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Research Article

PRONIOSOMAL PROVESICULAR SYSTEM FOR TRANSDERMAL DELIVERY OF HYDRALAZINE FOR HYPERTENSION

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ABSTRACT

The aim of the study was to develop a proniosomal carrier system for Hydralazine for prophylaxis and treatment of hypertension that is capable of efficiently delivering entrapped drug over an extended period of time. The potential of proniosomes as a transdermal drug delivery system for Hydralazine was investigated by encapsulating the drug in various formulations of proniosomal gel composed of various ratios of sorbitan fatty acid esters, cholesterol, lecithin prepared by coacervation-phase separation method. The formulated systems were characterized in vitro for size, vesicle count, drug entrapment, drug release profiles and vesicular stability at different storage conditions. Stability studies for proniosomal gel were carried out for 6 weeks. The mean vesicle size of niosome formed upon hydration of gel determined by DLS was found to be in range 1.67-3.142 um. Spontaneity determines the rate of niosome formation upon hydration, data obtained exhibit that Span 40, 60, and Tween 60 produce niosome more instantly. The method of proniosome loading resulted in an encapsulation yield of 93.08± 1.66%. Proniosomes were characterised by transmission electron microscopy. In vitro studies of hydralazine proniosome was carried by drug diffusion through cellophane membrane and In vitro skin permeation studies, the % drug released after 24 hrs and flux was found ranging in 46-56% and 5.62-8.7 ug/cm^2/hr respectively. At refrigerated conditions, higher drug retention was observed. It is evident from this study that proniosomes are a promising prolonged delivery system for hydalazine and have reasonably good stability characteristics.

Keywords: Proniosome, Transdermal drug delivery, Hypertension.

INTRODUCTION

Transdermal therapeutic systems are the recently developed devices, which are non invasive to skin as compared to other routes for administration of drugs. Although the skin, particularly the stratum corneum presents a barrier to most drug absorption, it provides a large (1-2 m^2) and accessible surface area for drug diffusion. Various types of transdermal therapeutic systems are utilized for long term continuous infusion of therapeutic agents, including antihypertensives, antifungal, analgesics, steroids and contraceptive drugs. Although transdermal delivery is currently limited to few drugs, it has achieved considerable commercial success. Some drugs which are used in transdermal delivery systems include nitroglycerine, scopolamine, estradiol, testosterone, nicotine, clonidine and estrogen-progestin combination into transdermal products[1].

Hydralazine hydrochloride (HH) has been commonly used as a antihypertensive drug in many countries for years since it lowers the blood pressure by vasodilating plain muscles of vessels, and decreasing arterial and peripheral vascular resistance. It has been classified in direct-acting peripheral vasodilators .The short half-life (3-4 hours), undesirable side effects and rapid absorption from GI tract make HH a good candidate for formulation in a sustained release dosage form. The aim of this study was to design matrix tablets of HH in order to maintain drug in the systemic circulation for a longer time period. Thus, its side effects can be minimized by lower dose administration, drug bioavailability can be increased and patient compliance can be improved[2].

The transdermal route of drug delivery has many advantages for administration of drugs in local and systemic therapy. The vesicular drug delivery is thus potentially beneficial as vesicles tend to fuse and adhere to the cell surface; this is believed to increase the thermodynamic activity gradient of the drug at vesicle stratum corneum interface thus leading to enhanced permeation rate[3].

Proniosomal gel is mixture of surfactants, lecithin and cholesterol which appears like the lamellar liquid crystals composed of vesiculating bilayer lamellas stacked together which are termed as compact noisomes. The Proniosomal Gels are becoming more popular due to ease of application and better percutaneous absorption, than other semi solid preparations. Gels can resist the physiological stress caused by skin flexion, mucociliary movement, adopting to the shape of the applied area and for controlling drug release. To achieve optimal drug action, functional molecules should be transported by a carrier to the site of action and released to perform their task[4].

Proniosome, a dry product either in powder or gel form may avoid many of the problems associated with aqueous noisome dispersions and minimize problems of physical stability (aggregation, fusion, leaking). The additional convenience of the transportation, distribution, storage and dosing would make 'dry niosomes' a promising industrial product. Proniosomes on hydration form multilamellar noisome suspension[5].

MATERIAL AND METHODS

Hydralazine HCL was provided by D.K. Pharmachem, Mumbai, India. Soya lecithin was a kind gift from Natter man phospholipid GMBH, Germany, and contained 93±3% phosphotidylcholine. All other chemicals used throughout this investigation were of analytical grade and no additional purification was carried out. Double distilled water was used throughout the study.

Reagent preparation

Saline pH 7.4, Phosphate-buffer

Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogenphosphate and 8.0 g of sodium chloride in sufficient water to produce 1000 ml. Adjust the pH if necessary.

Preparation of Proniosomal Gel

Proniosomal gel was prepared by phase separation coacervation technique. Precisely weighed amount of surfactant, soya lecithin and cholesterol in a specified ratio were taken in a dry, clean, wide mouth small test tube. A measured amount of ethanol (absolute alcohol) was added to test tube to dissolve the ingredients. The open end of test tube was covered with a lid to prevent loss of solvent from it and warmed over water bath at 67±3^{0}C for about 5 minute until the surfactant mixture was dissolved completely. Then the aqueous phase (40 mg/ml Hydralazine HCL in phosphate buffer saline (pH 7.4)) was added and warmed on a water bath till a clear solution was formed. The clear solution formed was cooled to room temperature to convert it to a gel known as Proniosomal gel. The gel obtained was preserved in the same glass tube in a dark for characterization.

Optimization of formulation

Various process variables, which could affect preparation and properties of the Proniosomal gel formulation, were identified and studied.

Following two process variables of proniosome gel formulation were selected for optimization of formulation.

- Type of surfactant
- Ratio of surfactant, lecithin and cholesterol

The formulation code and respective variables used in the preparation are given in table 1 (group 1) and 2 (group 2).

Table 1: Different variables used in the preparation of Hydralazine HCL (group 1)

Sr. NO.	Formulation code	surfactant	Ratio of S:L:C	Solvent
1	PNA 1	Span 20	9:9:2	Ethanol (1 ml)
2	PNA 2	Span 40	9:9:2	Ethanol (1 ml)
3	PNA 3	Span 60	9:9:2	Ethanol (1 ml)
4	PNA 4	Tween 20	9:9:2	Ethanol (1 ml)
5	PNA 5	Tween 60	9:9:2	Ethanol (1 ml)
6	PNA 6	Span 20:40	9:9:2	Ethanol (1 ml)
7	PNA 7	Span 40:60	9:9:2	Ethanol (1 ml)

Table 2: Different variables used in the preparation of Hydralazine HCL (group 2)

Sr. NO.	Formulation code	surfactant	Ratio of S:L:C	Solvent
1	PNB 1	Span 20	9:9:1	Ethanol (1 ml)
2	PNB 2	Span 40	9:9:1	Ethanol (1 ml)
3	PNB 3	Span 60	9:9:1	Ethanol (1 ml)
4	PNB 4	Tween 20	9:9:1	Ethanol (1 ml)
5	PNB 5	Tween 60	9:9:1	Ethanol (1 ml)
6	PNB 6	Span 20:40	9:9:1	Ethanol (1 ml)
7	PNB 7	Span 40:60	9:9:1	Ethanol (1 ml)

Characterization Of Proniosomal Gel

Thermal Analysis

Differential scanning colorimetry thermograms of proniosomes without drug and drug loaded proniosomes were recorded on a perkin elmer diamond DSC, USA calibrated with indium and zinc. The DSC runs were performed over a temperature range 50-250^{0}C at a heating rate of 10^{0}C per minute for proniosomes formulation and for Hydralazine HCL at temperature range of 90-290^{0}C.

Morphological evaluation

physical appearance

The prepared gel was viewed by naked eye to characterize color and physical state of gel. Proniosomes gel was also viewed by optical microscope at 40 X magnification, to observe crystal characteristics of gel by spreading a thin layer of Proniosomal gel on a slide and placing the cover slip on it.

Vesicle shape and surface characteristics

Proniosomes gel was hydrated with phosphate saline (pH 7.4) with slight agitation to produce noisome. A drop of noisome suspension was placed on a slide and after placing cover slip observed under microscope. To further evaluate the surface characteristics of vesicle, transmission electron microscopy was performed on a formulation. The noisome suspension was negatively stained with a 1% aqueous solution of phosphotungustic acid. Noisome suspension was dried on a microscopic carbon coated grid for staining. The excess solution was removed by blotting. After drying, the specimen was viewed under the microscope at 13.5, 22 kV old enlargement.

Vesicle size and size distribution

Hydrated proniosomes gel as noisome suspension was evaluated for noisome size. The dispersion was observed under optical microscope at 100 X magnification. The size of 200 vesicles was measured using a calibrated ocular and stage micrometer fitted in the optical microscope.

Vesicle size of noisome was also analyzed by differential light scattering method (Malvern Master Sizer). For this proniosomes was hydrated with phosphate buffer saline with slight agitation.

Rate of Spontaneity

The number of niosomes formed after hydration of proniosomes for 20 min. Approximately 20 mg of Proniosomal gel was transferred to glass bottle and spread uniformly around walls. 2ml of phosphate buffer was added along the walls and left aside for 20 min . then a drop was withdrawn and placed on Neubauers chamber to count the number of vesicles. The number of noisomes eluted from Proniosomes was counted.

Drug Entrapment efficiency

Proniosomal gel was dispersed in 10 ml phosphate buffer saline to produce niosome suspension. The niosome suspension was centrifuged at 18000 rpm in cooling centrifuge at 200^{o}c for 30 min to separate Hydralazine containing niosome from unentrapped drug. Then the sediment vesicle was resuspended in 1 ml 30 % PEG-400 and 1 ml 0.1 % Triton X-100 solution was added it. Resulting solution was filtered and diluted to 100 ml with phosphate buffer saline and analyzed. The percentage entrapment efficiency is calculated from the equation.

$$\% \text{ entrapment efficiency} = \frac{\text{Amount of entrapped drug} \times 100}{\text{Amount of total drug}}$$

In Vitro release

In Vitro release studies of hydralazine were carried out by drug diffusion studies through cellophane membrane and in vitro skin permeation studies.

Drug diffusion studies through cellophane membrane

In vitro release studies on proniosomal gel were performed using locally manufactured Franz-diffusion cell. The capacity of receptor compartment was 30 ml. The area of donor compartment exposed to receptor compartment was 2.54cm^2. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side of the dialysis membrane. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±0.5^{o}C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically (Shimadzu-1800) at 219 nm.

The release through cellophane membrane from hydro-alcoholic solution of drug (1mg/ml of hydro HCL in 5%v/v ethanol in water) was also performed by using above mention procedure.

In vitro skin permeation studies

Preparation of Human cadaver skin

The skin was stored at 0-4^{o}C after collection. The excised human abdomen skin was treated to remove hair and subdermal tissue. The subdermal fat was removed with help of scalpel and swapped with isopropyl alcohol. The treated skin was stored at 0^{o}C in deep freezer for not more than 2 days.

To actually mimic the in vitro permeation study, the permeation studies were performed using excised cadaver skin mounted on Franz cell. The release studies were carried out by above mention procedure. The release through cadaver skin from hydro-alcoholic solution of drug (2mg/ml of HCL in 5%v/v ethanol in water) was also performed by using above mentioned procedure.

Data Analysis

The cumulative amount of Hydralazine permeated through cellophane membrane and excised human abdomen skin was plotted as a function of time. The slope of the linear portion of the plot was derived by regression. The flux (permeation rate) was calculated from slope.

$$\text{Flux} = \frac{\text{Slope of cumulative amount of drug release v/s time curve}}{\text{Effective permeation area}}$$

The release profiles were fitted to the Zero order model (Eq. 1), First order (Eq. 2) and Higuchi square root model (Eq.3).

1. $Q_t = Q_0 - k_0 t$ .....Eq.
2. $Q_t = Q_0 - e^{-k_1 t}$ .....Eq.
3. $Q_t = k_H \sqrt{t}$ .....Eq.

Q_t is the total amounts of drug release after time t, Q_0 the initial amount of drug, and k_0, k_1, k_H are release rate constant for kinetic models, respectively.

Stability Studies

The ability of vesicles to retain the drug (Drug Retention Behaviour) was assessed by keeping the proniosomal gel at three different temperature conditions, i.e., Refrigeration Temperature (4-8^{0}C), Room Temperature (25±2^{0}C) and oven (45±2^{0}C). Throughout the study, proniosomal formulations were stored in aluminium foil-sealed glass vials. The samples were withdrawn at different time intervals over a period of six weeks and observed microscopically for change in consistency, liquid crystalline structure & solid drug crystals upon storage stored samples were also analysed for particle size & percent drug entrapment at 450 nm.

Statistical Analysis

The statistical significance of the difference in particle size, spontaneity and percentage entrapment between the two formulation groups prepared with different ratio of surfactant, lecithin and cholesterol was tested by one way analysis of variance. Differences were considered to be statistically significant at a level of $p \leq 0.05$.

RESULT AND DISCUSSION

Thermal Analysis

The DSC thermograms of proniosome without drug exhibits large area peak at 91.73^{o}C represent phase transition temperature of soya lecithin and surfactant (Span 60) (figure no.3). DSC of Hydralazine HCL shows a peak at 272.95^{o}C (figure no.1). The DSC thermograms of drug loaded proniosome exhibits two peaks, one at 101.42^{o}C which represent transition temperature of bilayer lamellas of proniosomal gel, and second peak at 269.42^{o}C is same or near to peak of drug shown in thermograms of Hydralazine (figure no.2). It suggests that no interaction has taken place between drug and other formulation ingredients. The results obtained suggest that the proniosome gel of Hydralazine consisted of homogeneous bilayer lamellas of surfactant and lecithin.

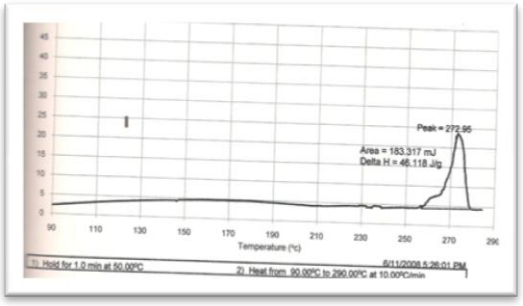

Figure 1: DSC Thermogram of Hydralazine HCl

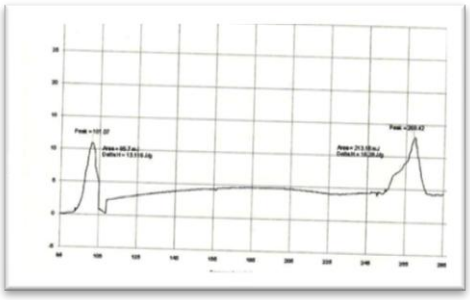

Figure 2: DSC Thermogram of PNA 3 loaded with Hydralazine HCl

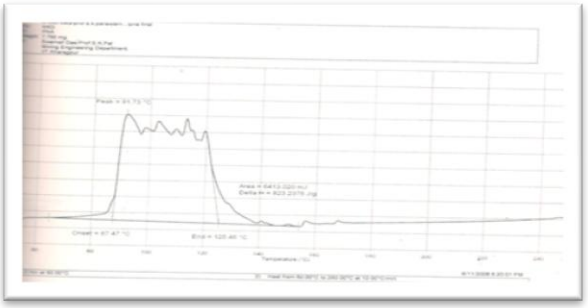

Figure 3: DSC Thermogram of PNA 3 without Hydralazine HCl

Morphological evaluation

Morphological evaluation show that proniosome gel of Hydralazine prepared with Span (40, 60) and combination of Span results in semisolid crystalline gel and proniosome gel of Tween appears as semisolid compact mass. Proniosome produced from Span 20 have thick sticky gel like appearance. The results of morphological evaluation are reported in table no. 3. TEM imaging of Hydralazine loaded niosome produced from proniosome revealed that vesicles are spherical in shape. TEM imaging of niosome produced from formulation PNA 3 shows in figure no. 4.

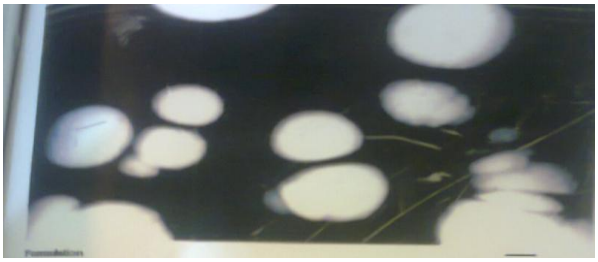

Figure 4: TEM imaging of niosome produced form formulation PNA3

Table 3: Characterization of various Proniosomal gel formulation loaded with Hydralazine HCl

S.No.	Formulation Code	Physical appearance	Vesicle Size(µm)		Spontaneity* (niosome/mm^3)	Percent Entrapment*
			Optical Microscope	DLS		
1.	PNA 1	Dark yellow semisolid thick sticky gel	Does not produce niosomes	--	--	--
2.	PNA 2	Yellowish semisolid crystalline gel	2.132	1.607	$(7.34\pm0.221)\times 10^3$	90.50±1.25
3.	PNA 3	Yellowish semisolid crystalline gel	2.347	1.702	$(9.687\pm0.883)\times10^3$	93.08±1.66
4.	PNA 4	Light yellow semisolid compact mass	3.673	2.322	$(5.93\pm0.441)\times 10^3$	75.58±4.83
5.	PNA 5	Yellow semisolid compact mass	2.896	2.004	$(9.687\pm0.312)\times10^3$	84.25±0.90
6.	PNA 6	Yellowish semisolid crystalline gel	3.569	3.123	$(6.406\pm0.662)\times10^3$	81.05±1.52
7.	PNA 7	Yellowish semisolid crystalline gel	3.173	2.323	$(6.875\pm0.883)\times10^3$	81.16±1.52
8.	PNB 1	Yellow semisolid thick sticky gel	Does not produce niosomes	--	--	--
9.	PNB 2	Yellowish semisolid crystalline gel	2.398	1.757	$(9.062\pm0.883)\times10^3$	87.25±0.66
10.	PNB 3	Yellowish semisolid crystalline gel	2.765	2.368	$(8.906\pm0.662)\times10^3$	91.83±0.94
11.	PNB 4	Yellow highly viscous liquid	3.464	2.641	$(7.031\pm0.221)\times10^3$	70.25±1.98
12.	PNB 5	Yellow semisolid compact mass	3.279	2.406	$(9.218\pm1.104)\times10^3$	83.91±1.90
13.	PNB 6	Yellowish white semisolid crystalline gel	4.741	2.555	$(8.281\pm0.662)\times10^3$	81.88±2.62
14.	PNB 7	Yellowish semisolid crystalline gel	2.975	2.508	$(8.437\pm1.325)\times10^3$	81.25±1.14

***Value represented as, Mean ± SD (n=3)**

Vesicle size and size distribution

Effect of type of surfactant and ratio of cholesterol has an immense effect on vesicle size and distribution. In study it was found that the vesicle size of niosome formed from proniosome containing S:L:C ratio of 9:9:2 did not significantly differ (p=0.47) from the niosome produced from proniosome gel containing S:L:C ratio 9:9:1. Formulation PNA 2 produces niosome of smallest size range. The vesicle size distribution of PNA 2 and PNA 3 formulation are reported in Figure no. 5 and 6 respectively.

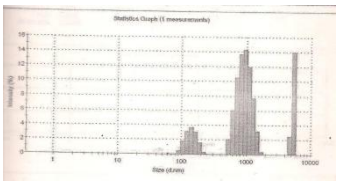

Figure no. 5: Size distribution Data of PNA 2 Formulation

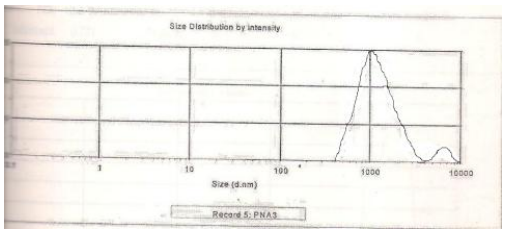

Figure 6: Size distribution Data of PNA 3 Formulation

Rate of Spontaneity

The results of study given in table no. 3 show that formulation prepared with Span 40, Span 60 and Tween 60 form niosome more spontaneously. The rate of hydration of group 1 proniosome was not significantly differing (p=0.28) from group 2 proniosome.

Drug entrapment

As shown in table no. 3, all the proniosome gel showed very good entrapment efficiency. Hydralazine is a hydrophilic drug and must be entrapped in aqueous core of niosome. Niosome composed of Span 40, 60 have shown highest entrapment because the drug leaching from the vesicle is low due to its high phase transition temperature and low permeability.

In vitro release study

Drug diffusion studies through cellophane membrane

Drug diffusion study was conducted through cellophane membrane with selected optimized formulations (PNA2, PNA3, PNA5, PNB2, PNB3 & PNB5) & hydro alcoholic solution of drug using locally fabricated franz diffusion cell. The cumulative amount of hydralazine release from proniosome formulations through an artificial membrane at various sampling time were reported in Table No. 4 & Figure No.7.

The result indicate that the proniosome with increased ratio of cholesterol have high release rate. The percent cumulative release in 24hrs (Table No. 6) was found ranging in 46% -56%, PNA2 formulation has shown highest percentage release in 24hr.

Table 6: Data of % release in 24 hr and flux from cellophane membrane

Sr. No.	Formulation Code	% release in 24 hr	Flux (µg/cm^2/hr)
1	PNA 2	56.06	8.70
2	PNA 3	52.40	7.79
3	PNA 5	46.17	6.15
4	PNB 2	50.10	6.56
5	PNB 3	46.48	6.90
6	PNB 5	45.66	5.62
7	Hydro alcoholic solution	22.84	3.18

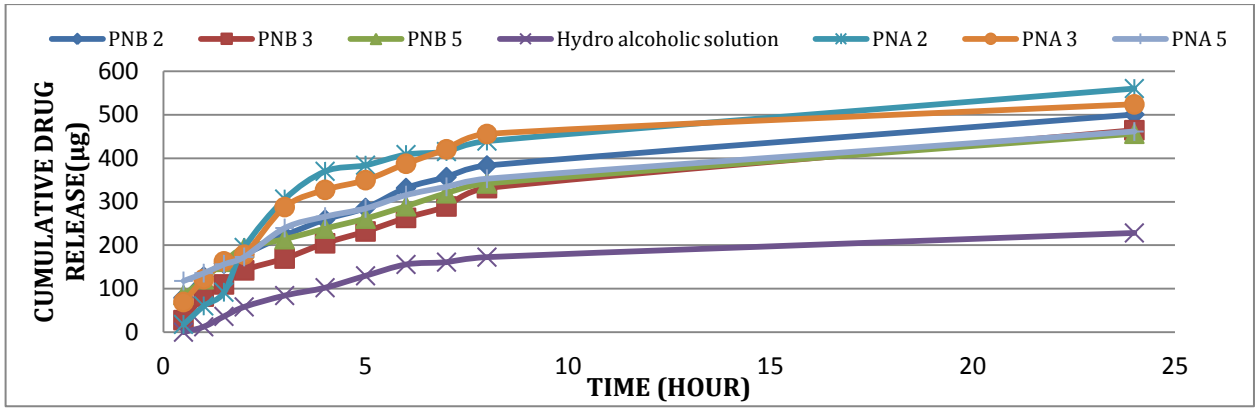

Figure 7: Cumulative amount of drug release through cellophane membrane from different Proniosome formulation

Table 4: Data of Cumulative amount release from proniosomal gel (50 mg) and hydro alcoholic solution of same concentration through cellophane membrane at various time point of sample collection

Sr. No.	Time	Cumulative amount release (µg)						
		PNB 2	PNB 3	PNB 5	PNA 2	PNA 3	PNA 5	Hydro alcoholic solution
1	0.5	79.2	27.8	85.4	18	69.5	118	0
2	1	127.91	81.97	121.87	60.6	122.3	135.93	12.6
3	1.5	157.87	109.98	162.04	92.02	163.03	156.33	36.41
4	2	178.52	142.39	193.65	195.07	178.43	173.21	58.26
5	3	222.09	169.52	214.08	306.43	287.94	239.77	84.37
6	4	259.2	204.64	238.49	370.21	327.59	265.99	102.45
7	5	285.9	231.81	261.44	384.34	349.91	284.86	129.88
8	6	331.73	263.07	289.91	408.81	387.66	315.49	155.92
9	7	356.85	289.34	319.72	415.62	420.92	334.51	161.218
10	8	383.53	331.53	342.27	439.85	456.03	353.15	172.73
11	24	501.08	464.8	456.65	560.66	524.2	461.77	228.46

The data obtain from permeability studies using cellophane membrane with hydralazine proniosome revealed a flux of 5.62-8.70 µg/cm2/hr. The flux (Figure No. 9) obtain from PNA formulations(S: L: C::9: 9: 2) was found to significantly higher than PNB formulations (S: L: C::9: 9: 1).

The data of release of hydralazine (Table No. 4, Figure No. 7) from hydro alcoholic solution revealed that proniosomal formulations have shown significantly increase percent release & flux with comparison to same concentration of hydro alcoholic solution of hydralazine.

From the data(Table No. 8) it was found that value of correlation coefficient of higuchi equation is nearer to 1.0 for all formulations, and hence it can be predicted that the release from proniosom follow higuchi kinetics.

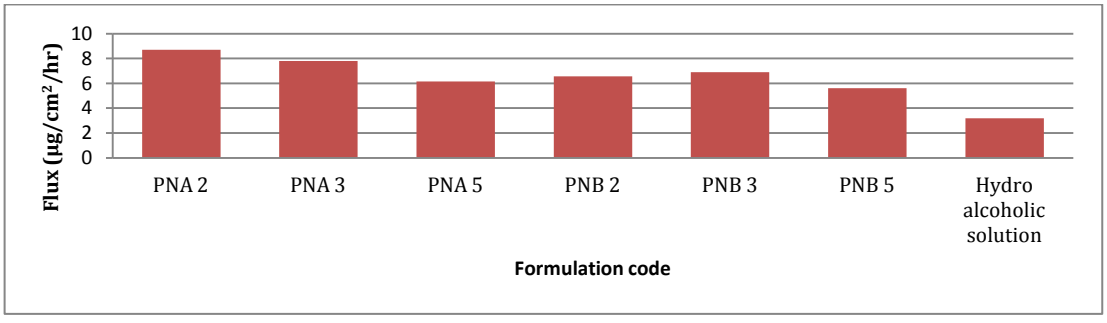

Figure 9: Graphical representation of flux (permeation rate) from cellophane membrane

Table 8: Correlation coefficient and release rate constant of various kinetic equation (through cellophane membrane)

S.No.	Formulation	Zero order		First order		Higuchi	
		K_0	R^2	K_1	R^2	K_H	R^2
1.	PNA 2	-2.061	0.7636	-0.0177	0.5760	13.835	0.8988
2.	PNA 3	-1.767	0.7864	-0.0117	0.6642	11.745	0.9167
3.	PNA 5	-1.414	0.8759	-0.0097	0.7788	8.9068	0.9676
4.	PNB 2	-1.667	0.8753	-0.0116	0.7468	10.524	0.9685
5.	PNB 3	-1.709	0.898	-0.0115	0.6894	10.627	0.9790
6.	PNB 5	-1.443	0.889	-0.0106	0.7587	9.0217	0.9753

In Vitro Skin permeation Studies

Formulation PNA2 & PNA3 were utilized to predict release profile from proniosomal gel through human skin. Proniosomal gels have shown excellent slow sustained release (Table No. 5, Figure No. 8) with 75.44, 71.163% release in 24hr (Table No. 7). The transdermal flux calculated was sufficiently high, this exhibit that there were no or very small lag time to reach hydralazine in the receptor solution (Figure No. 10).

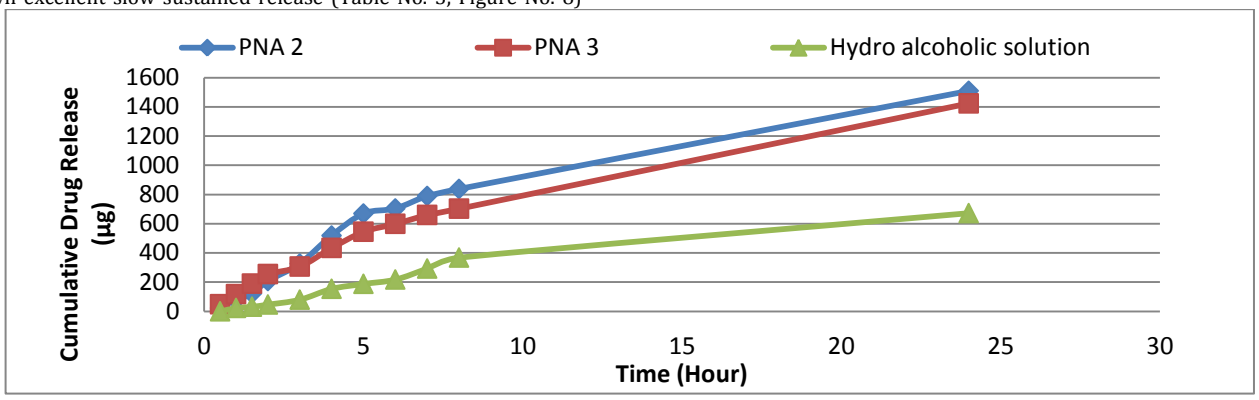

Figure 8: Cumulative amount of drug release through excised human skin from Proniosome formulation (PNA 2, PNA 3, Hydro alcoholic solution)

Table 5: Data of Cumulative amount release from proniosomal gel (100 mg) and hydro alcoholic solution of same concentration through excised human skin at various time point of sample collection

Sr. No.	Time (hr)	Cumulative amount release (µg)		
		PNA 2	PNA 3	Hydro alcoholic solution
1	0.5	22.6	48.4	0
2	1	79.71	117.37	23
3	1.5	122.26	189.56	31.6
4	2	207.07	254.78	46.47
5	3	326.37	306.31	79.84
6	4	518.14	433.75	154.05
7	5	668.95	544.64	188.02
8	6	703.58	598.87	217.53
9	7	789.24	659.6	292.63
10	8	837.43	702.58	367.8
11	24	1508.8	1423.26	671.85

Table 7: Data of % release in 24 hr and flux from excised human skin

Sr. No.	Formulation Code	% release in 24 hr	Flux (µg/cm²/hr)
1	PNA 2	75.44	15.51
2	PNA 3	71.16	13.88
3	Hydro alcoholic solution	33.59	5.9

The percent release of hydralazine through human skin in 24hrs from hydro alcoholic solution was found to be 33.59% which was significantly very lower than percent release from any proniosomal formulation (Table No. 5, Figure No. 8).

The release rate data obtained from in vitro skin permeation studies was fitted to various kinetic model & found that for both the formulation, value of correlation coefficient (Table No. 9) of higuchi equation is nearer to 1.0, & hence it can be predicted that the release of hydralazine from proniosome through skin follow higuchi kinetics.

Table 9: Correlation coefficient and release rate constant of various kinetic equation (through excised human skin)

S.No.	Formulation	Zero order		First order		Higuchi	
		K_0	R^2	K_1	R^2	K_H	R^2
1.	PNA 2	-3.133	0.9360	-0.0246	0.688	18.908	0.9907
2.	PNA 3	-2.834	0.9712	-0.0205	0.755	16.609	0.9984

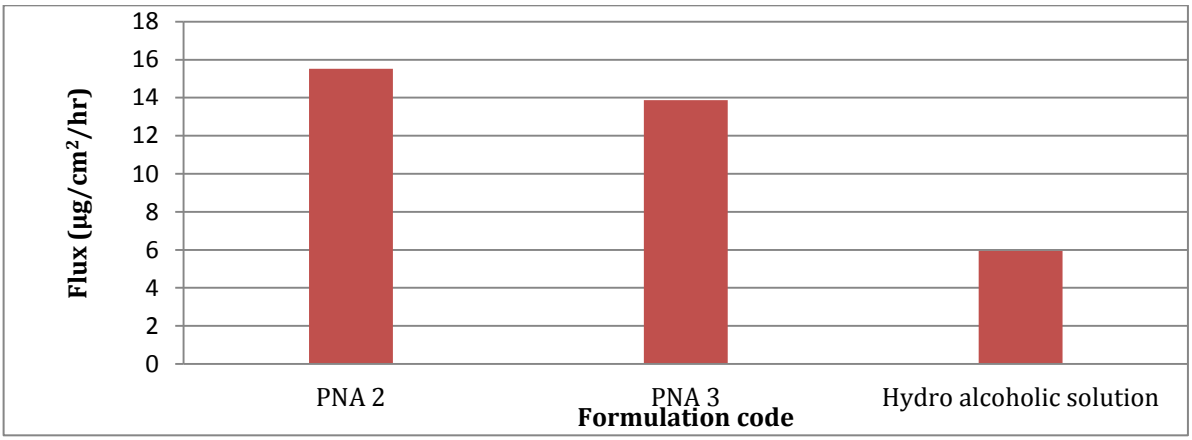

Figure 10: Graphical representation of flux (permeation rate) from excised human skin

Stability Studies

From the results of vesicular size and shape and drug content (Table no.10) of the optimised formulation (PNA 3) it was concluded that the formulation was stable at refrigeration and room temperature as well.

Table 10: Data of Stability Testing of PNA 3 formulation at various Storage Temperature

Characteristics	Before storage	After 6 weeks storage		
		4-8^{0}c	25±2^{0}C	45±2^{0}C
Vesicle size (µm)	2.347	2.152	2.743	1.795
% Entrapment	93.08±1.66	91.46±1.08	87.59±0.961	78.83±1.26
% Drug content	95.02±0.835	92.60±01.34	90.84±2.04	86.72±1.16

CONCLUSION

Antihypertensive treatment requires prolonged and controlled release of Hydralazine HCl which can be achieved through proniosomal gel as a transdermal drug delivery system. All the proniosomal gel formulations were evaluated for the encapsulation efficiency, vesicle size and shape and the results were found in the acceptable range. Vesicle size and drug permeation was greater for formulation containing Span 40, 60 due to its high hydrophobicity which resulted in smaller size of vesicle. Thus proniosomal gel will be suitable drug delivery system for Hydralazine HCl due to ease of preparation and incorporation of less no. of excipients.

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