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Research Article

FORMULATION DEVELOPMENT & EVALUATION OF PRONIOSOMAL GEL OF CARVEDILOL

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ABSTRACT

The present investigation aimed at formulation development and performance evaluation of proniosomal gel as a vesicular drug carrier system. Carvedilol has oral bioavailability 25% to 30% due to high first pass metabolism (80%) and presence of food decreases its absorption rate in gastrointestinal tract. Short half life of Carvedilol (4 to 6 hr) indicates need of controlled release delivery. To overcome these limitations carvedilol was incorporated in proniosome for transdermal delivery. Proniosome drug delivery was preferred due to improved stability of the system than niosomes.

To optimize the formulation, various proniosome gels composed of various ratios of sorbitan fatty acid esters, polysorbates, cholesterol, lecithin were prepared by coacervation-phase separation method. Proniosomal gel (PNG) formulations of carvedilol were characterized for vesicular shape & size, entrapment efficiency, permeation study, stability study, pH and viscosity of gel. The effects of cholesterol, lecithin and different non-ionic surfactants on transdermal permeability profile of Carvedilol were also assessed. The percent encapsulation of carvedilol in proniosome with tween surfactants revealed a very high entrapment efficiency than the span surfactants.

Optimized formulation gave flux 3.416µg/cm^2/hr up to 12 hrs. with highest permeation (65%) of carvedilol among all formulations. Penetration enhancers, non-ionic surfactants and vesicle-skin interaction may contributed to the enhanced carvedilol permeation. Thus Proniosome was found to be a promising carrier system for Carvedilol because of ease in preparation and stability for prolonged period.

Keywords: Carvedilol, Proniosomes, Non ionic surfactants, Permeation

INTRODUCTION

Transdermal delivery of drugs through the skin to the systemic circulation provides convenient route of administration for a variety of clinical indications. For transdermal delivery of drugs, stratum corneum is the main barrier layer for permeation of drug. Hence to increase the flux through skin membrane, different approaches of penetration enhancement are used. Drug-vehicle based enhancement methods such as liposomes, prodrugs and ion-pair are used in transdermal research as better alternative methods to enhance permeation of drugs through skin. Many approaches involving chemical penetration enhancement are extensively studied but vesicle based enhancement approach is not exploited much.[1,2,3.]

Proniosomes are dry formulations of water-soluble carrier particles that are coated with surfactant and hydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The advantages of niosomes over liposomes are low toxicity due to non ionic nature , no requirement of special precautions and conditions for their formulation and preparation. Stability is of prime concern in the development of any formulation. Though, niosomes are advantageous as drug carriers, because of low cost and chemically stable as compared to liposomes,they too are associated with problems related to physical instability such as fusion, aggregation, sedimentation, leakage on storage, hydrolysis and oxidation due to presence of water in noisome. So there is need to prepare pronisome formulation to improve stability than noisome due to absence of water[3,4,5,6.] Carvedilol comes under the class of alpha and beta blocker antihypertensive agent. It shows low oral bioavailability of about 20 to 25% due to high first pass effect (80%) and food decreases its absorption rate. So there is need that drug is given through the transdermal drug delivery system. Carvedilol has short biological half life about 4 to 6 hr. and frequency of dosing is high. Carvedilol being BCS class-II drug (Mol.Wt.406Da) and is suitable for transdermal delivery. Thus transdermal proniosome drug delivery system for carvedilol was developed and assessed for permeation characteristics. Use of non-ionic surfactant and cosolvents are necessary to prepare proniosome which also essential to improve the solubility of drug. Presentation of Carvedilol in the form of proniosome might confer additional advantage of photostability to carvedilol[6,7,8.] Proniosome formulation was prepared by coacervation phase separation method. The coacervation-phase separation is specially reported for preparation of proniosomal gel by simply dissolving the drug and surfactants in cosolvents and adding the gelling agent and it is used for small scale production.[9]

Proniosome gels were evaluated for entrapment efficiency, in vitro permeation, stability, viscosity, pH, vesicular size and shape etc. The results revealed that the entrapment efficiency was better when tween surfactants were used in the formulation but permeation was more when spans were used as surfactants. Hence the optimized formulation was prepared by using span 60 and gave 65% permeation up to 12 hrs. with flux 3.416µg/cm^2/hr. The stability of proniosomal form of optimized formulation was confirmed by Scanning electron microscope as the vesicular size and shape was unchanged even after the 2 months.

MATERIALS AND METHODS

Materials

Carvedilol was obtained as a gift sample from Cipla pharmaceuticals pvt.ltd. Kurkumbh (Pune,India). Span-20, Span-40, Span-60, Tween-20, Tween-80, Cholesterol and Lecithin were purchased from Research Lab (Mumbai, India) and were of pharmaceutical grade. Chloroform, Ethanol and Potassium dihydrogen phosphate were of analytical reagent grade and obtained from Research lab (Mumbai, India)

Methodology

Drug – Excipients Compatibility Study

The Drug – Excipients Compatibility Studies were performed in order to confirm absence of any interaction between drug and excipients.The differential scanning calorimetry (DSC) of plain drug and its physical mixture with various excipients was obtained using Mettler Toledo Instrument and results are reported in fig no.1.FT-IR(FT-IR 8400S, Shimadzu) spectrum of drug sample and its physical mixture with excipients was recorded as KBR pellets at resolution of 4 cm$^{-1.}$ The results are reported in Fig no.2 and Table no.1

Formulation of Proniosomal gel

Proniosomal gels were prepared by Coacervation Phase Separation method, Precisely weighed amounts of surfactant, lipid phase and

drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (2.5 ml) was added to it. All the ingredients were mixed well with a glass rod; the open end of the glass vial was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 50-60°C for about 5 minutes until the drug is dissolved completely in surfactant mixture. Then the aqueous phase phosphate buffer pH 7.4(1.6ml) was added and warmed on a water bath until a clear solution was formed. Preliminary the composition of these formulations is reported in Table no.2 and they are referred as F1 to F5. Carbopol (1.5%) was added and the mixture was converted into proniosomal gel on cooling and referred as F6 to F10 respectively.[10,11.]

Table 2: Compositions of Preliminary formulations

Qty. in Mg Sr.No.	Drug	Span 20	Span 40	Span 60	Tween 20	Tween 80	Cholesterol	Lecithin
F1	12.5	90	-	-	-	-	10	140
F2	12.5	-	90	-	-	-	20	130
F3	12.5	-	-	90	-	-	30	120
F4	12.5	-	-	-	90	-	40	110
F5	12.5	-	-	-	-	90	50	100

F1 : Span 20 , F2 : Span 40 , F3 : Span 60 , F4: Tween 20 , F5: Tween 80.

Evaluation of formulations

1) Encapsulation Efficiency

Each preliminary proniosomal formulation (F1-F5) was centrifuged at 25,000rpm at 25^{0}C for 30 min to separate untrapped drug as supernatant. Supernatant was separated, filtered and sufficiently diluted with methanol to determine the concentration of untrapped drug spectrophotometrically[12].The percentage of drug encapsulation was calculated by equation no.1:

EE (%) = [(*C*t-*C*f)/*C*t] ×100 ----------Eq.(1)

Where,

*C*t is the concentration of total drug,

*C*f is the concentration of free drug.

Results of encapsulation efficiency of Various Formulations are reported in Table no.3

2) All proniosomal gel formulations (F6 to F10) were evaluated for following parameters:-

a) pH Determination

The pH of each Proniosomal gel was determined using pH meter (Model GMPH, Labindia, Mumbai.). The electrode first calibrated with pH 4.0 and pH 7.0 solution then readings were recorded on pH meter[13]. For each formulation (F6 to F10) results are reported in Table no.4

b) Vesicle size and shape

Vesicle size and shape for each formulation(F6 to F10) was determined by electron microscope(Motic Image,Germany).2 gm of each formulation(F6 to F10) was spread uniformly on glass slide and observed under electron microscope for vesicular shape[14]. The results are reported in Table no.4

c) Viscosity Determination

Viscosities of the formulated Proniosomal gels were determined using Brookfield Viscometer (DV-II + Pro) with T-Shaped spindle set using Spindle no.92 at 2 rpm at 25°C [14,15]. Results are reported in Table no.4

d) Drug Content

The drug content was determined by 2gm proniosomal gel sample was withdrawn from container and dissolved in 100 mL ethanol. From this 1 mL solution was diluted up to 10 ml with ethanol. Then the absorbance was measured by U.V (Jasco 630-V) spectrophotometer against blank at λ max-241 nm and the drug content was calculated. Results are reported in Table no.4

e) In vitro drug permeation

The permeation of drug from Proniosomal gel formulations was determined by using Franz (vertical) diffusion cell (Orchid Scientifics, Mumbai) with area 1.59 cm^2. The dorsal abdominal skin of the Wistar rat (7–9 weeks old) was shaved 24 h before study then mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The top of the diffusion cell was covered with paraffin paper. The donor compartment was filled with the proniosome formulation. A 20ml aliquot of 4:6 (v/v) Ethanol: pH 7.4 phosphate buffer was used as receptor medium[16,17]. The available diffusion area of cell was 2.54cm^2. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 300 rpm. Aliquots equivalent to 0.5ml of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution at appropriate interval of an hour up to 12hr.

These formulations were critically evaluated for in vitro flux (Jss) and permeability coefficient (Kp). The in vitro flux(J_{ss}) was determined by Fick's law of diffusion, considering the transport of drugs across the skin barrier as a process of passive diffusion. The skin flux(µg/cm^2/h),was determined from the slope of linear portion of the cumulative amount permeated per unit area versus the time plot. The permeability coefficient (K_p) (cm/h), was determined from the equation number 2:

$Kp = J_{ss}/C_0$ ---------- Eq.(2)

Where C_0 is the concentrationof drug in donor compartment.

The skin flux can be experimentally determined from the following equation:

$J_{ss} = (dQ/dt)_{ss} \times 1/A$ ---------- Eq.(3)

Where,

J_{ss}**:** Steady-state flux (mg/cm^2 per h), A**:** Area of skin tissue (cm^2) through which drug permeation takes place, $(dQ/dt)_{ss}$**:** The amount of drug passing through the skin per unit time at a steady-state (µg/h).

The results for permeation are reported in Table no.5 and Fig.3. The results of flux and permeability coefficient are reported in Table no.6.

3) Evaluation of Optimised Formulation

Formulation F8 was optimised on the basis of permeation studies and evaluated for following parameters

1) Stability study

Stability studies were carried out by storing the optimized formulation (F8) at various temperature conditions as per ICH guidelines i.e. at refrigeration temperature (2°-8°C), room temperature (25°± 0.5°C) for a period of two months[18,19,20]. Drug content and variation in the average vesicle diameter were determined before and after the completion of 2 months. Surface morphology of optimized formulation (F8) by SEM image is shown in Fig no.4 and the results are reported in Table no.7

2) Skin irritation study

The albino Wistar rats were housed in polypropylene cages, with free access to standard laboratory diet and water. Animals were acclimatized for at least 7 days before experimentation.The dorsal abdominal skin of rats was shaved 24 h before study. Transdermal gel was applied and site of application was occluded with gauze and covered with a nonsensitizing microporous tapes. Formalin (0.8 %w/v) solution was applied as standard skin irritant. The formulation was removed after 24 h and score of erythema was recorded and was compared with standard[21,22,23,24]. Results are shown in Fig no.5

RESULTS AND DISCUSSION

1) Drug – Excipients Compatibility Study

The drug excipients compatibility study by DSC showed broadening and early onset of endotherm for Carvedilol (at 108^{0}C) than endotherm for pure Carvedilol **(**117^{0}C). These results indicated partial amorphisation and solubilisation of carvedilol due to the processing and absence of any additional peak indicated that there was no interaction between the drug and excipients used in the formulation. FTIR studies clearly indicated principle peaks of Carvedilol were retained in physical mixture with other excipients. Results are reported in Fig no.1and Fig no.2 and Table no.1

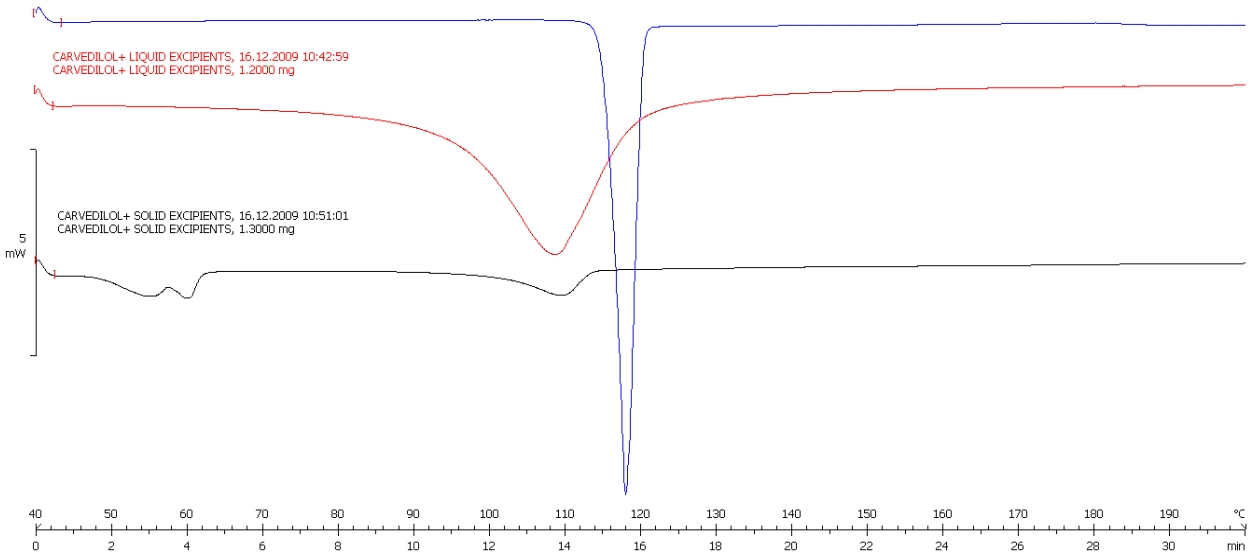

Fig. 1: DSC Spectra of Carvedilol and Physical Mixture of Excipients with Drug

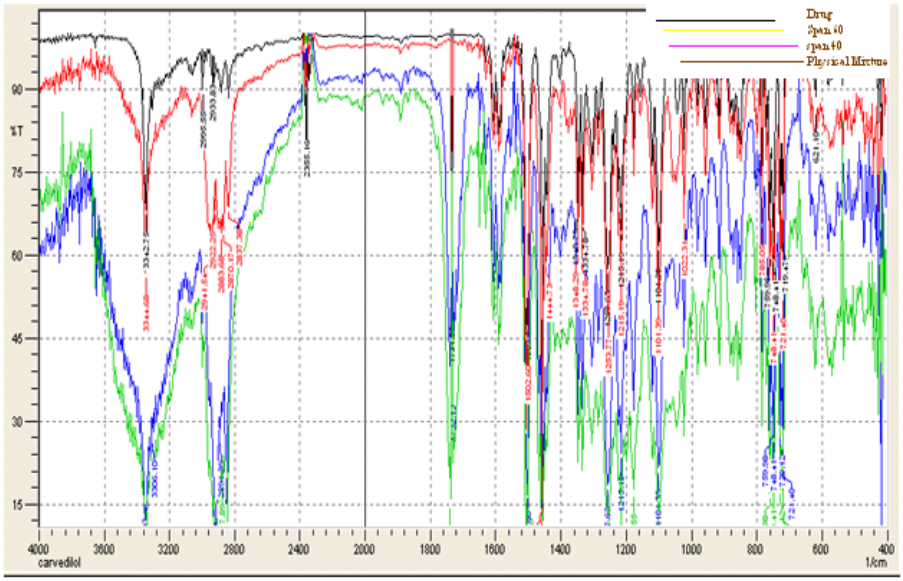

Fig. 2: FTIR spectra of drug and drug with excipients

Table 1: Data of IR Peaks of Carvedilol

Reported IR peaks of Carvedilol	Observed IR Peaks of Carvedilol
3331 cm^{-1} N-H stretching	3361.69 cm^{-1}
3200 cm^{-1} O-H aromatic	3069.05 cm^{-1}
3063 cm^{-1} C-H aromatic	3053 cm^{-1}
1015 cm^{-1} C-O ether	1016.45 cm^{-1}

2) Encapsulation Efficiency

The preliminary proniosomal formulations (F1 to F5) prepared as per the procedure mentioned earlier and compositions reported in Table no.2 met all the necessary specifications of vesicular size and shape which was confirmed under electron microscope. So all the formulations were tested for entrapment efficiency for carvedilol.

The results of encapsulation efficiency determination **(**Table no.3) indicated that formulation containing Tween-80 (F5) had high encapsulation efficiency than formulations containing other surfactants. It was also observed that as cholesterol content in formulation increased there was proportional increase in encapsulation efficiency.

Table 3: Encapsulation efficiency of various preliminary formulations

Sr.No.	Formulations Code	(%) Encapsulation
1	F1	92.18 ± 0.70
2	F2	94.55 ± 1.23
3	F3	97.74 ± 0.17
4	F4	98.03 ± 0.11
5	F5	98.81 ± 0.12

* Represents mean ± S.D. (n = 3)

3) pH of Formulation

Skin compatibility is the primary requirement for a good topical formulation, it was found that the pH of all the formulations were in the range of 5.24 to 7.60 that suits the skin pH, indicating skin compatibility. The results of pH determination are reported in Table no.4

4) Vesicle size and shape

The results of vesicle size measurement are reported in Table no.4.From these results it could be concluded that the entrapment efficiency of formulations containing tweens was high and might be due to there larger vesicular size. These could be also explained on the basis that the highly lipophilic portion of the drug is expected to be entrapped completely within the bilayer of proniosomes. Tweens being more hydrophilic than spans exhibited higher entrapment efficiency as formulation containing Span-60(F8) had lower size among all the tested formulations (F6 to F10). It was clear that span hydrophobicity had attributed to the smaller vesicle size.

5) Viscosity Determination

Viscosity measurement of all the formulations revealed optimum consistency and the results are reported in Table no.4

6) Drug Content

Uniformity in content of proniosomal gel (F6 to F10) were confirmed to assure uniformity in dosages. The results are reported in Table no.4.

7) In vitro drug Permeation Study

The proniosomal gel formulations (F6 to F10) were characterized for their drug permeation and the results are reported in Table no.5. The drug permeation was maximum from formulation containing Span 60(F8) among all tested formulations. From the permeation profile it was clear that the gel showed optimum drug release up to 12hrs. from the formulation F8 which was 65.39%,results are shown in Fig no.3. The permeation data fitted in zero order drug release indicating controlled release of the carvedilol from the formulations. It was but obvious that the smaller vesicular size of F8 enabled it to penetrate easily through the skin as smaller vesicles tend to fuse readily with the skin. Thus proniosome gel prepared by using span-60 exhibited better permeation and optimum entrament efficiency when compared with the formulations contaning tweens.

This could also be due to the emulsification effect of the surfactant after the hydration of the proniosome by the dissolution medium and formation of elution channels within the gel structure due to loss of lipid bilayer that resulted in higher flux value[24]. (Table no.5 and 6).Hence formulation F8 had selected as optimum formulation and stability testing studies were carried out .these observations were in accordance with earlier reports saying that incorporation of cholesterol was known to influence vesicle stability, permeability and entrapment efficiency. Increase in the cholesterol content resulted in a more intact and ordered lipid bilayer as a barrier for drug release and helped as a controlled release polymer and also decreased drug leakage by improving the fluidity of the bilayer membrane and reducing its permeability[23]

Table 4: pH, vesicle size, viscosity and drug content of proniosome gel formulation

Sr.No.	Formulations Code	pH	Vesicle size(µm)	Viscosity (centipoise)	Drug content(%)
1	F6	5.24±0.05	10.30±0.05	46115±1.11	85.55 ± 0.59
2	F7	6.98±0.12	8.50±0.9	45223±0.92	86.90 ± 0.63
3	F8	6.23±0.6	5.61±0.04	47889±1.13	90.43 ± 0.43
4	F9	7.11±0.04	12.10±1.11	45778±0.11	88.05 ± 0.38
5	F10	7.60±0.02	15.13±1.12	43667±0.34	89.08 ± 1.11

* Represents mean ± S.D. (n = 3)

Table 5: Cumulative Percent drug permeation of proniosomal gel formulations

Time (hr.)	Cumulative Percent drug permeation				
	F6	F7	F8	F9	F10
1	2.995±1.23	1.123±1.12	10.933±1.14	10.933±1.22	10.933±1.66
2	3.584±1.67	2.243±1.13	13.893±1.56	14.159v1.28	14.159±1.57
3	4.708±1.34	4.614±1.56	16.263±1.66	16.732±1.34	16.113±1.45
4	5.482±1.25	7.821±1.23	22.638±1,69	19.113±1.39	19.113±1.65
5	7.807±1.67	11.59±1.78	27.456±1.74	22.027±1.45	22.027±1.45
6	11.42±1.33	15.739±1.88	32.378±1.45	24.872±1.67	24.872±1.78
7	17.274±1.65	20.449±1.45	38.678±1.89	27.859±1.11	27.859±1.91
8	21.753±1.58	25.45±1.99	43.231±1.97	30.305±1.58	30.305±1.93
9	27.273±1.20	31.225±1.95	47.541±1.94	34.392±2.15	34.392±1.94
10	34.915±1.11	37.307±2.67	53.429±1.77	40.153±1.33	40.153±1.95
11	43.786±2.45	44.541±1.77	59.786±2.11	46.292±1.94	46.292±1.96
12	53.773±2.87	56.212±2.68	65.397±2.16	52.098±1.84	51.339±1.99

* Represents mean ± S.D. (n = 3**)**

Table6: Results of Flux,permeability coefficient of formulations

Formulation code	Flux (µg/cm²/hr)	Permeability coefficient(cm²/hr)
F6	2.099±0.09	0.273±0.13
F7	2.290±0.12	0.167±0.03
F8	3.416±0.15	0.183±0.15
F9	2.62±0.15	0.209±0.13
F10	2.45±0.13	0.203±0.17

* Represents mean ± S.D. (n = 3**)**

8) Stability Studies

From the results of vesicular size and shape and drug content (table no.7) of the optimised formulation (F8) it was concluded that the formulation was stable at refrigeration and room temperature as well. Results for vesicular shape are shown in Fig No.4

9) Skin irritation study

No erythema was found after 12 hrs. when optimized formulation F8 was applied dorsal skin of rat and compared with standard irritant. Thus the formulation was found to be suitable for transdermal application. The resuts are reported in Fig no.5.

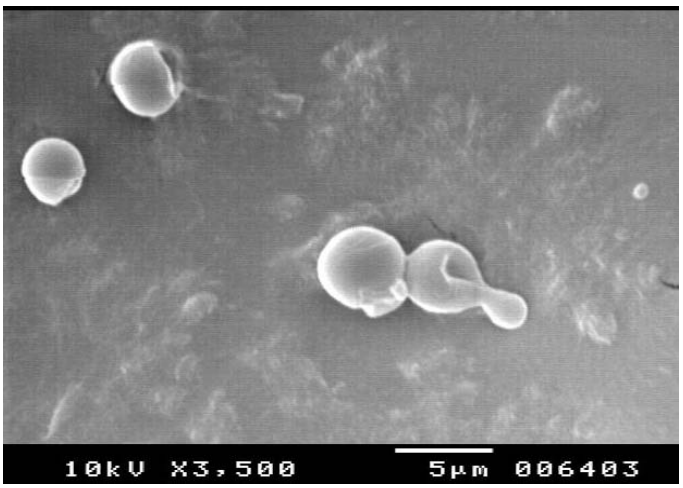

Fig. 4: a) SEM of optimized formulation F8 before 2 month

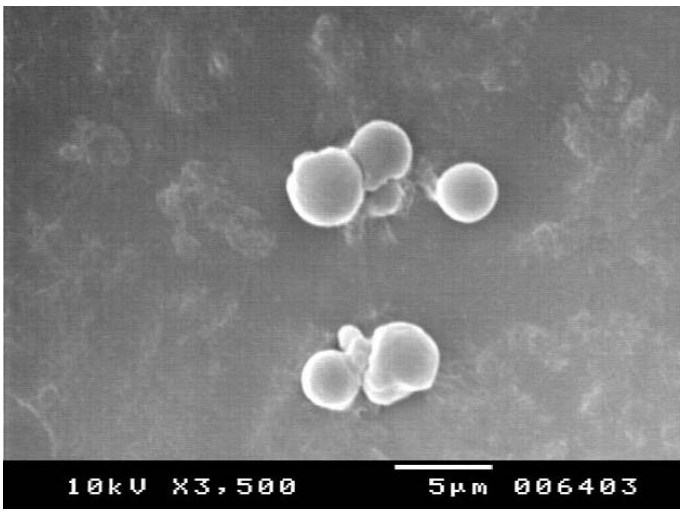

Fig. 4: b) SEM of optimized formulation F8 after 2 month

Table 7: Results of stability studies of optimized formulation (F8)

Sr. No.	Temp.	Initial		After 2 months	
		Drug content	Vesicle size	Drug content	Vesicle size
1	2⁰c	90.43±0.43	5.61±0.04	90.23±0.22	5.92±0.02
2	25⁰c	90.43±0.43	5.61±0.04	90.19±0.12	5.77±0.03

* Represents mean ± S.D. (n = 3**)**

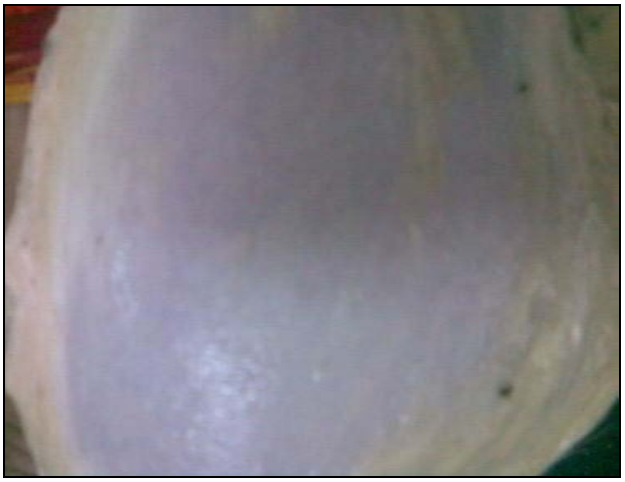

Fig. 5: a) Photograph of dorsal skin of rat showing no erythema after application of opimised formulation (F8)

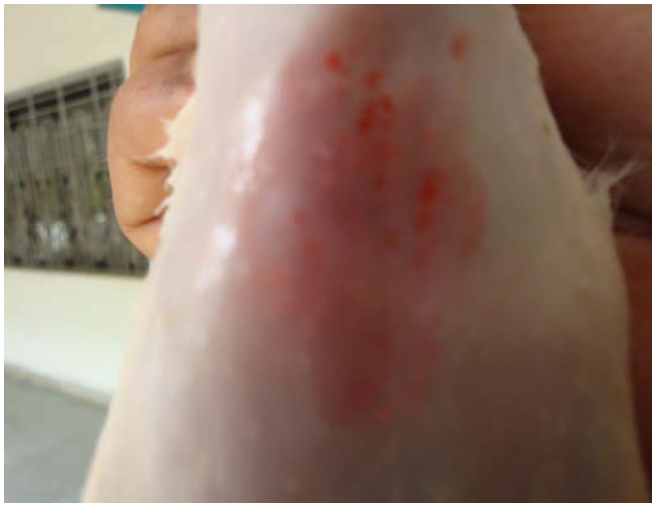

Fig. 5: b) Photograph of dorsal skin of rat after application of standard irritant

CONCLUSION

Antihypertensive treatment demands prolonged and controlled release of Carvedilol which can be achieved through proniosomal gel as a 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 60 due to its high hydrophobicity which resulted in smaller size of vesicle. Thus proniosomal gel will be suitable drug delivery system for Carvedilol due to ease of preparation and incorporation of less no. of excipients.

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