Research Article

Transdermal Delivery of Prepared Inclusion Complexes of Carvedilol with Cyclodextrins

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ABSTRACT

Cyclodextrins are cyclic (α-1, 4)-linked oligosaccharides β-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface, which have been extensively used to increase aqueous solubility of carvedilol, (CVD). In present study 1:1 M and 1:2 M solid inclusion complexes of drug with β-cyclodextrin (β-CD) and 2-hydroxy-propyl-β-cyclodextrin (HP-β-CVD) have been prepared by Lyophilization method. The liquid complexes were used to prepare the aqueous gels according to 32 factorial design, using Carbopol-940, propylene glycol and polyethyleneglycol-400. These formulations were evaluated for drug content, pH, stability, partition coefficient between stratum corneum and buffer (pH 6.4) and release rate through rat skin and cellophane membrane. Thus, the cyclodextrins provided the increment in aqueous solubility stability and rapid release of drug molecule. Anti hypertensive activity of prepared formulations was conducted for the inhibition of the isoprenaline induced tachycardia in conscious rabbits.

Keywords: Cyclodextrins, Solubilization, Carbopol, Gels, Carvedilol, Transdermal.

INTRODUCTION

Carvedilol (CVD), an antihypertensive drug which has selected as model drug is a β -adrenergic receptor antagonist with α_2-adrenergic receptor antagonist activity that has been approved for the treatment of essential hypertension and symptomatic heart failure. The ratio of α_2 to β -adrenergic receptor antagonist potency for CVD is 1:10.[1] Limited efficacy of this agent, coupled with the strong predisposition to cause postural hypotension dizziness, gastrointestinal (GIT) disturbances, dry eyes, vivid dreams and nephrotoxicity. Optimization of the systemic profile of CVD via transdermal delivery has been shown to reduce GIT and cardiovascular system (CVS) related side effects.[2, 3] Carvedilol increases cutaneous blood flow by over 60%, and this effect results from a dramatic reduction in cutaneous vascular resistance[4] and comparative studies to evaluate the suitability of Carvedilol for transdermal delivery based on their biopharmaceutical characteristics have shown the need for a permeation enhancer. They act by modulating the barrier properties of skin. In this regard carrier based system using cyclodextrins are used, which facilitate transdermal delivery by extracting the lipid of the stratum corneum.[5] Cyclodextrins are cyclic oligosaccharides which have recently been recognized as useful pharmaceutical excipients.[6] These are cyclic (α-1,4)-linked oligosaccharides containing a relatively hydrophobic central cavity and hydrophilic outer surface, which have been extensively used to increase aqueous solubility of poorly soluble drugs or insoluble drugs.[7] β-cyclodextrin (β-CD) and hydroxy-propyl-β-cyclodextrin (HP-β-CD) were selected for present work due to numerous advantages like : they have a well defined chemical structure, which provides a number of potential sites for chemical modification or conjugation, availability of different cavity sizes, low toxicity and low pharmacological activity, good aqueous solubility and protect the included conjugated drugs from biodegradation.[8] These have no erethema, edema, non-irritant and non mutagenic to skin.[9] Therefore it was proposed to develop inclusion complexes of drug using these Cyclodextrins by lyophilization method for increasing the solubilization of its water insoluble property. The present study evaluates the preparation of cyclodextrin based solid complexes, their evaluation and development of transdermal gel using carbopol-940. This evaluated for drug permeation and diffusion studies through hairless rat skin and cellophane membrane (in vitro drug release profile) and pharmacological evaluation of prepared gel.

MATERIALS AND METHODS

Materials

The sample of Carvedilol used in this study was generously provided by Cipla Limited Mumbai, as a gift sample. β-CD and HP-β-CD were used provided by Himedia, Mumbai. All chemicals and solvents used in this study were of analytical reagent grade. Freshly distilled water was used throughout the work.

Estimation of Carvedilol

For estimation of Carvedilol ultraviolet (UV) spectrophotoscopic method was used, which was based on measurement of absorbance at 242.0nm in methanol and at 245.0nm at in polyethyleneglycol-400 (PEG-400): water (2:1) medium. The concentration was obtained from corresponding absorbance using calibration curve prepared in the concentration range 2-20 µg/ml in methanol and 1-10µg/ml in PEG-400: water (2:1). The

absorbance can be determined by using equation developed by these methods as y = 0.0519 x + 0.014 for methanol and y = 0.1044x + 0.0172 for PEG 400: water medium.[10]

Preparation of Inclusion Complexes

By phase solubility study it was found that the drug can complex with cyclodextrins (CDs) in the molar ratio of 1:1 and 1:2 for maximum solubilization in water. Thus solid inclusion complexes of carvedilol and cyclodextrins (β-CD and HP-β-CD) were prepared in 1:1 and 1:2 molar ratios by lyophilization method.[11]

These were stored in amber colored vials for further studies. The prepared complexes were characterized and evaluated by FTIR, DSC, UV absorption and Electron microscopy.[12] For analysis of prepared complexes and aqueous solubility determination of complexes the most commonly used method is to extract the guest from the complex. Amount equivalent to 10 mg of drug-β-CD and HP-β-CD-drug complex (1:1 and 1:2 M) were added to a series of screw-capped test tubes. 10 ml water was added in each tube. The tubes were shaken and placed in a water bath at 60^0C for about one hour. The tubes were shaken periodically during the incubation period. The mixture was cooled and dichloromethane (DCM) was added. Cyclodextrins were soluble in water whereas drug was soluble in DCM. DCM was evaporated under vacuum and volume made up with methanol for analysis of drug by spectroscopic method. On the basis of this analysis the aqueous solubility of the prepared complexes were determined.

Preparations and Characterization of Aqueous Carvedilol Gel

Carvedilol aqueous gel was prepared using the liquid complexes of β-CD/HP-β-CD (1:2 molar ratio) with carbopol-940, propylene-glycol (PG), polyethyleneglycol-400 (PEG-400) and water. The formulae for preparing gel using these ingredients were optimized according to 3^2 factorial designs. In this design three levels of variables PEG-400 (20, 30, 40% w/w) and PG (15, 20, 25% w/w) were taken and the concentrations of carbopol-940 (1.5% w/w) and liquid complex (1.0% w/w) were kept constant and the release rate of drug through cellophane membrane of each of nine batches was analyzed and the batch having maximum release was used for the preparation of aqueous gels.[13, 14]

Characterization of Carvedilol Gel

Determination of pH

For pH determination 500 mg gel was dissolved in distilled water and volume made up to 10 ml then pH was determined by dipping calibration pH electrode of pH-meter inside the mixture. For release rate studies phosphate buffer pH: 6.4 were prepared by I.P. (1996) method.

Assay and content uniformity

For assay and content uniformity drug concentration in aqueous gel was measured in a spectrophotometer. Known amount of aqueous gel (500 mg) was dissolved in phosphate buffer (pH 6.4), suitably diluted with PEG-400: Water (2:1) medium and the absorbance was measured at 245.0 nm using PEG-400 water mixture as blank. The drug content was then computed from calibration curve. Random sampling of gel at different points from the bulk was carried out for the content uniformity in gel by the above procedure.[15]

Determination of partition coefficient between stratum corneum and buffer (pH 6.4)

For determination of partition coefficient between stratum corneum (SC) and buffer (pH 6.4) partitioning of Carvedilol from aqueous gel was carried out by mounting known amount of the sample (1.0 g) on the hairless rat skin and allowed to equilibrate for 5 hours. Receiver compartment was 20 ml buffer (pH 6.4). After 5 hours gel was scrapped off and drug content was analyzed. Amount thus partitioned into skin was calculated.[16]

Stability Studies

For stability studies the β-CD and HP-β-CD gels were selected for in vitro stability studies. The formulations were stored in amber colored glass vials at 4±1°C, room temperature (25°C) and 50°C for 60 days. After 10, 30 days and 60 days they were evaluated for the following parameters:

(1) Residual drug content - Drug content of stored cyclodextrin formulations was determined after 10, 30, 60 days and percent residual drug content was calculated by spectrophotometry. Known amount of aqueous gel (500 mg) was dissolved in phosphate buffer pH 6.4, suitably diluted with PEG-400: water (2:1) mixture and absorbance was measured using Shimadzu UV-1601 spectrophotometer at λ_{max} 245.0 nm. Initial drug content was taken as 100% for each of the formulations.

(2) pH of the formulations - pH of the cyclodextrin formulations (aqueous gel) were checked using calibrated pH-meter. Formulations were placed in a beaker and electrode was dipped in it and measurement of pH was carried out and recorded.

Diffusion studies across cellophane membrane

The Diffusion Studies using cellophane membrane ware carried out. Treated cellophane membrane was tied on the mouth of dialysis tube (surface area 4.52 cm^2) and diffusion conditions were maintained. 50 ml of phosphate buffer (pH 6.4) was used as receptor fluid at 37°C at 100 rpm on magnetic stirrer. One gram of gel sample was placed on cellophane membrane and this assembly was touched onto dissolution medium surface. Samples were withdrawn (2 ml) at regular intervals and replaced with same amount of buffer (pH 6.4). The samples were suitably diluted with PEG-400: water mixture and absorbance was noted at 245.0 nm using PEG-400: water

mixture as blank. The percentage drug released was determined.

Diffusion Studies through hairless rat skin

Male wistar rat skin (back portion of ear) was used in present study. Dorsal fur was removed with mechanical hair clipper and depilatory cream was applied to remove small hair. The skin was immersed in distilled water maintained at 60^0C for 2 min. Fatty layers were removed and washed with saline and distilled water for use.[17] The permeation studies were carried out by using hairless rat skin. Prepared skin was tied on the mouth of dialysis tube (surface area 4.52 cm^2). 50 ml phosphate buffer (pH 6.4) was used as receptor medium in a beaker on magnetic stirrer at 100 rpm at 37^0C. One gram of sample was placed on the rat skin and assembly was touched on the surface of dissolution medium. Samples (2 ml) were withdrawn at regular intervals and replaced with same amount of buffer. These samples were diluted suitably with PEG-400: water medium. Drug content was measured at 245.0 nm using UV spectrophotometer.[18] The steady state flux of carvedilol through rat skin was determined from the slope of the linear portion of the amount permeated per cm^2 (Q) versus time graph.

Pharmacological Studies of Gel

Pharmacological profile of carvedilol was determined on rabbits after taking permission by relevant ethical committee for conducting this study. For this study twelve rabbits were divided into four groups having three rabbits per group. One group of rabbits was kept as control, second group for delivery of plain drug, third and fourth group for application of gel formulation. β-blocking activity was quantified by the inhibition of the Isoprenaline-induced tachycardia in conscious rabbits. Plain drug was administered orally to the second group and the fall in heart rate was observed and recorded by experts. After that isoprenaline injection diluted with 0.9% NaCl (0.001mg/kg) given I.P. after every two hours up to 5 hrs. Different gel formulations were applied on back portion of ear (tample) to the third and fourth groups. After every 2 hr intervals isoprenaline injection was given I.P. up to 9 hrs. After half-an-hour intervals fall in heart rate were observed and heart rate was recorded up to 10 hrs.[19]

RESULTS AND DISCUSSION

From Phase solubility study the optimized molar ratio for complexation were 1:1 and 1:2 M for further studies (Figure 1). The solid inclusion complexes were prepared by lyophilization method. Pure sample and their physical mixtures were characterized for identification, confirmation and purity determination using FTIR, DSC, UV and SEM analysis methods. Thus all of above studies showed that complexes were formed in both 1:1 and 1:2 Molar ratios of β-CD/HP-β-CD carvedilol systems. [12]

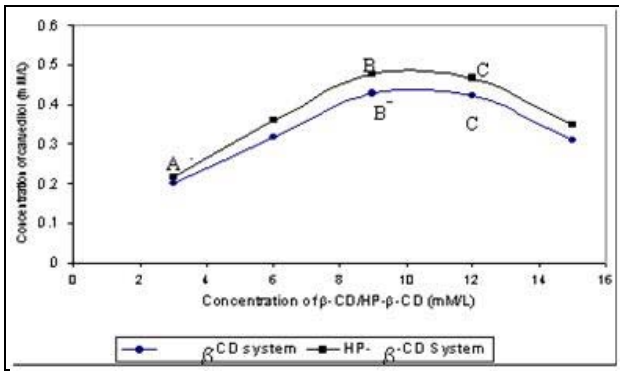

Figure 1: Phase solubility diagram of β-CD and HP-β-CD-drug complex system

Prepared solid inclusion complexes were analyzed for drug content using extraction method and concentration of drug computed from its calibration curve. It was found that in β-CD-CVD-complex and HP-β-CD-CVD complex 1:2 M has greater drug content than 1:1 M and the highest drug content was found in HP-β-CD-CVD complex 1:2 M. It was found that the aqueous solubility's were computed using calibration curve. Data suggested that the aqueous solubility of inclusion complexes were found to be 15.0 and 30.83 times increase in 1:1 M of β-CD and HP-β-CD complex respectively. 17.5 and 34.16 times increase in solubility was found for 1:2 M complexes of drug with β-CD and HP-β-CD respectively. It is concluded that the aqueous solubility of optimized inclusion complexes carvedilol β-CD/HP-β-CD was more than the pure carvedilol. The 1:2 Molar complexes of β-CD/HP-β-CD had greater solubility than 1:1 Molar complexes of β-CD/HP-β-CD.

On the basis of the solubility determination further gels were prepared using the liquid complexes of β-CD/HP-β-CD of 1:2 molar ratios. The formula for development of gel was optimized according to 3^2 factorial designs (Table 1) and results revealed that, there is maximum drug release in batch No.5 in both cases of β-CD and HP-β-CD system after two hours. The optimized formula thus comprises of - Polyethylene glycol (PEG-400) (30% w/w); Propylene glycol (PG) (20% w/w); β-CD/HP-β-CD liquid complex (1.0% w/w); Carbopol-940 (1.5% w/w); Distilled water q. s. to (100.0% w/w). PEG-400, PG, β-CD/HP-β-CD liquid complex and water were mixed in required amount and they were mixed properly. Weighed amount of Carbopol-940 was added and stirred with a glass rod and this was allowed to left for 24 hrs for proper swelling of polymer. These formulations were stored in amber colored wide mouth glass containers for further studies. A second order polynomial equation was derived from the results of all nine batches. $y = bo + b_1 + x_1 + b_2x_2 + b_3x_1^2 + b_4x_2^2 + b_5 x_1 x_2$. In the equation y is the response (drug release at 2 hr). The main effect (x_1, x_2) represents the average results of changing one factor at a time from its low to high value. The interaction (x_1x_2) shows the response changes with combined effect of factors. The coefficients corresponding to linear effects (b_1, b_2) interaction (b_5) and quadrate effect (b_3, b_4) were

determined from the results of the experiments (Table 2). The fitted equation relating to percent drug released in 2 hrs. (y) to transformed factor as follows: for β-CD-gel the equation is : $Y(\beta) = [5.2 - 6.98 \times 10^{-2} x_1 - 1.43 x_2 \times 10^{-1} + 1.62x_1^2 + 3.78 \times 10^{-2} x_2^2 - 1.04 x_1x_2] \times 10^{-2}$ and for HP-β-CD-gel the equation is : $Y(HP) = [5.5 - 1.28 x_1 - 0.511 x_2 + 0.129 x_1^2 + 0.806 x_2^2 - 0.250 x_1x_2] \times 10^{-2}$

The samples prepared were evaluated for percent drug content and uniformity of drug content as per the pharmacopoeial specification for topical preparation. The drug content in the gel was found to be 96 to 98% w/w. The drug was found uniformly distributed in gels (95 to 98% w/w).

Table 1: Detail layout of 3^2 factorial designs to study the effect of variables on transdermal permeation using cellophane membrane

Batch No.	Values for variables		% drug released within 2 hrs. with	
	Propylene glycol (% w/w) (x_1)	PEG-400 (% w/w) (x_2)	β-CD gel	HP-β-CD gel
1	15 (-1)	20 (-1)	51.5	50.5
2	15 (-1)	30 (0)	39.5	48.5
3	15 (-1)	40 (1)	47.0	51.0
4	20 (0)	20 (-1)	59.25	54.5
5	20 (0)	30 (0)	59.75	63.0
6	20 (0)	40 (1)	54.50	52.5
7	25 (1)	20 (-1)	46.75	58.4
8	25 (1)	30 (0)	52.5	54.75
9	25 (1)	40 (1)	44.75	50.5

Table 2: Analyzed values for different variables

	Values		P value		Std. Error	
	β-CD gel	HP-β-CD gel	β-CD gel	HP-β-CD gel	β-CD gel	HP-β-CD gel
b_0	0.05275	0.05483	0.00124	0.000821	0.00439	0.00397
b_1	-0.000698	-0.01267	0.791	0.01007	0.00241	0.00217
b_2	-0.00143	-0.00511	0.593	0.100	0.00241	0.00217
b_3	0.01620	0.00129	0.03021	0.755	0.00417	0.00377
b_4	0.000378	0.00806	0.934	0.122	0.00417	0.00377
b_5	-0.01041	-0.00250	0.03862	0.417	0.00295	0.00266

Table 3: Effect of Storage on the residual drug content (n=3)

Parameters	Formulation Code	4±1°C storing after days			room temperature (25°C) storing after days			50°C storing after days		
		10	30	60	10	30	60	10	30	60
% residual drug content	β-CD gel	92.8±.08	90.2±.58	87.8+.42	97.2±.08	96.0±.14	95.0±.03	96.3±.09	95.1±.11	94.8±.04
	HP-β-CD gel	94.9±.63	91.7±.92	89.1+.04	97.8±.03	97.1±.06	96.2±.12	97.2±.23	96.4±.08	95.2±.07
pH	β-CD gel	6.4	6.2	6.0	6.4	6.4	6.4	6.4	6.3	6.2
	HP-β-CD gel	6.4	6.3	6.1	6.4	6.4	6.4	6.4	6.4	6.3

The stratum corneum (SC), is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an inter-cellular lipid-rich matrix, which acts as permeation barrier for the transdermal delivery of most drugs.[20] Because the SC is the first and main barrier for drug permeation through skin, it is interesting to verify the partitioning of the drug. Carvedilol alone has partition 10.5 ± 0.81l; β-CD-CVD-gel (1:2 M) has 1.26 ± 0.03 and HP-βCD-CVD Gel has 1.06 ± 0.04. Data suggested that the $K_{Sc/buffer}$ had decreased by complexation suggesting that complexation might be decreasing the affinity of carvedilol for the SC, probably due to enhancement of the aqueous solubility of drug. This decrease in $K_{Sc/buffer}$ is due to decreased partition coefficient was predicted with increasing cyclodextrins concentration.[21]

The storage stability testing was carried out for the cyclodextrin formulations by the measurement of residual drug content after storing at 4±1°C, room temperature (25°C)and 50°C after 10, 30 and 60 days. The percent residual drug content of formulations were found to be

87.8%, 89.1% at 4±1°C; 95.0%, 96.2% at room temperature and 94.8%, 95.2% at 50^{0}C after 60 days for β-CD-gel and HP-β-CD gel formulation, respectively (Table 3). It can be concluded that the residual drug content of formulations stored at room temperature were found higher in comparison to formulations stored at 4±1°C and 50^{0}C and the HP-β-CD gel formulation was more stable than β-CD-gel formulation.[22]

pH of formulations were determined with pH-meter after 10, 30 and 60 days. After 60 days pH of formulations of β-CD and HP-β-CD was found to be at 6.0 and 6.1 for storage at 4±1^{0}C; 6.4 at Room temperature; 6.2 and 6.3 at 50^{0}C respectively (Table 3). The pH of formulations stored at 4±1^{0}C and 50^{0}C were lower in comparison to formulation stored at room temperature due to presence of aqueous base in the formulations. The pH of HP-β-CD gel formulations was found to be more stable than β-CD gel formulations as per the skin pH for topical preparations. This indicated that the ideal storage condition for the formulations is room temperature where the potency and therapeutic efficacy of formulations will remain constant and pH will remain around 6.4.

In vitro release rate studies were performed for prepared gel across cellophane membrane and rat skin membrane. The percentage cumulative drug released was determined for pure drug and β-CD/HP-β-CD drug complexed gel system within two hours. The purpose of this study was to determine the amount of carvedilol, which penetrates into the different layers of the skin, from aqueous gel containing an inclusion complex.[22] Figure 2 shows the *in vitro* diffusion study through cellophane membrane. The percent cumulative released of carvedilol alone was 44.0 % after twenty minutes. However, when complexed with β-CD and HP-β-CD then 86.5% and 92.5 % were found respectively. Figure 3 shows the *in vitro* permeation profile of carvedilol and carvedilol-CDs-complexes through hairless rat skin. A linear relationship was obtained when percentage cumulative release was plotted against time up to twenty minutes indicating that the hairless rat skin is permeable to carvedilol and that the percutaneous transport can be described by firstly first order kinetics than zero order kinetics. It is seen that complexation with carvedilol/CDs increased the amount of carvedilol transferred across the hairless rat skin. The % cumulative release of carvedilol alone was 37.5% after 20 minutes through rat skin. However, when complexed with β-CD and HP-β-CD then 81.0% and 91.5% were transferred, respectively.

The amount of drug permeated per cm^2 (Q) against time revealed the flux. The slope of the linear portion of graph revealed flux up to 30 minutes. The flux increased when carvedilol was in a complexed form. The percutaneous absorption of carvedilol was improved when it was complexed with CDs. The permeation study through hairless rat skin gave the flux. Flux is the amount of material flowing through a unit cross section of a barrier in unit time (Figure 4). Results concluded that the complexation with β-CD/HP-β-CD of carvedilol increased the amount of carvedilol permeated across the hairless mouse skin. These observations were found to be similar to that reported in literature.

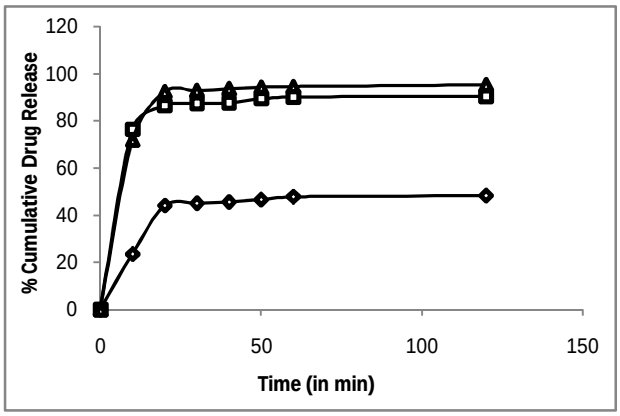

Figure 2: *In-vitro* percentage cumulative drug released through cellophane membrane

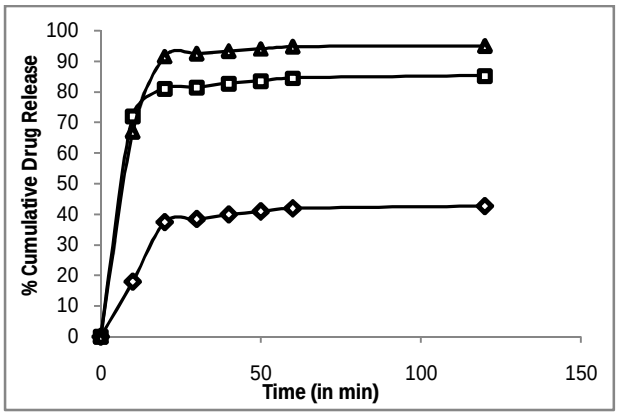

Figure 3: *In-vitro* percentage cumulative drug released through rat skin

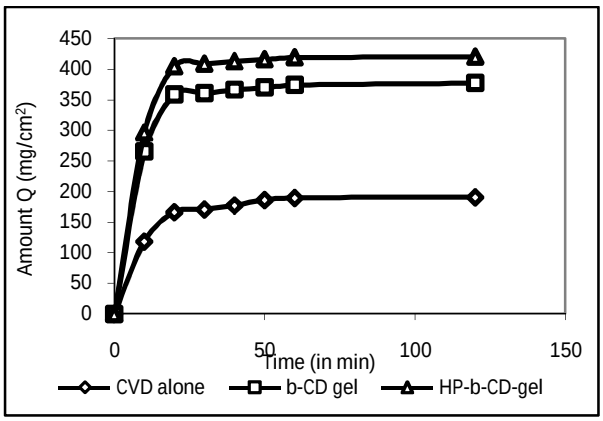

Figure 4: Amount Q (µg/cm^2) of the formulations across hairless rat skin

In vivo pharmacological response was measured in rabbits with plain drug and different gel formulations. After administering plain drug (orally) the effect of carvedilol has been observed. There was fall in heart rate within one hour. After one hour, isoprenaline injection was given to increase the heart rate inhibiting the effect of carvedilol. Isoprenaline injections were given on 3rd and 5th hours to produce the increase in heart rate. In case of gel formulations, β-CD gel had the constant effect on heart

rate. There was reduction in heart rate within one hour after gel application. The fall in heart rate was lower than HP-β-CD gel due to less penetration in dermal delivery. After application of HP-β-CD gel the effect of fall in heart rate was greater than β-CD-gel application. After one hour of delivery isoprenaline injection was given I.P. in every 2 hours up to 9 hours. The purpose of this delivery was to challenge the effect of carvedilol after completion of the effect of drug. Thus the fall in heart rate (pharmacological response) was better in HP-β-CD gel formulation than β-CD-gel (Figure 5). Therefore, such system can be suitably used for the transdermal delivery of such potent cardioactive drugs for effective and safer control of heart rate.

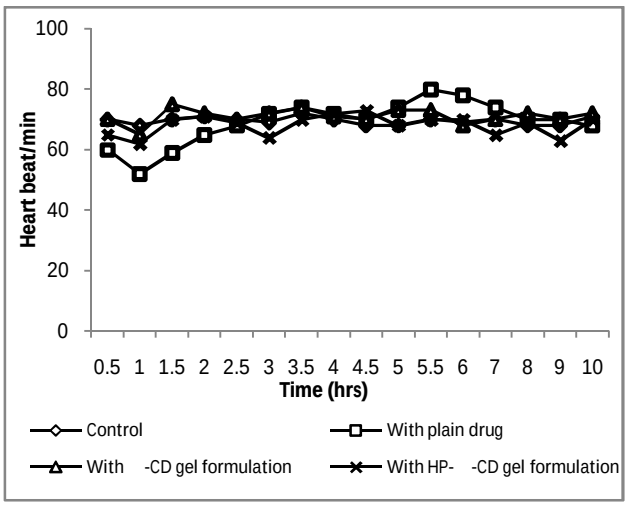

Figure 5: Effect of drug on heart rate

CONCLUSION

In conclusion, the results show that the prepared CVD-β-CD-Complex and HP-BCD-CVD-Complex was effectively incorporated under polymer. This system increases drug solubility, release rate and gives stability in transdermal drug delivery.

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