

Proniosome based Transdermal Gels of Valsartan: Formulation, In-vitro and Ex-vivo Characterization

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Abstract: The present study is focused to evaluate the feasibility of proniosomes for transdermal delivery of valsartan. Proniosomes were prepared by varying the molar ratios of sorbitan monopalmitate (span 40) and cholesterol and were characterized for morphology, size, entrapment efficiency and rheological behavior. The niosomes formed upon hydration of proniosomes were spherical and multilamellar. In-vitro release studies across cellophane membrane indicate that the drug release follows zero order kinetics by diffusion. Further ex-vivo permeation study carried out using rat skin reveal a significant improvement in the permeation kinetics of valsartan from proniosomes compared to control. Among the formulations tested, the formulations containing low amounts of cholesterol showed a two to three fold enhancement in the permeation. The formulations are more stable when stored at refrigerated conditions compared to room temperature. In conclusion, proniosomes offer potential and provide suitable carriers for improved transdermal delivery of valsartan.

Keywords: Cholesterol, Proniosomes, Span 40, Transdermal, Valsartan.

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I. Introduction

Transdermal drug delivery offers potential and serves as a surrogate for oral route of administration in improving the bioavailability of many drugs. The main advantages associated with transdermal delivery include: avoidance of first pass metabolism, enormous surface area of the skin, ease of administration and termination of action and also able to deliver the drug in a controlled manner makes the route more attractive for drug delivery [1]. However the major concern in transdermal delivery is limited facilitation of the drug to permeate across the skin because of the presence of stratum corneum (SC) which acts as a barrier and hinders the drug absorption [2].

In the recent past, researchers have explored various strategies so as to overcome the barrier function of SC. Among them the promising delivery strategy includes the use of colloidal vesicular carriers like liposomes, niosomes, ethosomes, transferosomes and elastic liposomes etc. [3-5] which exhibited a significant improvement in the transdermal permeation of drugs. The mechanisms underlying for enhancement in permeation from vesicular systems include a) the vesicles in contact with SC can fuse and provide a better contact with skin surface leading to higher vesicle-skin interactions b) the intercalation of vesicles into the intercellular lipid layers of the skin may also lead to disorganization of the regular structure of the skin with altered permeability characteristics of SC thus impede the barrier function. However the size and composition of vesicles [6], nature of drug and biophysical factors [7,8] are the important factors to be taken into consideration which contribute for efficient vesicle-skin interaction.

Niosomes, nonionic surfactant based vesicles have been used in cosmetic applications and experimentally as drug carriers. In contrary to liposomes, they offer several advantages such as higher chemical stability, intrinsic skin penetration enhancing properties and lower costs [9]. However, several problems were unresolved for niosomes dispersion with respect to their physical instability, vesicle aggregation, fusion, leaking or hydrolysis of encapsulated drugs which ultimately affect the shelf-life of the product [10]. The proniosomes approach has provided a major breakthrough in the research of vesicular systems for improving the stability. The proniosomes; semisolid liquid crystal (gel) product of nonionic surfactant contains minimal amount of ethanol as solvent and least quantity of water [11]. The gel upon hydration converts into niosomes [12-14]. Proniosomes when applied onto the skin surface transform into niosomes due to the hydration by water from the skin itself which would provide an occlusive condition and offer potential for drug delivery via the transdermal route [10].

Since the nature of the drug can influence the efficient transdermal delivery, the selection of drug is of prime importance. Valsartan is used in the management of hypertension and also used in the patients with heart

failure who cannot tolerate ACE inhibitors [15,16]. Valsartan is rapidly absorbed following oral administration; however the bioavailability is limited to 20% due to extensive first pass metabolism. Moreover, the higher log partition coefficient value [4.5], lower biological half-life (7.5 h), favorable molecular weight (435.5) and with minimum skin irritation makes the valsartan a suitable candidate for transdermal delivery. The present research encompasses the formulation and characterization of valsartan loaded proniosomes using various molar ratios of sorbitan monopalmitate (Span 40) and cholesterol. Further ex-vivo permeation study was carried out to evaluate the feasibility of proniosomes for efficient and sustained delivery of valsartan.

II. Materials And Methods

Valsartan was a kind gift sample from Dr. Reddy's laboratories, Hyderabad. Sorbitan monopalmitate (Span 40) was purchased from Sigma chemical co., St. Louis, MO, USA. Cholesterol was obtained from E. Merck, Mumbai, India. Sodium azide and dialysis tube (DM-70; MW cut off 12,000 to 14,000) were purchased from Himedia, Mumbai, India. All other chemicals and solvents used were of analytical grade. Freshly collected double distilled water was used all throughout the study.

2.1 Preparation of proniosomes

Proniosomes were prepared according to the method reported elsewhere in the literature with slight modification [17, 18] and the composition of different proniosomal formulations is represented in "Table 1". In brief, accurately weighed amounts of lipid mixture (1mmol) comprising of sorbitan monopalmitate (Span 40) and cholesterol at various ratios (10:0, 9:1, 8:2, 7:3, 6:4, 5:5 and 4:6) respectively were taken in a clean and dry, wide mouth glass vial. The drug was added to the lipid mixture followed by the addition of 400mg of absolute ethanol. After ensuring the homogenous dispersion of the ingredients, the vials were tightly sealed in order to prevent the evaporation of the solvent and warmed in a thermostatic water bath at 55-60°C for about 5 min with intermittent shaking until the ingredients were dissolved. To the resultant transparent solutions, about 0.16 mL double distilled water maintained at the same temperature was added stream wise while warming in the water bath till a clear or translucent solution was obtained which upon cooling formed a white creamy proniosome gel. The obtained gels were stored in the same closed glass vials in dark for characterization.

2.2 Characterization of proniosomes

2.2.1 Microscopic evaluation

The morphology of the niosomes was examined by optical microscopy. The proniosomes were hydrated with 7 mL of phosphate buffered saline (pH 7.4); mixed gently and final volume was adjusted to 10 mL with the same vehicle. The niosomes formed after hydration was observed at a magnification of 450X through an optical microscope (Coslabsmicro, India) and photomicrograph was taken.

2.2.2 Determination of vesicle size and entrapment efficiency

The proniosomes were hydrated with phosphate buffered saline (pH 7.4) and subjected to bath sonication (Sonica, Italy) for 3 minutes. The resultant dispersion was used for the determination of size and entrapment efficiency measurements.

The mean size and size distribution of niosomes was determined by photon correlation spectroscopy using Zetasizer 3000 HSA (Malvern Instruments, Malvern, UK). Each sample was suitably diluted and size analysis was performed at 25°C with an angle of detection of 90°C. Size, polydispersity index of niosomes were obtained from the instrument. The entrapment efficiency of the system was determined by reported method [19]. In brief, ultra-filtration was carried out using Centrisart (Sartorius AG, Gottingen, Germany) at 3500 rpm for 15 minutes, which consist of filter membrane (Molecular weight cut-off 20 KDa) at the base of the sample recovery chamber. The amount of the valsartan in the aqueous phase was quantified by HPLC. Entrapment efficiency was calculated from the difference between the initial amount of valsartan added and that present in the untrapped form and was expressed as a percentage of the total amount of valsartan added.

2.2.3 Rheological studies

The spreadability of the topical formulations meant to be applied onto the skin depends on the degree of consistency and resistance offered by the preparation which can be measured in terms of viscosity. The rheological behavior of proniosomal formulations was studied by using a controlled stress rheometer with the cone (24 mm) and plate geometry (Brookfield Programmable DVIII+ Digital Rheometer, MA, USA). Before carrying out the measurement the sample was allowed to equilibrate for 5 min and the torque sweep was in the range of 10 to 110%. The measurements were performed in triplicate at ambient temperature. The rheological properties were calculated using Rheocalc 32 software.

2.3 *In vitro* release study

In vitro release studies through artificial cellophane membrane was performed using fabricated vertical Franz diffusion cells with an effective diffusional surface area of 4.153 cm² and 14 mL of receptor cell volume. After soaking the cellophane dialysis membrane in the diffusion medium, the proniosomal formulation or control (drug suspended in the same solvent of formulations) equivalent to 10 mg of valsartan was placed in the donor compartment. The receptor compartment consisting of 40% (v/v) ethanolic phosphate buffered saline, pH 7.4 was maintained at 37±2°C under constant stirring upto 24 h [20]. The donor chamber and the sampling port were covered by parafilm to prevent evaporation during the study. An aliquot of 200 µL was withdrawn periodically and replaced with equal volume to maintain constant receptor phase volume. At the end of the study, the samples were suitably diluted and the amount of drug was quantified by HPLC. The obtained data was fitted into mathematical equations (zero order, first order, Higuchi and Korsmeyer Peppas models) [21] in order to describe the kinetics and mechanism of drug release from the proniosomal formulations.

2.4 *Ex vivo* permeation study

Male albino wistar rats weighing between 180-200 gm used in the study were obtained from Mahaveer Enterprises (146-CPCSEA no: 199; Hyderabad, India). The animals were housed in separate cages and maintained under controlled condition of temperature and the rats had free access to water and food until they were sacrificed for skin harvesting. The study was conducted with the prior approval of Institutional Animal Ethical Committee, Trinity College of Pharmaceutical Sciences. Euthanasia and disposal of carcass was in accordance of the guidelines.

The rats were sacrificed with excess ether inhalation. The abdominal skin was exposed and hair was removed with hair clipper taking extreme precautions not to damage the skin. The rat abdominal skin was isolated, excised and the adhering subcutaneous fat, tissue and capillaries were removed with a pair of scissors. The heat separation technique [22] was adopted to prepare the epidermis which involves soaking of the entire abdominal skin in water at 60°C for 45 seconds. The epidermis was washed with water, wrapped in aluminium foil and stored at -20°C till further use [23] (used within 2 weeks of preparation).

The permeation of valsartan from proniosomes was determined by using fabricated vertical Franz diffusion cells with an effective diffusion area of 4.153 cm². The skin was brought to the room temperature and mounted between the donor and receiver compartment of the Franz diffusion cell, where the stratum corneum side faced towards the donor compartment. The skin was allowed to equilibrate and after ensuring no interference with the skin components in the receptor phase, the donor compartment was challenged with proniosomal formulation or control (drug suspended in the same solvent of formulations) equivalent to 10 mg of valsartan. A 14 mL aliquot of 40% (v/v) ethanolic phosphate buffered saline, pH 7.4 [20] (containing 0.003% w/v of sodium azide to retard microbial growth) was used as receptor medium in order to maintain sink condition.^[20] The donor chamber and the sampling port were covered by parafilm to prevent evaporation during the study. The receptor compartment was maintained at 37±1°C under constant stirring up to 24 h. At predetermined time intervals an aliquot of 200 µL was withdrawn and replenished with an equal volume fresh diffusion buffer. At the end of the study, the samples were suitably diluted and the amount of drug was analyzed by HPLC. The experiment was conducted in triplicate for all the formulations.

2.5 Permeation Data Analysis

The cumulative amount of drug permeated (Q) was plotted against time. The steady state flux (J_{ss}) was calculated from the slope of linear portion of the cumulative amount permeated per unit area vs. time plot. The permeability coefficient (K_p) of the drug was calculated by dividing steady state flux with initial concentration of valsartan in donor compartment. The enhancement ratio (ER) was calculated by using the following equation ER = Transdermal flux from Proniosomal gel/Transdermal flux from control.

2.5.1 HPLC analysis of valsartan

The samples were assayed for valsartan by using HPLC method reported earlier.^[24] HPLC was equipped with LC-10 AT solvent delivery unit, SPD-10 AVP UV-Spectrophotometric detector, Spinchrom software, Rheodyne injector fitted with 20 µL capacity (Shimadzu Co., Tokyo, Japan) and a Lichrospher C18 column (5 µm, 4.6mm×250mm). Isocratic elution was carried out at a flow rate 1 mL/min. The mobile phase consisted of 45:55 (v/v) acetonitrile and phosphate buffer (pH 2.7) respectively and the detection wavelength was set at 265 nm. Aliquots of 20 µL of each sample were spiked onto the column. The assay was linear (r²=0.9989) in the concentration range of 1–10 µg/mL with the lowest detection limit at 0.5 µg/mL.

2.6 Determination of drug deposited into the skin

The extent of drug deposited in the epidermal layers was determined as described earlier in reports [25]. After 24 h of *in vitro* permeation study, the skin was removed from the diffusion cell and washed briefly in methanol (25 mL) for 15 sec to remove the adhering formulation. The skin was allowed to dry at room

temperature for 10 min, chopped into pieces, homogenized in 10 mL of 40% (v/v) ethanolic phosphate buffered saline (pH 7.4) and sonicated for 30 min using bath sonicator (Sonica, Italy) to leach out the drug. The samples were centrifuged and the supernatant layer was passed through 0.45 μm membrane filter and assayed for valsartan by HPLC.

2.7 Skin irritation test

The skin irritancy potential of the proniosome formulations was evaluated in albino rats. The study protocol was approved by the Institutional animal ethical committee. The hair was removed on the back of the animal and the formulations was applied topically covering a surface area of 6 cm^2 once daily for a period of seven days (n=3). Control group rats were covered with proniosomes without drug. Before application of the formulation, the animals were examined for any signs of skin irritation each day throughout the treatment period and evaluated based on the irritation score as reported earlier [26]. Scores between 0 and 4 were used to grade erythema and oedema which range from no response to a severe response.

2.8 Stability studies

The formulations were stored in glass vials covered with aluminium foil at room temperature and in refrigerator (4°C) for a period of 30 days. At definite time intervals (10, 20 and 30 days), samples were withdrawn and hydrated with phosphate buffered saline pH (7.4) and observed for any sign of drug crystallization under optical microscope. The samples were also evaluated for particle size and % retention of valsartan.

2.9 Statistical analysis

Statistical analysis of the data obtained was performed using one-way ANOVA followed by Neuman keuls post test with GraphPad Prism software (version 4.00; GraphPad Software, San Diego California). The level of statistical significance was chosen as less than $P < 0.05$.

III. Results And Discussion

3.1 Preparation and characterization of proniosomes

The vesicular systems have been exploited in improving the dermal and/or transdermal bioavailability of drugs and substances. Among them, nonionic surfactant vesicles proved to be an alternate to phospholipid vesicles because of their low cost, greater stability and ease of storage [27]. Valsartan loaded proniosomes with nonionic surfactant i.e. span 40 were prepared by a simple method reported in the literature which involves the principle of coacervation-phase separation [28]. Several techniques were used earlier in order to improve the stability of the vesicular formulations which is a primary concern in the production scale-up. Cholesterol is the common additive used as a structural lipid to improve the stability and entrapment efficiency of vesicular formulations [29,30]. The effect of cholesterol was investigated by varying the composition of span 40 to cholesterol ratio keeping the total lipid constant at 1mmol. The different span types have the same polar head group with varied alkyl chain [31]. In earlier report, a span of varied alkyl chain length were used in the preparation of proniosome gels and among them span 40 was used as representative because it gives the vesicles of large size with higher drug load [32]. Further, in case of span 40 the drug leaching was also less. Highest entrapment could be observed with an increase in phase transition temperature of span [31]. The phase transition temperatures for span 20, 40, 60 and 80 are 16, 42 and 53°C respectively and span 80 having the lowest phase transition temperature at -12°C [33]. Due to the high phase transition temperature and low permeability span 40 was used in our study to facilitate stable vesicle formation and to improve the skin permeation of valsartan from proniosomes [13].

The niosomes were formed upon hydration of proniosome gel and the microphotograph was depicted in “Fig.” 1. The niosomes were multilamellar with spherical in shape. With increase in the cholesterol concentration the abundance of the vesicles was reduced and with higher amount of cholesterol (>60%) the system tends to show crystallization which might be due to the decreased solubility of the drug. Henceforth the optimized formulations contain 0 to 60% of cholesterol. Vesicle size and size distribution is an important parameter for the topical administration of vesicular systems [6]. The mean size of the vesicles was in the range of 1.5 to 2.5 μm . No significant change was observed with addition of 10% cholesterol. However further increase in cholesterol concentration led to a significant increase in the size of the vesicles (“Table 2”). Small value of polydispersity index (PI) (<0.1) indicates a homogenous population, while a PI (>0.3) indicates a higher heterogeneity. The PI used as a measure of a unimodal size distribution was within the acceptable limits for all the proniosomal formulations. Among all the formulations tested, the PI was lower for the formulations containing no and 10% of cholesterol (PN-0 and PN-1 respectively) (“Table 2”).

The entrapment efficiency was found to be between 68 to 85%. The drug entrapment was poor in case of proniosome formulation (PN-0) which is devoid of cholesterol (68%) whereas the same has been increased to

84% with addition of 10% cholesterol (PN-1) into the formulation. This could be well explained based on the fact that, upon addition of cholesterol the bilayer hydrophobicity as well as stability is greatly improved with reduced permeability of the bilayer, thus leading to the effective intercalation of hydrophobic drug within the hydrophobic core of the bilayer with an enhanced drug pay load [34]. However, further increase in cholesterol led to decline in the entrapment values. The higher amounts of cholesterol may compete with the drug for the packing space available in the bilayer during the niosome formation and also because of disruption of linear regular structure of bilayer [35]. Our results also support the hypothesis explained in the literature reports.

The rheological behavior is an important parameter to be evaluated for percutaneous application [36]. The rheograms of different proniosomal formulations are depicted in “Fig.” 2. It is apparent from the rheograms that with increase in shear rate the viscosity was decreased and the formulations exhibit good flow behavior. Further the thixotropic degree (n) of the formulations was determined from the rheological data using by Ostwade power equation [37]. The thixotropic degree (n value) was higher for the proniosomal formulations and it was decreased with increase in cholesterol concentration from 0 to 60% (“Table 2”). The rheological behaviour of the formulations clearly indicates their ease of application onto the skin.

3.2 In vitro release study

The *in vitro* release behaviour of valsartan from proniosomes was studied. The percentage drug release from different formulations was represented in “Fig. 3”. The release of valsartan from control was nearly 80% within 8 h which clearly suggest the permeability of the membrane and prevalence of the sink condition for the drug. A typical biphasic release pattern was observed with proniosomal formulations. Initially a rapid burst release followed by sustained release for a period of 24 h was observed (“Fig. 3”). The high concentration gradient prevailing at the initial time points might have contributed for the rapid drug release and also may be due to the faster release of untrapped drug from the proniosome formulations. The amount of drug release from the proniosome formulation containing lower amounts of cholesterol (10 to 20%) was less. This could be due to the more ordered and compact structure of the bilayer, thus retarding the drug release. However further increase in cholesterol concentration led to an increase in drug release which could be due to the disordered bilayer or more rigidity of the membrane. The *in vitro* release data is in correlation with the entrapment efficiency results. The data was subjected to mathematical treatment to know the kinetics and mechanism of drug release from the proniosomal formulations. The data suggest that the drug release from proniosome formulations is diffusion controlled following zero order kinetics. The diffusional exponent ‘n’ values are lower than 0.5 indicating that the drug release mechanism involves fickian diffusion (“Table 3”).

3.3 Ex vivo permeation study

The objective of the formulation of proniosomes for valsartan is to check the feasibility of transdermal delivery so as to improve the bioavailability. The *ex-vivo* permeation study was conducted across the rat abdominal skin and the cumulative amount of drug permeated is depicted in “Fig. 4”. The significant permeation of valsartan results only when the drug is released from the niosomes formed after the hydration of proniosomes with the skin fluids [38]. No lag time was observed in all the formulations which clearly indicate the formation of niosomes. Moreover, the drug was detected within 0.5 h which explains the procedures of diffusion of water from the receptor fluid to the skin membrane, release of drug and permeation across the skin occurred rapidly [12].

The permeation was very much less for the formulation devoid of cholesterol which might be due to the improper formation of vesicles, less stability and poor entrapment of the drug. A significant improvement in permeation was observed with inclusion of cholesterol (10%) into the formulation. However further increase in cholesterol from 10% to 60% the permeation found to decrease which could be because of the increased hydrophobicity of the vesicular membrane, thus reduced partitioning of the drug into the skin layers (“Fig.” 4). The maximum flux obtained was 40.72 and 38.22 $\mu\text{g}\cdot\text{h}\cdot\text{cm}^2$ within 1h from control and proniosome formulation without cholesterol (PN-0) respectively. The maximum flux was significantly higher ($p<0.001$) for the formulation containing 10% cholesterol and decreased with increase in cholesterol [39]. However the flux was higher at all time points for the proniosome formulations compared to control and no cholesterol which indicates the controlled delivery of valsartan from proniosome formulations (“Fig. 5”). The permeation enhancement assessed in terms of permeation parameters (flux, permeability coefficient and enhancement ratio) were calculated and shown in “Table 4”. The steady state flux and permeability coefficient were very much higher for the proniosomal formulation (PN-1) containing 10% of cholesterol ($p<0.001$). The enhancement ratio well above 1 indicates improved permeation and in our findings we could notice an ER greater than 1 for all proniosomal gel formulations compared to control. Such an improvement is statistically significant (“Table 4”). Based on the permeation parameters the proniosomal formulations can be ranked in the following decreasing order PN-1 > PN-2 > PN-3 > PN-4 > PN-5 > PN-6 > PN-0 > Control.

Interestingly, the amount of drug released across the cellophane membrane was significantly higher than the permeation across skin, indicates the barrier properties of the skin. This also suggests that the vesicle skin interaction and direct contact of vesicle with skin are important attributable factors for improved transdermal delivery of valsartan [40]. The results are in consistent with the reports [12]. Overall it is evident from the results that the permeation of valsartan has been greatly improved from proniosome formulations. In the literature, several mechanisms have been put forward to explain the factors contributing for the improved permeation of drugs from proniosomes. Nonionic surfactants and phospholipids are known to interact with intercellular lipids present in the stratum corneum and there upon modify the structural and permeability characteristics making it more permeable to the drugs [41,42]. Earlier in reports, fusion of niosomes on the surface of skin is demonstrated by electron microscopy which can also lead to direct transfer of intact vesicle into the skin layers and impede the barrier function of stratum corneum. In support to this, it is also suggested that the high estradiol fluxes encapsulated in niosomes is because of direct transfer of drug from the vesicles [12]. To summarize, several mechanisms in combination might have played a significant role in enhancing the permeation of valsartan from proniosomes.

3.4 Estimation of drug deposited in rat skin

The extent of drug deposited in the skin (DCS) layers upon treatment of rat skin with all the formulations and drug solution was shown in “Fig. 6”. Since the permeation was increased with all the proniosomal formulations, obviously the drug in the skin layers was also increased. This can be well explained based on the fact that the saturation of skin layers at the termination point of the experiment i.e. 24 h could have led to the higher DCS values. The higher deposition of valsartan in skin layers with proniosomal gels compared to control reveals the potential of these formulations in avoiding the barrier function of the stratum corneum and delivering the drug efficiently into the viable regions of the skin for improved bioavailability.

3.5 Skin irritation studies

After application of proniosomal gel formulations we could not notice any marked erythema, oedema or erosion during seven days. However, a slight reddening of the skin was observed but it was subsided and normalized after 24 h of the study.

3.6 Stability studies

The microscopical studies indicate that the vesicles formed from proniosomes were multilamellar and we could not observe any appreciable change in the morphological behavior. However after 20 days, we could notice drug crystallization in the preparation which is devoid of cholesterol (PN-0). The niosomes formed after hydration was evaluated for size analysis and % retention of valsartan in the vesicles (“Fig. 7 & 8” respectively). The data clearly indicates the influence of temperature on the stability of proniosomes. The formulations stored in refrigerator at 4°C were comparatively more stable than the formulations stored at room temperature. Among the formulations, the proniosome containing span 40 alone exhibited a significant reduction in the % retention which indicates the instability and disordered structure of the vesicular membrane (“Fig.” 8).

IV. Conclusion

Valsartan loaded proniosomal gels could be successfully developed. The rheological studies indicate high thixotropic degree for the proniosomal formulations with good flow behavior. The results indicate that span 40 with inclusion of 10% cholesterol yielded stable vesicles with optimum size, entrapment efficiency and enhanced permeation kinetics. The permeation study revealed the potential of proniosomes in delivering valsartan across the skin in a controlled manner with zero order kinetics by diffusion. In conclusion, the proniosomes prove to be efficient carriers for transdermal delivery of valsartan. However, further *in vivo* studies need to be conducted to optimize the transdermal delivery of valsartan.

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TABLE LEGENDS

Table 1. Composition of Valsartan Loaded Proniosomal Gels.

Table 2. Size, Entrapment Efficiency and Thixotropic Degree of Valsartan Proniosomal Formulations.

Table 3. *In Vitro* Release Kinetics for Valsartan from Proniosomal Gels Across Cellophane Membrane.

Table 4. Permeation Parameters of Valsartan from Different Proniosomal Formulations Across Rat Skin (mean±SD; n=3).

FIGURE LEGENDS

Figure 1: Microphotograph of niosomes formed upon hydration of proniosomal gel (PN-1).

Figure 2: Rheograms of various valsartan proniosomal gel formulations.

Figure 3: *In vitro* percentage drug release profiles of valsartan from different proniosomal gel formulations (mean±SD; n=3).

Figure 4: Cumulative amount permeated vs. time profiles of valsartan from different proniosomal gel formulations across rat skin (mean±SD; n=3).

Figure 5: Mean flux of valsartan from different proniosomal gel formulations across rat skin (mean±SD; n=3).

Figure 6: Drug deposited in the skin layers after 24 h following treatment with different proniosomal gel formulations (mean±SD; n=3);

*, ** represents significant difference at p<0.05 and p<0.01 respectively.

Figure 7: Change in size of proniosomal formulations upon storage in A) Refrigerator and B) Room temperature.

Figure 8: Percentage retention of valsartan in proniosomal formulations upon storage in A) Refrigerator and B) Room temperature.

Table 1: Composition of valsartan loaded proniosomal gels

Formulation Code	Ratio		Ethanol (mg)	Water (µL)
	Span 40	Cholesterol		
PN-0	10	--	400	160
PN-1	9	1	400	160
PN-2	8	2	400	160
PN-3	7	3	400	160
PN-4	6	4	400	160
PN-5	5	5	400	160
PN-6	4	6	400	160

Total 1mM lipid mixture was used in all the preparations

All formulations contained 10 mg Valsartan

Table 2: Size, entrapment efficiency and thixotropic degree of valsartan proniosomal formulations

Formulation code	Size (nm)	P.I	Entrapment efficiency (%)	n
PN-0	1574±39	0.189	68.45±2.2	0.559
PN-1	1596±26	0.194	84.56±1.2	0.512
PN-2	1874±41	0.255	79.56±2.5	0.488
PN-3	2105±22	0.249	75.49±3.8	0.451
PN-4	1985±45	0.269	77.51±1.1	0.459
PN-5	1789±26	0.238	72.15±2.6	0.411
PN-6	2254±38	0.261	71.56±2.4	0.409

n, indicates thixotropic degree

Table 3: *In vitro* release kinetics for valsartan from proniosomal gels across cellophane membrane

Formulation code	Zero order		First order		Higuchi	Peppas
	K ₀ (h ⁻¹)	R ²	K (h ⁻¹)	R ²	R ²	'n' value
Control	7.2151	0.634	2.693	0.459	0.778	0.354
PN-0	2.21	0.913	2.45	0.788	0.988	0.431
PN-1	2.138	0.956	2.476	0.794	0.998	0.451
PN-2	2.146	0.902	2.461	0.763	0.985	0.468
PN-3	2.339	0.906	2.454	0.771	0.986	0.444
PN-4	2.294	0.913	2.433	0.771	0.988	0.387
PN-5	1.908	0.884	2.397	0.801	0.977	0.276
PN-6	1.865	0.866	2.396	0.763	0.959	0.278

Table 4: Permeation parameters of valsartan from different proniosomal formulations across rat skin (mean±SD; n=3)

Formulation code	Q24	Jss (µg/cm ² /h)	Kp (cm/h)×10 ⁻³	ER
Control	1066±79	9.48±0.78	0.97±0.80	1.00
PN-0	1258±58	12.17±0.89	1.22±0.98	1.28
PN-1	3055±99	30.71±0.35	3.14±0.03	3.24
PN-2	2536±85	25.39±1.30	2.60±1.29	2.68
PN-3	2265±70	23.14±0.84	2.37±0.85	2.44
PN-4	2236±30	21.93±1.12	2.24±1.14	2.31
PN-5	1881±94	18.14±0.90	1.855±0.92	1.91
PN-6	1698±98	16.68±1.28	1.71±1.31	1.76

Jss, Kp, ER represents steady state Flux, Permeability coefficient, Enhancement ratio respectively

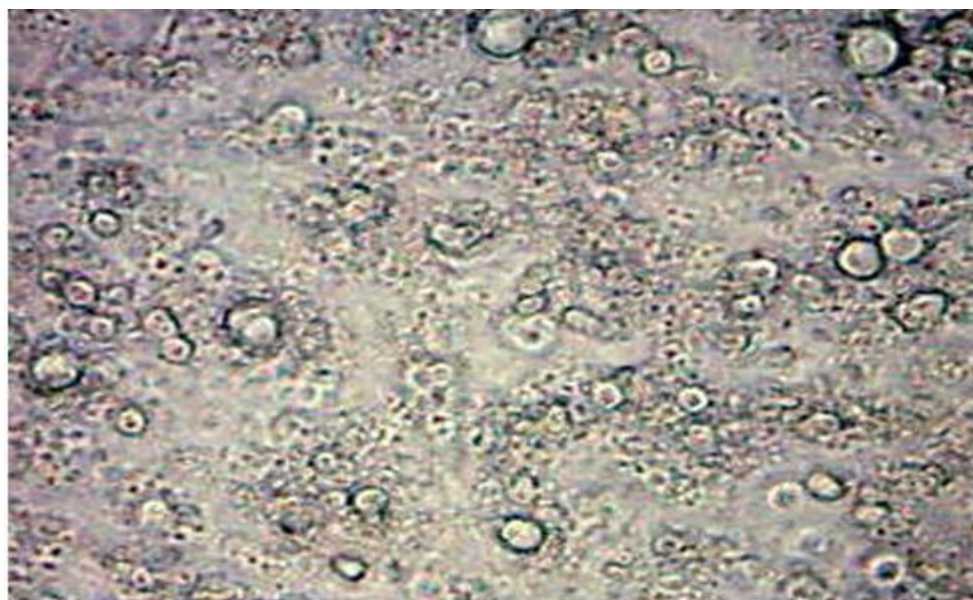


Figure 1: Microphotograph of niosomes formed upon hydration of proniosomal gel (PN-1)

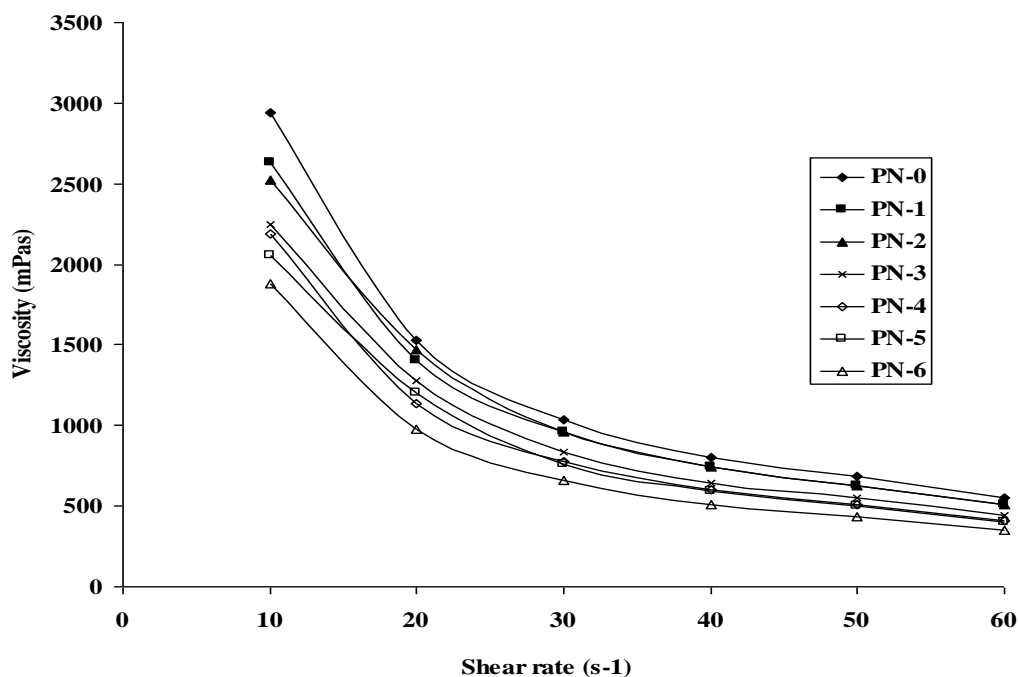


Figure 2: Rheograms of various valsartan proniosomal gel formulations

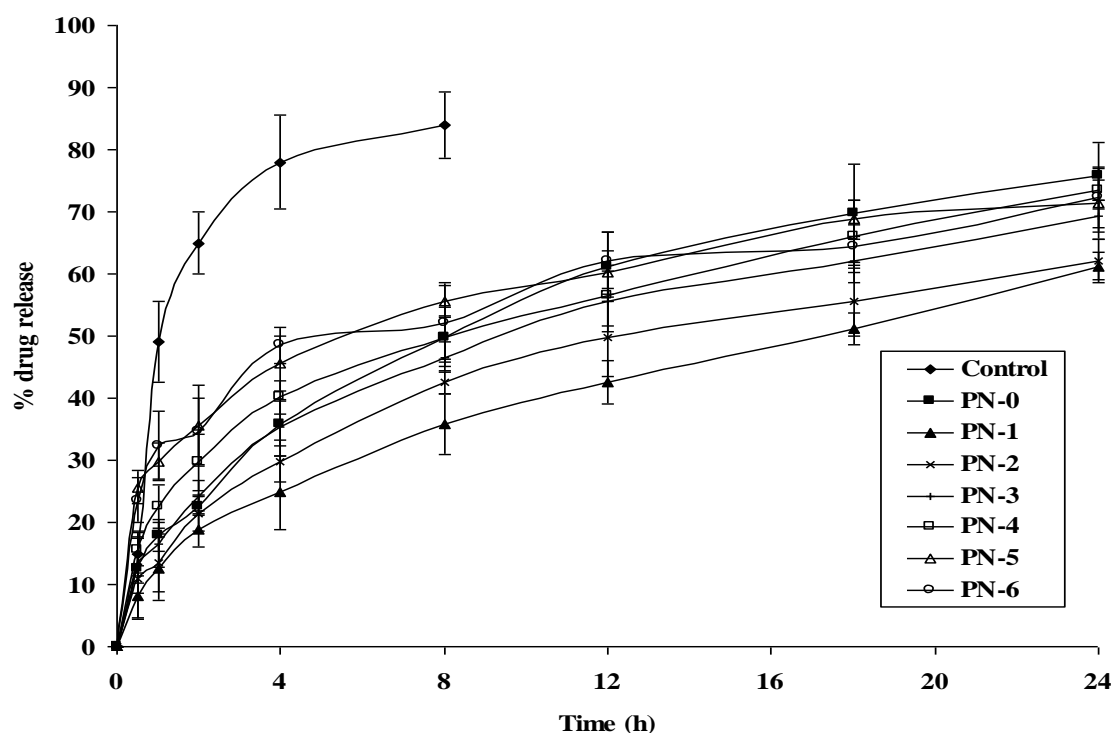


Figure 3: *In vitro* percentage drug release profiles of valsartan from different proniosomal gel formulations (mean±SD; n=3)

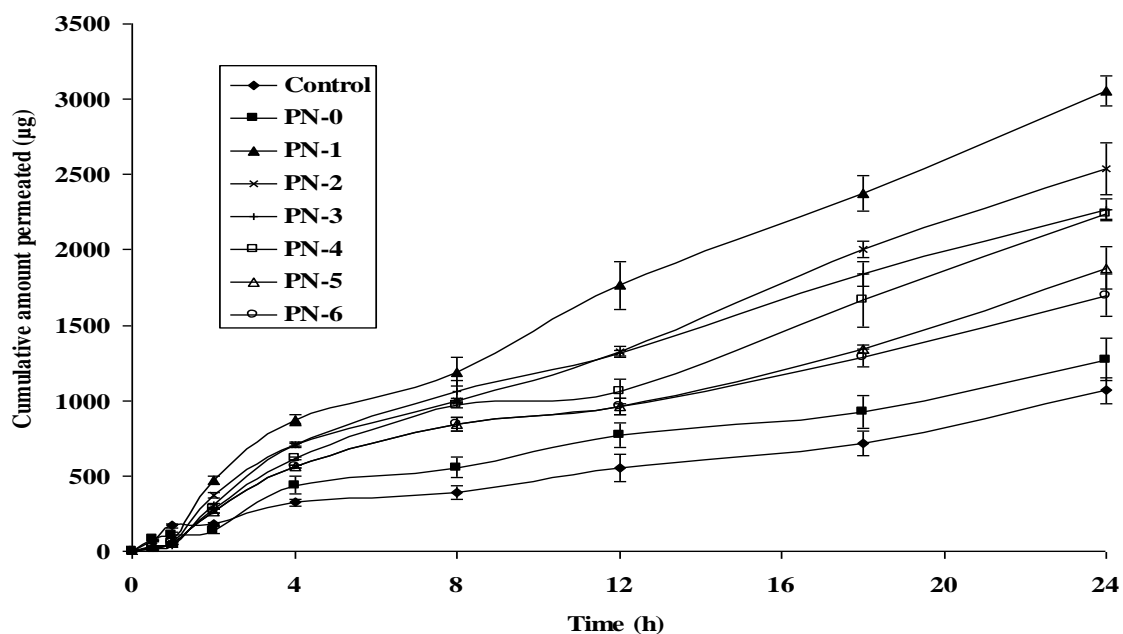


Figure 4: Cumulative amount permeated vs. time profiles of valsartan from different proniosomal gel formulations across rat skin (mean±SD; n=3)

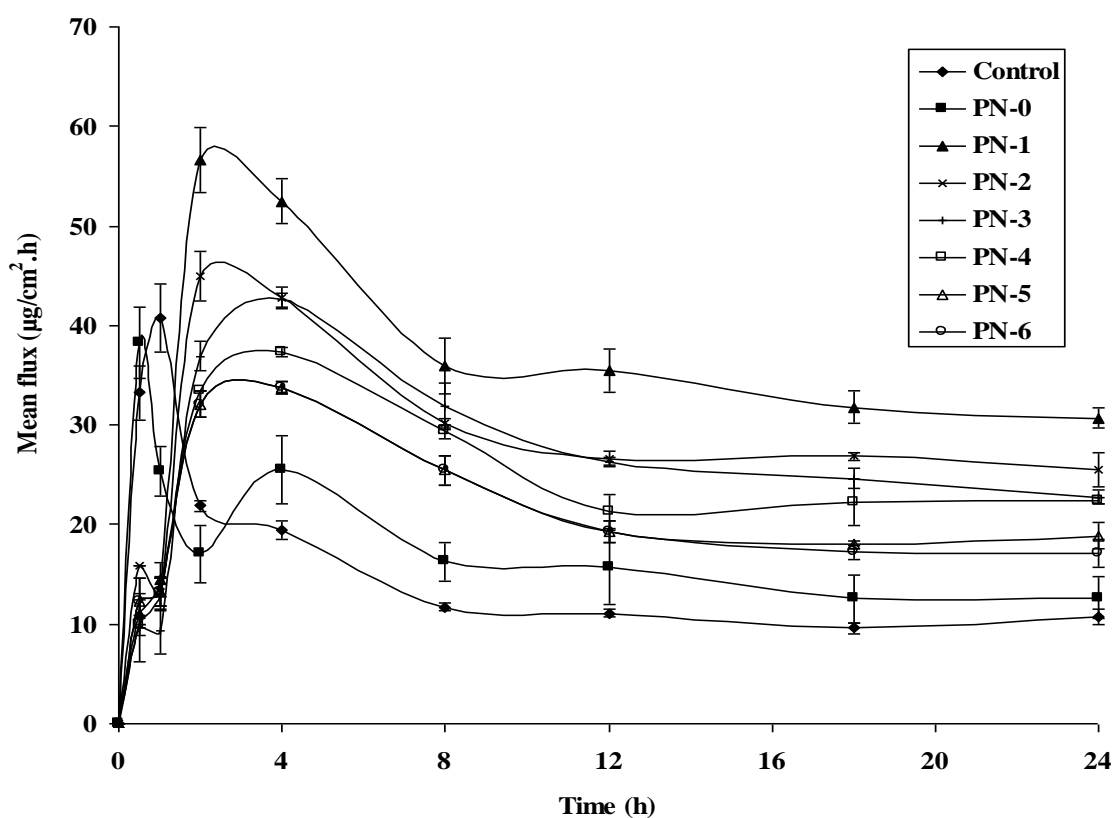


Figure 5: Mean flux of valsartan from different proniosomal gel formulations across rat skin (mean±SD; n=3)

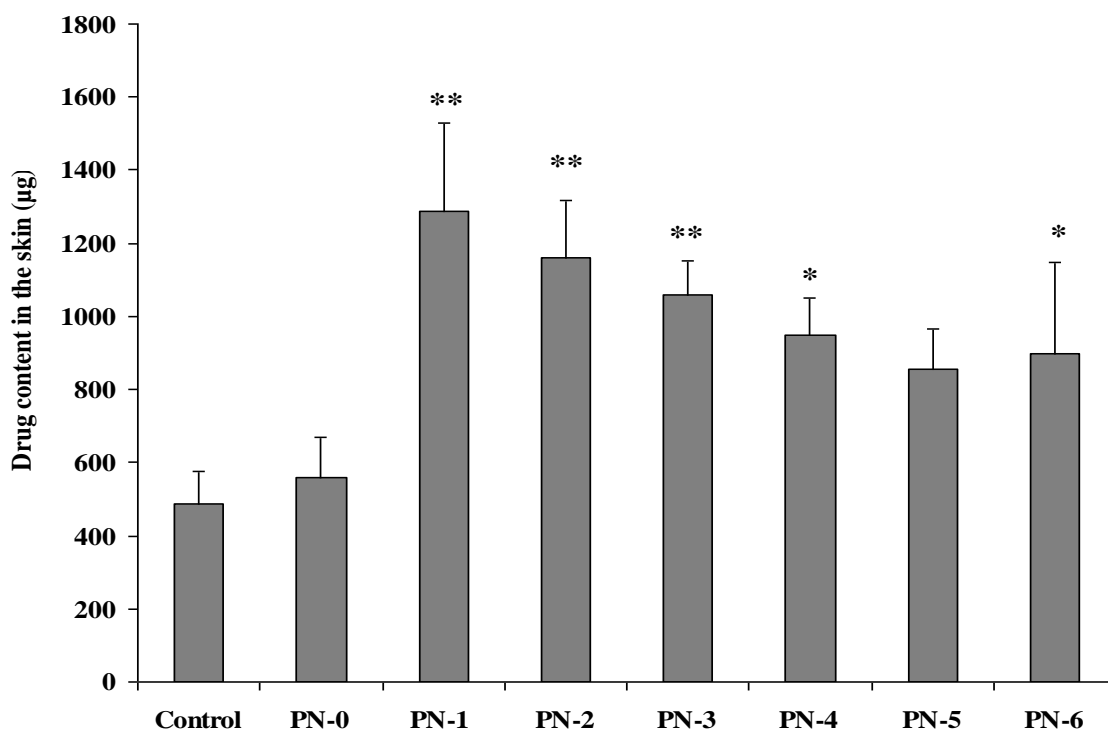


Figure 6: Drug deposited in the skin layers after 24 h following treatment with different proniosomal gel formulations (mean±SD; n=3);

*, ** represents significant difference at $p < 0.05$ and $p < 0.01$ respectively

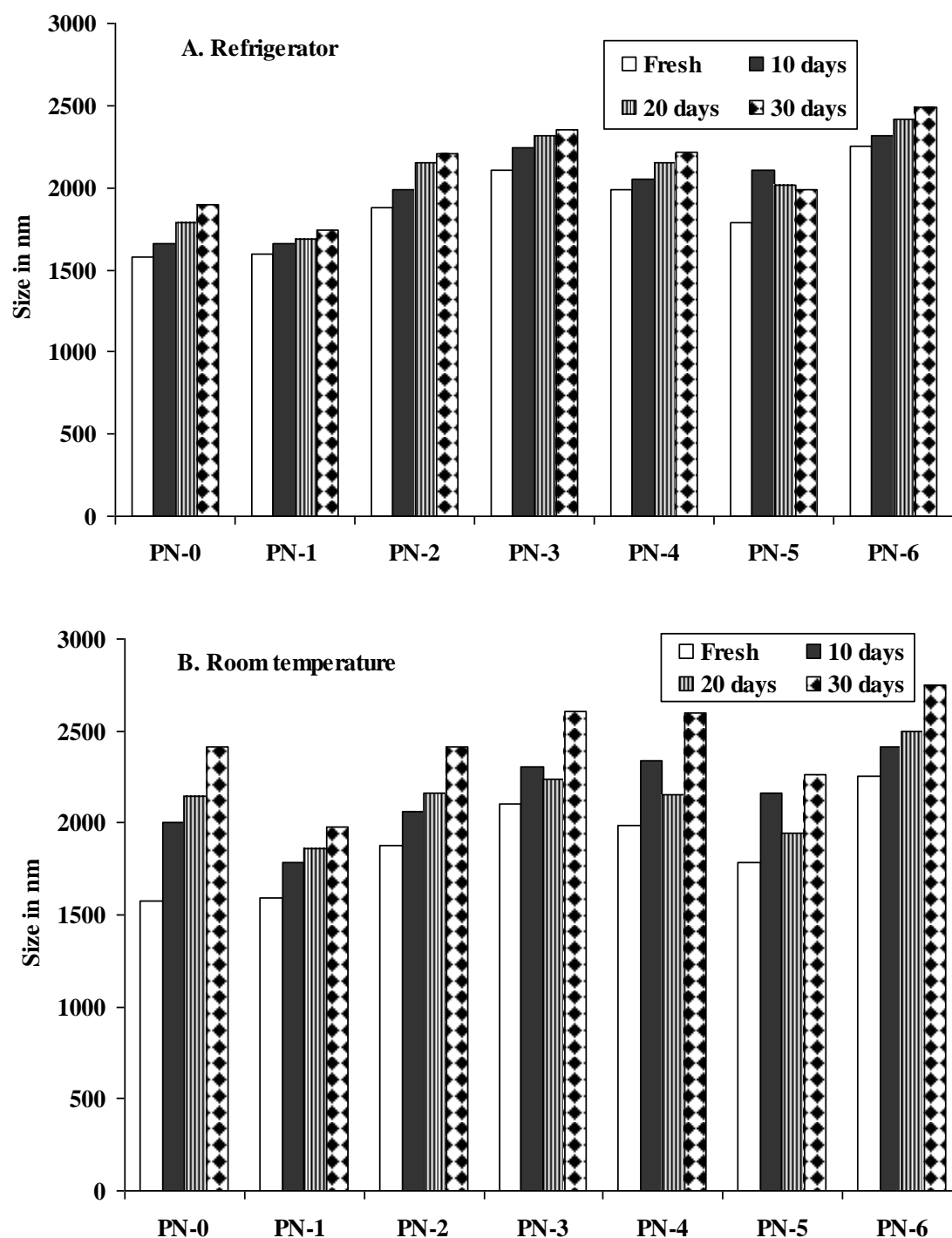


Figure 7: Change in size of proniosomal formulations upon storage in A) Refrigerator and B) Room temperature

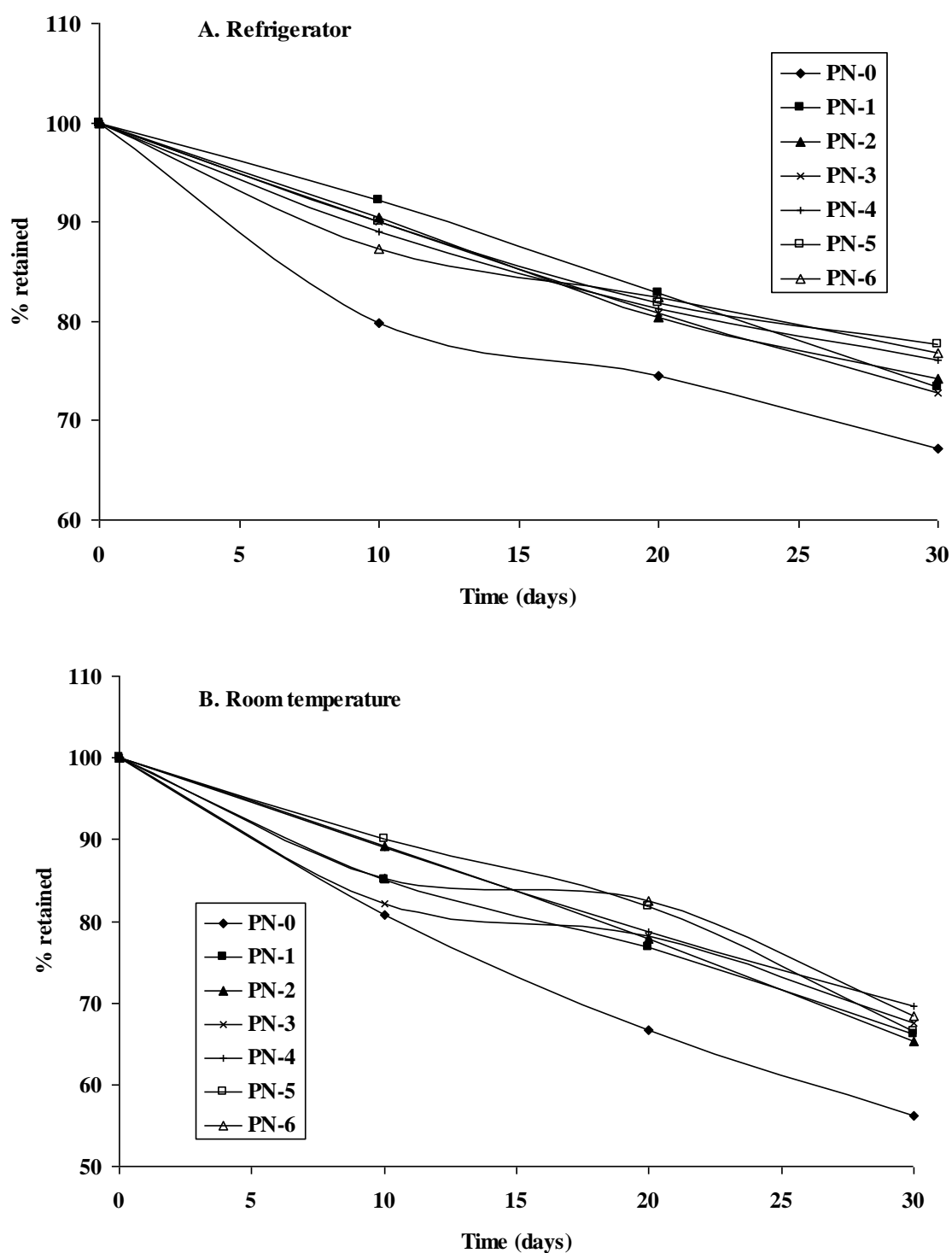


Figure 8: Percentage retention of valsartan in proniosomal formulations upon storage in A) Refrigerator and B) Room temperature

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