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Formulation and Evaluation of Carvedilol Proniosomal Gel

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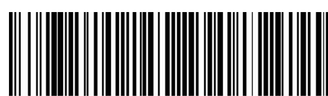

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Keywords: Carvedilol, Encapsulation efficiency, Vesicle size, Surface morphology, *In-vitro* drug release

ABSTRACT

The present work deals with the preparation of carvedilol proniosomal gel by coacervation phase separation method by using different surfactants like spans 20, 60, 80, tween 20, 80, soya lecithin and cholesterol. The prepared proniosomal gel formulations were evaluated for vesicle size analysis, surface morphology studies, encapsulation efficiency, and *in-vitro* drug release. The results showed that carvedilol in all the formulations was successfully entrapped and changes in the release rate. The encapsulation efficiency was found high in spans than compared to the tweens. Vesicles formed with the spans were smaller in size than vesicle formed with tweens. From all the formulations span 60 shows maximum entrapment efficiency (95%), and drug release (98%). It is evident from this study that proniosomes are a promising prolonged delivery system for carvedilol.

1. 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 [1].

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. Such as fusion, aggregation, sedimentation, leakage on storage, hydrolysis and oxidation due to presence of water in noisome. So there is need to prepare proniosome formulation to improve stability than niosome due to absence of water [2, 3].

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. 406 Da) 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. Proniosome formulation was prepared by coacervation phase separation method [4].

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 in spans than tween. Surfactants were used in the formulation but permeation was more in spans when compared with the tweens. Hence the encapsulation efficiency and *in-vitro* permeation studies show better results in spans.

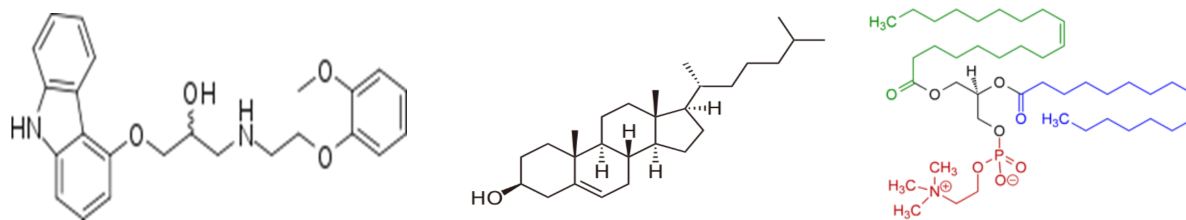

Fig. No. 1. Structure of Carvedilol Cholesterol Lecithin

2. MATERIALS AND METHODS

Carvedilol was obtained as a gift sample from Aurobindo Pharma Pvt. Ltd [5]. 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. Methanol and Potassium dihydrogen phosphate, sodium chloride and sodium hydrogen phosphate were of analytical reagent grade and obtained from Research Lab (Mumbai, India).

Preparation of 7.4 Phosphate Buffer (As per I.P-2007)

2.38 g of disodium hydrogenphosphate, 0.19 g of potassium di hydrogen phosphate and 4 g of sodium chloride was dissolved in a few ml of water and make up to the final volume to 1000 ml and adjust pH to 7.4

Compatibility Studies

The compatibility between drug and excipients is determined by using FTIR spectrophotometer.

Preparation of calibration curve

Weigh accurately 100 mg carvedilol was dissolved in a small quantity of methanol and make up to volume with 100 ml 7.4 phosphate buffer (i.e. primary stock solution). Take 10 ml of primary stock solution were dissolved in 100 ml of buffer solution (i.e. secondary stock solution). From 2nd stock solution we take 2, 4, 6,8,10 µg/ml and make up to volume upto10 ml and measure the absorbance in U.V. at 241 nm.

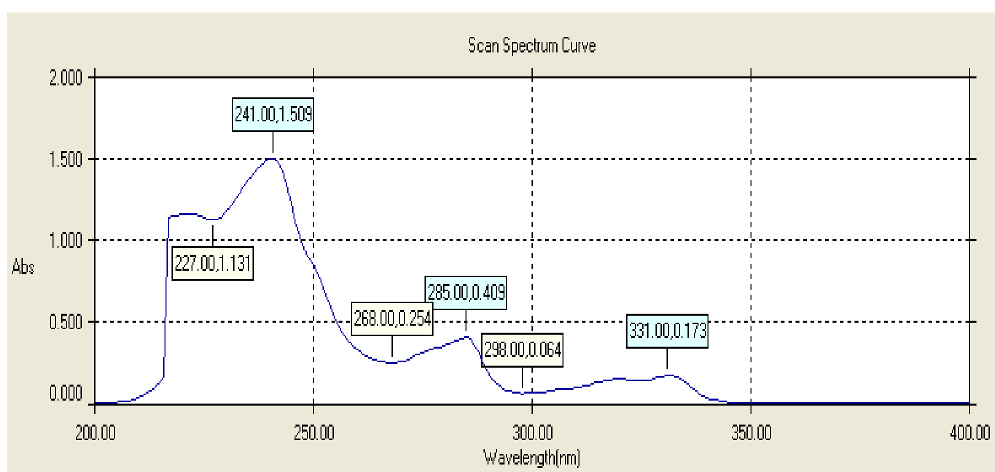

Fig. No. 2. Spectrum of carvedilol drug

Preparation of Proniosomal Gel

Proniosomal gel was prepared by coacervation phase separation method. Weighed amount of surfactants, lipid, and drug are added in a clean and dry wide mouthed glass vials and alcohol is added, after warming mix all the ingredients with a glass rod. The open end of the glass bottle is covered with a lid to prevent the loss of solvents from it and warmed over water bath at 60-70^{0}C for about 5 min until surfactants mixture is dissolved completely and then 0.1% glycerol solution is added and warmed on a water bath till a clear solution was formed and that clear solution is converted to proniosomal gel after cooling. The obtained gel was preserved in the same glass bottle in dark condition for characterization [6].

Table No. 1. Composition of Carvedilol Proniosomal Gel

Formulation code	Drug (mg)	Surfactants	Quantity (mg)	Cholesterol (mg)	Lecithin (mg)
F1	12.5	Span 20	1000	100	100
F2	12.5	Span 60	1000	100	100
F3	12.5	Span 80	1000	100	100
F4	12.5	Tween 20	1000	100	100
F5	12.5	Tween 80	1000	100	100
F6	12.5	T20:T60	500:500	100	100
F7	12.5	S20:S60	500:500	100	100
F8	12.5	S60:S80	500:500	100	100
F9	12.5	S80:S20	500:500	100	100

3. Evaluation Parameters of Proniosomal Gel

pH and Viscosity

Accurately weighed gel was taken and then diluted with the pH 7.4 phosphate buffer and checked the pH by using pH meter and Brook field viscometer is used to determine the viscosity of the gel [7].

Vesicle size analysis

The vesicle size was determined by adding proniosomal gel (100 mg) was hydrated with saline solution (0.9% NaCl) and placed in a small glass vial with occasional shaking for 10 mins, The dispersion was observed under optical microscope 100X magnification. The number of vesicles was measured by using stage micrometer.

Rate of Spontaneity

After hydration of proniosomes for 15-20 min, the niosomes are formed. Approximately 10 or 20 mg of proniosomal gel was transferred in to a glass bottle and spread uniformly around walls, then add 2 ml of saline was added along the walls of the glass bottle and left aside for 20 min. Place a drop of hydrated proniosomal gel in a Neubauers chamber and count the number of vesicles and determine the number of niosomes eluted from proniosomes was counted.

HUMAN

Encapsulation efficiency

Accurately weighed 100 mg of proniosomal gel was dispersed in isopropranol, warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 25000 rpm for 40 minutes 5^0C. To determine the clear fraction of free drug was checked in U.V spectrometer at 241 nm. The percentage of encapsulation efficiency was calculated from the equation.

$$\% \text{ encapsulation efficiency} = \frac{\text{Total drug - unencapsulated drug*100}}{\text{Total drug concentration}}$$

Scanning electron microscopy (SEM)

Scanning Electron Microscopy (SEM), the niosomes are mounted on a aluminium stub using double sided adhesive carbon tape. The vesicles are sputter coated with a gold palladium

(Au/Pd) by using a vacuum evaporator and examined in a Scanning electron microscopy equipped with a digital camera at accelerating voltage.

Zeta potential analysis

Zeta potential analysis was determining for the colloidal properties for a prepared formulations. The proniosomes derived from noisome dispersion will be determined using zeta potential analyser based on the Electrophoretic light scattering and laser Doppler Velocimetery method. The temperature was set at 25°C and measures the charge on vesicles and means zeta potential values.

***In-vitro* permeation studies**

In-vitro permeation studies of proniosomal gel can be done by using modified diffusion cell. The proniosomal formulation was adhering to furry side of the skin. This formulation was applied on the skin mounted and clamped between the donor and receptor compartment with furry side facing upward (donor side). The receptor compartment was surrounded by a water jacket for maintaining the temperature at 37^0C. The temperature was maintained using a thermostatic hot plate temperature control available on the magnetic stirrer. The receptor fluid was stirred by magnetic bead operated on a magnetic stirrer. The top of the donor compartment was open for air circulation. At each sampling interval of 1 hour samples were withdrawn from sampling port and were replaced with same volume of the fresh receptor fluid every time. Samples withdrawn were analysed by U.V. spectrophometrically at 241 nm [8].

Stability studies

The stability studies are conducted according to the ICH guidelines. The stability of the proniosomal gel was keeping at three different temperature conditions like refrigeration temperature (4-8^0C), room temperature (25±2^0C) in aluminium foil sealed glass vials. The samples were withdrawn at different intervals of time over a period of one month and they were observed visually and under optical microscope for the change in consistency and appearance of drug crystals upon storage point and drug leakage from the formulations by analysing drug content.

Drug release kinetics

Depending upon R^2 and slope values obtained from different models, the best-fit model was selected.

Zero Order Release

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented as:

$$Q = Q_0 + K_0t$$

Where Q is the amount of drug released or dissolved (assuming that release occurs rapidly after the drug dissolves), Q_0 is the initial amount of drug in solution (it is usually zero), and K_0 is the zero order release constant. The plot made: cumulative % drug release Vs time (zero order kinetic model). Zero order drug release mechanism is mainly applicable to dosage forms like transdermal systems, coated forms, osmotic systems as well as matrix tablets with low soluble drugs.

First Order Release

To study the first order release rate kinetics the release rate data were fitted into the following equation,

$$LogC = LogC_o - kt / 2.303$$

Where C is the amount of drug released at time t, C_o is the initial amount of drug in the solution and k is the first order release constant. This model is applicable to study of hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water-soluble drugs in porous matrices.

Higuchi Model

This model is based on the hypotheses that (i) initial drug concentration in the matrix is much higher than drug solubility; (ii) drug diffusion takes place only in one dimension (edge effect must be negligible); (iii) drug particles are much smaller than system thickness; (iv) matrix swelling and dissolution are negligible; (v) drug diffusivity is constant and (vi) perfect sink conditions are always attained in the release environment, Higuchi described the release of

drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion

$$Q = K_t^{1/2}$$

Where, K_t is the constant reflecting the design variables of the system.

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

Korsmeyer-Peppas Model

It derived a simple relationship which described drug release from a polymeric system. To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer-Peppas model [9].

$$M_t/M_\infty = Kt^n$$

Where M_t / M_∞ is fraction of drug released at time t, K is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms as given in following table for cylindrical shaped matrices.

Table No. 2. Diffusion exponent and release mechanism

Diffusion exponent (n)	Diffusion mechanism
< 0.5	Fickian diffusion
0.5-1	Non- Fickian transport
1	Case-II transport
> 1	Super Case-II transport

Fickian (case I) behavior indicates that, drug partially diffuses through the swollen polymer matrix and partly through the water filled pores and channels in the matrix channel. While non fickian (anomalous) indicates that, drug partially diffuses through the swollen polymer matrix and also partly through the gradually expanding hydrated and eroding matrix with increasing diffusional path. Case-II transport and Super case-II transport indicates when drug diffusion is rapid compared to the constant rate of solvent–induced relaxation and swelling in the polymer [10].

4. RESULTS AND DISCUSSION

Preformulation studies showed the absorption maxima for carvedilol at 241 nm and the developed Spectrophotometric method obeyed beer's law with linearity range of 20 -100 µg/ml (Figure 2). Drug along with the ingredients showed no change in any characteristic peak in preliminary compatibility studies revealed that there no interaction between carvedilol and excipients which was as evident from FTIR spectral studies.

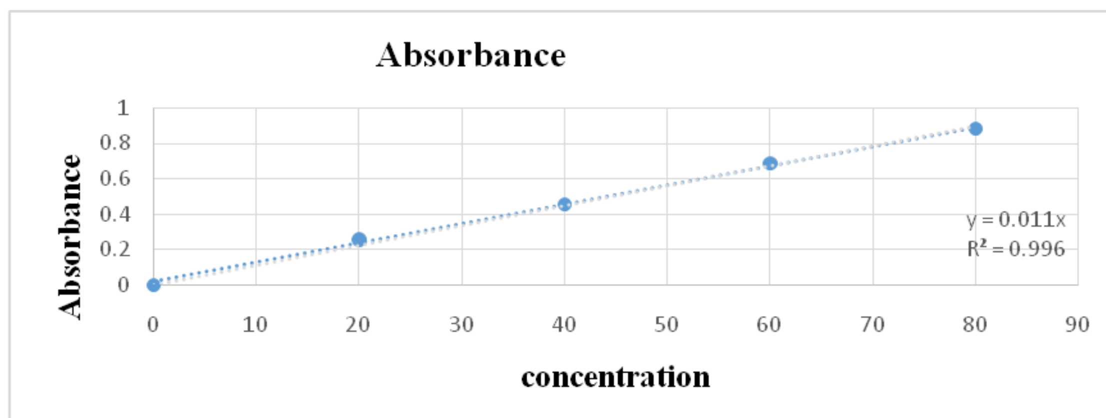

Fig. No. 3. Calibration curve of carvedilol

Proniosomal gels were prepared by coacervation phase separation method. The formulations were studied for physical characteristics like determination of pH, Viscosity and Encapsulation efficiency is found to be within the acceptable limits shown in Table 4.

Table No. 3. Physicochemical characterization studies of carvedilol proniosomal gels

S.no	Formulation code	pH	Vesicle size	Encapsulation efficiency
1	F1	7.31	12.6	92.5
2	F2	7.12	3.7	95.3
3	F3	7.35	15.4	90.9
4	F4	7.55	16.6	85.7
5	F5	7.63	18.8	83.3
6	F6	7.51	14.5	87.7
7	F7	7.22	5.0	91.7
8	F8	7.41	5.0	90.5
9	F9	7.56	11.2	89.6

Table No. 4. Viscosity Results

Formulation code	Viscosity(c^P)
F1	7245
F2	9317
F3	7647
F4	623.1
F5	609

The physical characteristics of proniosomal gel formulation were showed good spreadability, and viscosity. Determination of vesicle size was found to be 3.7-19 µm. while increasing in Encapsulation efficiency the vesicle size was decreases, Vesicle Size was reduced during the dispersion was agitated. For spontaneity studies, the gel formulation was treated with ethanol, propanol, butanol and isopropanol. Niosomes are more found in isopropanol than the niosomes containing propranol and ethanol faster phase separation, isopropanol and butanol due to their lower solubility in water.

In-vitro permeation studies of Proniosomal Gel showed the cumulative percentage permeation 93.36 %, 99.4 %, 91.5 %, 85.8 %, 82.9 %, 83.6 %, 93.8%, 94.8%, 93% in 12 hrs from the formulations F1, F2, F3, F4, F5, F6, F7, F8, F9 respectively as showed in Table 6. The stability studies showed that proniosomal gels were stable at 4 to 80^0C and 25 ± 20^0C and there are no leakage of drug from proniosomes and were found stable at these temperatures. All formulations from F1 to F9 showed zero order drug permeation kinetics by drug release mechanism though diffusion of drug from proniosomal gels. Graphical representation was given in Figure 5. The above results indicate that the proniosomal gels could be formulated for controlled release.

Vesicle Size Determination

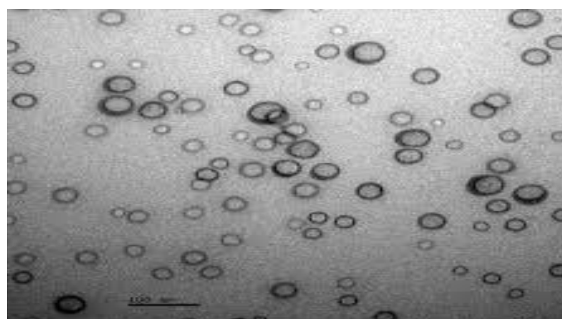

Fig. No. 4. Represents the best formulation (F2) in carvedilol proniosomal gel under optical microscope with uniform size

SEM Analysis

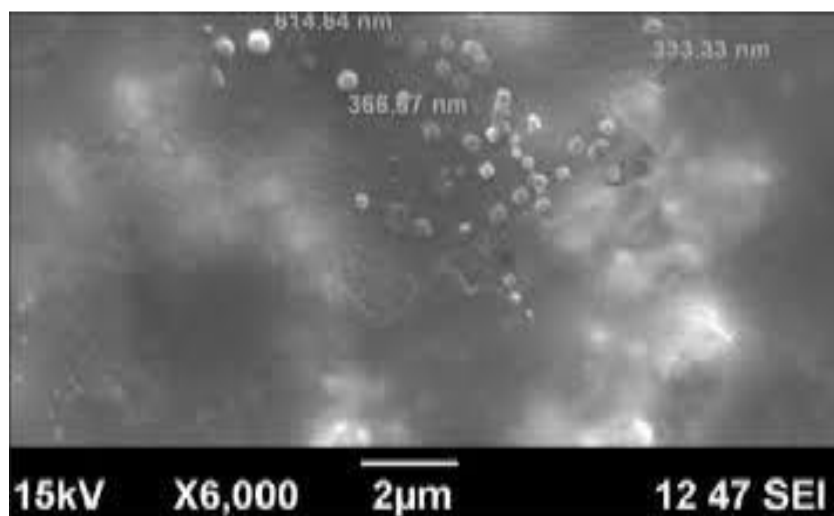

Fig. No. 5. Scanning Electron microscopy for F2 Formulation

Table No. 5. *In-vitro* diffusion studies and drug release studies

Formulation code	% Drug permeated	Regression Zero order	First order	Higuchi	Korsemeyer Slope	Regression
F1	93.6 %	0.961	0.174	0.774	0.353	0.951
F2	99.4%	0.979	0.134	0.777	0.334	0.961
F3	91.5%	0.905	0.179	0.664	0.992	0.891
F4	85.8%	0.983	0.179	0.804	0.981	0.945
F5	82.9%	0.979	0.182	0.835	0.654	0.934
F6	83.6%	0.980	0.181	0.821	0.633	0.913
F7	95.8%	0.986	0.167	0.807	0.781	0.958
F8	94.8%	0.989	0.167	0.812	0.941	0.940
F9	93.0%	0.973	0.167	0.787	0.694	0.880

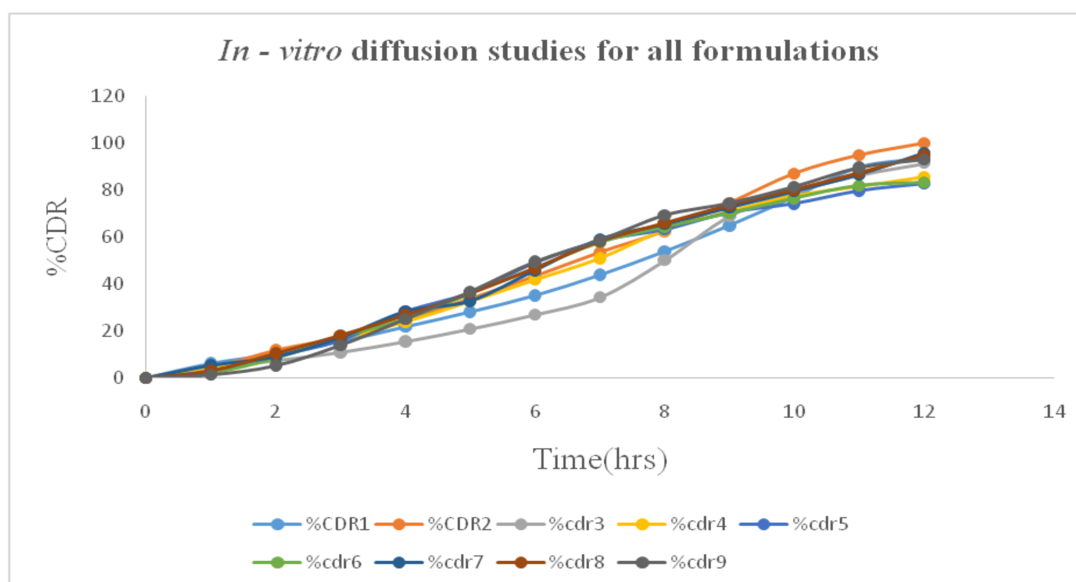

Fig. No. 6. *In vitro* drug diffusion of carvedilol from various proniosomal gel formulations

5. CONCLUSION

The present study developed proniosomal gel for transdermal delivery of carvedilol by using different types of non – ionic surfactants. Proniosomal gel formulations were evaluated for the vesicle size, shape, entrapment efficiency, *in-vitro* permeation studies and *in- vitro* drug release mechanism and the results are found in acceptable range. Vesicle size was decreased with increasing in the entrapment efficiency. Among all the formulation F2 (Span60) was selected as an optimised formulation, due to low vesicle size, high entrapment efficiency, and released the drug in a controlled manner for extended period of time. Overall, these findings indicate that proniosomal gel will be promising drug delivery system for carvedilol**.**

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