion rate of drug from the vehicles. Hereby, a reverse relationship was observed between the viscosity of liposomal gels and the percent of atenolol released.

The comparative low skin permeability of atenolol than through cellulose membrane due to barrier properties of skin, while the high permeability of proniosomes resulted

in improved skin penetration of atenolol. Better transdermal flux without lag phase of proniosomal gel might be the result of high association of drug with vesicle bilayers, increased partitioning of vesicles into the stratum corneum and elasticity of vesicle membrane. Fusion of niosomes vesicles to the skin surface and direct transfer of drug from vesicles to the skin. All these factors would help achieving a sustained release of atenolol into blood circulation.

One of the most useful aspects of niosomes is that they greatly enhance the uptake of drugs through the skin. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug. Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonial encapsulated within niosomes are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence, decrease both in dose and toxicity. Enhanced atenolol penetration in skin might be due to the larger surface area of Nano sized, which eventually make close contact with superficial junctions of corneocyte clusters, furrows between corneocyte islands, and hair follicles. This subsequently enhances the accumulation of particles on the skin surface as well as within the hair follicles for longer duration with concomitant achievement of sustained drug release⁴⁹.

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 and the results were found in the acceptable range. Formulation A8 (Span 40 & 60, cholesterol, lecithin and distilled water as aqueous phase) has been selected as an optimized therapeutic system of atenolol. It provides the highest entrapment efficiency, small Nano size, suitable PDI and Zeta potential. The formula gave sustained *in vitro* and *ex vivo* release, optimum rheology and spreadability characters. More stable at low temperature (4 °C) and have high skin permeability. 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.

ACKNOWLEDGEMENTS

The experimental work of the research was facilitated and completed in research lab of Pharmaceutics and Pharmaceutical Technology department, Future University.

REFERENCE

- Ozge I, Muge K, Nuray A, Tamer B. In Vitro and In Vivo transdermal Studies of Atenolol using Iontophoresis. *Acta Pol Pharm Drug Res* 2008; 65(1): 29-36.
- Jun-S C, Sang- S. Enhanced bioavailability of Atenolol by transdermal administration of the ethylene-vinyl acetate matrix in rabbits. *Eur J Pharm Biopharm* 2003; 56: 439-443.
- Sastry SV, Reddy IK, Kahn MA. Atenolol gastrointestinal therapeutic system: Optimization of formulation variables using response surface methodology. *J Control Release* 1997; 45: 121-130.
- Jug M, Becirevic-Lacan M, Benghez S. Novel cyclodextrin-based film formulation intended for buccal delivery of Atenolol. *Drug Dev Ind Pharm* 2009; 35(7): 796-807.
- Hadgraft J, Guy R H. Transdermal drug delivery: a perspective. *J Control Rel* 1987; 237-251.
- Elias P M, Friend D S. The permeability barrier in mammalian epidermis. *J Cell Biol* 1975; 65: 180-191.
- Thong H-Y, Zhai H, Maibach, H I. Percutaneous penetration enhancers: an overview. *Skin Pharmacol Physiol* 2007; 20: 272-282.
- Betageri G, Habib M. Liposomes as drug carriers. *Pharm Eng* 1994; 14: 76-77.
- Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. *J Control Rel* 1994; 30: 1-15.
- Hu C J, Rhodes D G. Proniosomes. a novel drug carrier preparation. *Int J Pharm* 1999; 185: 23-35.
- Blazek-Welsh A, Rhodes D G. Maltodextrin based proniosomes. *AAPS Pharm Sci* 2001; 3: 1-8.
- Alsarra I A, Bosela, A A, Ahmed S M, Mahrous G M. Proniosomes as a drug carrier of ketorolac. *Eur J Pharm Biopharm* 2005; 59: 485-490.
- Barry B W. Novel mechanisms and devices to enable successful transdermal drug delivery. *Eur J Pharm Sci* 2001; 14: 101-114.
- Kirjavainen M, Urtti A, Jääskeläinen I, Suhonen T M, Paronen P, ValjakkaKoskela R, Kiesvaara J, Mönkkönen J. Interaction of liposomes with human skin in vitro the influence of lipid composition and structure. *Biochim Biophys Acta* 1996; 1304: 179-189.
- Frkjaer S, Hjorth E, Wfrtis O. Stability testing of liposomes during storage. In: Gregoriadis G. (Ed.), *Liposome Technology*. CRC Press, Boca Raton, FL 1984; 1:235-245.
- Hunt C, Tsang S. Tocopherol retards auto-oxidation and prolongs the shelf-life of liposomes. *Int J Pharm* 1981; 8: 101-11.
- Wong M, Thompson T. Aggregation of dipalmitoyl-phosphatidyl-choline vesicles. *Biochemistry* 1982; 21: 4133-4139.
- Vora B, Khopade A J, Jain N K. Proniosome based transdermal delivery of levonorgestrel for effective contraception. *J Control Rel* 1998; 54: 149-165.
- Hatziantoniou S, Rallis M, Demetzos C, Papaioannou G. The Pharmacological activity of natural lipids on a skin barrier disruption model. *Pharmacol Res*. 2000; 42:55-59.
- Niemiec S M, Ramachandran C, Weine N. Influence of non-ionic liposomal composition on topical delivery of peptide drugs into pilosebaceous units: an in vivo study using the hamster ear model. *Pharm Res* 1995; 12: 1184-1188.
- Arunothayanun P, Bernard MS, Craig DQM, Ucheghu IF, Fluorence AT. The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether. *Int J Pharm* 2000; 201: 7-14.
- Ibrahim A A, Bosela AA, Ahmed SM, Mahrous GM. Proniosomes as a drug carrier for transdermal delivery of ketorolac. *Eur J Pharm Biopharm* 2005; 59: 485-490.
- Mahmoud M, Ahmed I, Omaira S A, Mohamed H A, Nagia M A. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. *Int J Pharm* 2008; 361: 104-111.
- Khalid A, Reena T, Mohammad SS, Asgar A, Roop K K, Faiyaz S, Ehab T I. Proniosomal transdermal therapeutic system of losartan potassium. Development and pharmacokinetic evaluation. *J Drug Target* 2009; 17(6): 442-449.
- Bhowmik BB, Nayak BS, Chatterjee A. Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. *Int J Pharm Pharm Sci* 2009; 1:240-57.
- Rawlins EA. *Rheology*. London. Bailliere Tindall 1977; 123-29.
- Ourique AF, Melero A, De Bona da Silva C. Improved photo-stability and reduced skin permeation of tretinoin: development of a semisolid nanomedicine. *Eur J Pharm Biopharm* 2011; 79:95-101.
- Fontana MC, Rezer JF, Coradini K. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. *Eur J Pharm Biopharm* 2011; 79: 241-9.
- Pardakhty A, Varshosaz J, Rouholamini A. In vitro study of polyoxyethylene alkyl ether niosomes for delivery of insulin. *Int J Pharm* 2007; 328: 130-141.
- Van-Abbé, N J, Nicholas P, Boon E. Exaggerated exposure in topical irritancy and sensitization testing. *J Soc Cosmet Chem* 1975; 26: 173.

32. Nair VB, Panchagnula R: The effect of pretreatment with terpenes on transdermal iontophoretic delivery of arginine vasopressin. *II Farmaco* 2004; 59(7):575–81.
33. Dubes A, Parrot-Lopez H, Abdelwahed W, Degobert G, Fessi H, Shahgaldian P. Scanning electron microscopy and atomic force microscopy imaging of solid lipid nanoparticles derived from amphiphilic cyclodextrins. *Eur J Pharm Biopharm* 2003; 55(3):279–82.
34. Escobar-Chavez JJ, Merino-Sanjuán V, López-Cervantes M, Urban-Morlan Z, Piñón-Segundo E, Quintanar-Guerrero D. The tape-stripping technique as a method for drug quantification in skin. *J Pharm Pharm Sci* 2008; 11(1):104.
35. Chandra A and Sharma P K . Proniosome based drug delivery system of piroxicam. *Afr J Pharm Pharmacol* 2008; 2(9): 184-190.
36. Plasencia I, Norlen L: Bagatolli LA. direct visualization of lipid domains in human skin stratum corneum's lipid membranes. effect of pH and temperature *Biophys J*. 2007; 93:3142–55.
37. Miner PE: Rheological properties of cosmetics and toiletries. United Kingdom. Marcel Dekker Inc 1993; 313-362.
38. Devaraj GN, Prakash SR, Devaraj R, Apte SS, Rao BR, Rambhav D. Release studies on niosomes containing fatty alcohols as bilayer stabilizers instead of cholesterol. *Journal of Colloidal and Interface Science* 2002; 251:360- 365.
39. Kandasamy R ,Veintramuthu S. Formulation and Optimization of Zidovudine Niosomes. *AAPS Pharm. Sci. Tech* 2010; 11(3): 1119-1127.
40. Ahmed A A , Doaa AE, Aliaa N E. Comparative study on the effects of some polyoxyethylene alkyl ether and sorbitan fatty acid ester surfactants on the performance of transdermal carvedilol proniosomal gel using experimental design. *AAPS Pharm. Sci. Tech* 2010; 11(4): 1591-1602.
41. Nagasamy D , Swetha V , Tulasi K , Kalyani K: Proniosomes. A Superior Drug Delivery System. *International Journal of Pharmaceutical Sciences and Drug Research* 2014; 6(3): 178-182.
42. Singh CH, Jain CP, Kumar BN. Formulation, characterization, stability and in vitro evaluation of Nimesulide niosomes. *Pharmacophore*. 2011; 3: 168-185.
43. Shah PP, Desai PR, Patel AR, Singh MS. Skin permeating Nanogel for the cutaneous co-delivery of two anti-inflammatory drugs. *Biomaterials* 2012; 33:1607–17.
44. Lee CH, Moturi V, Lee Y. Thixotropic property in pharmaceutical formulations. *J Control Release* 2009 ; 136:88–9.
45. Duggin G. Softening Skin with Emollient Ingredients. *Manufacturing Chemist* 1996; 67(6): 27–31.
46. Subczynski WK, Wisniewska A. Physical properties of lipid bilayer membranes relevance to membrane biological functions. *Acta Biochim Pol* 2000; 47: 613–625.
47. Barakat HS, Darwish IA, El-Khordagui LK, Khalafallah NM. Development of naftifine hydrochloride alcohol-free niosome gel. *Drug Dev Ind Pharm* 2009; 5: 631-637.
48. Jadon PS, Gajbhiye V, Jadon RS, Gajbhiye KR, Ganesh N. Enhanced Oral Bioavailability of Griseofulvin via Niosomes. *AAPS PharmSciTech* 2009; 4: 1186-1192.
49. Abd-Elbary A, El-laithy HM, Tadros MI. Sucrose stearate-based proniosome-derived niosomes for the nebulisable delivery of cromolyn sodium. *Int J Pharm* 2008;357: 189–198.
50. Lv Q, Yu A, Xi Y, Li H, Song Z, Cui J . Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery. *Int J Pharm* 2009; 372(1–2):191–198.