4.0 EXPERIMENTAL (CARVEDILOL)

4.1 MATERIALS:

Carvedilol was kindly supplied by Cipla Ltd, Mumbai.

Carbopol 934P, (Noveon, USP) was obtained from BF Goodrich (Cleveland OH) USA.

HPMC K4M was supplied by COLORCON as a gift sample.

Hydroxypropyl cellulose (Klucel HXF) was obtained from Hercules, Aqualon, USA. Ethyl cellulose (14 cps) purchased from S.D. Fine chemicals, Mumbai, India.

Chitosan were gifted by Primex, Iceland.

Tablettose 100 was gifted by Meggle, Germany.

Magnesium stearate, talc, 1-Octanol, potassium dihydrogen phosphate, potassium bromide, propylene glycol, dichloromethane, potassium dihydrogen phosphate, methanol, sodium phosphate, sodium chloride, potassium cyanide, potassium phosphate, potassium chloride, sodium carbonate, sodium lauryl sulphate, osmic acid and glutaraldehyde were procured from Qualigens, Mumbai.

Formalin, sodium citrate, sucrose, fructose, hematoxylin eosin were obtained from Loba chemie, Mumbai.

Diazepam and ketamine were obtained from Troika labs, Ahmedabad.

Buffers:

Phosphate buffer pH 6.8

Phosphate buffer solution was prepared by mixing 50 ml of 0.2M potassium dihydrogen orthophosphate with 46.80 ml of 0.2M sodium hydroxide and diluting to 200ml with distilled water (BP 2000).

Saccharose buffer

It was prepared by dissolving 10 gm sucrose in 100 ml distilled water and adjusting pH to 6.8 by 0.2M sodium hydroxide.

Artificial Saliva

Table 4.1 Composition of artificial saliva (Oliviera A.G., 2005)

Ingredients	Concentration / g L^{-1}
Na_2HPO_4	0.26
NaCl	6.70
KSCN	0.33
KH_2PO_4	0.20
KCl	1.20
$NaHCO_3$	1.50

All the above listed ingredients were accurately weighed and dissolved in 1.0 Lit. water and stirred well to form a clear solution.

4.2 Preformulation studies:

4.2.1 Determination of n-octanol: buffer partition coefficient:

n-octanol was used to represent the biomembrane. The partition coefficient between n-octanol and phosphate buffer (pH 6.8 ±0.2) at 37^{0}C was determined by the shake-flask method (Takahashi et al., 2001). n-octanol and buffer were cosaturated with each other for 24 h at 37^{0}C before use. Then n-octanol (5 mL) was shaken with 5 ml buffer containing Carvedilol (2 mg/mL). The mixture was then separated using separating funnel and the concentration of drug in aqueous phase was determined spectrophotometrically by measuring absorbance at 242 nm. The partition coefficient (Kp) was calculated from the following equation:

$$\mathbf{Kp = C_1\text{-}C_2/C_2}$$

Where C1 is the original concentration of drug in the aqueous phase and C2 is the final concentration of drug in the aqueous phase.

4.2.2 Compatibility studies using FT-IR spectroscopy:

Infrared spectra of Carvedilol, physical mixtures of the Carvedilol and polymers such as Carbopol 934P, HPMC K4M and Chitosan were obtained using FT-IR 8300 (Shimadzu). For analysis of Carvedilol alone, a pellet was prepared by mixing approx. 2 mg of drug and 200 mg of KBr and compressing the mixture under high pressure. The physical mixtures of polymer and drug were prepared by mixing approx. 2 mg of drug, 2 mg of polymer, 200 mg of KBr and compressing the mixtures under high pressure (Liu et al., 2004; Jug and Becirevic-Lacan, 2004).

4.2.3 Differential scanning calorimetry (DSC): (Govender et al., 2005)

DSC thermograms of Carvedilol alone and physical mixture of drug and polymers (1:1) (Carbopol 934P, HPMC K4M and Chitosan) were recorded. The thermograms were recorded with Differential Scanning Calorimeter (DSC) Shimadzu, connected to a thermal analysis operation system (Thermal Analyst 60, TA Instruments), Japan.

For the DSC studies, about 2 mg of sample was weighed into an aluminum pan and the pan was crimped. The sample was heated in the DSC from 30 to 300^{0}C, at a heating rate of 10^{0}C/min.

4.2.4 X-Ray Diffraction (XRD): (Sinha et al., 2004)

Powder X-ray diffraction pattern of Carvedilol (5mg) was evaluated to analyze the crystallinity of the molecule. XRD was carried out using a Philips PW 1730 X-ray generator,

Holland. The operation conditions were as follows: X-ray source; Ni-filtered; Cu Kά radiation; voltage 40 kV; current 20 mA; time constant 2 s; scanning rate 2^0 cm^{-1}.

4.2.5 *In vitro* permeation studies: (Jasti et al., 2000; Artusi et al., 2003)

The sheep buccal mucosa was collected from local slaughter house and washed repeatedly with phosphate buffer pH 6.8. The buccal mucosa, with a part of sub-mucosa, was carefully separated from fat and muscles using a scalpel.

The experiments were conducted using modified Franz-type diffusion cells at 37°C. Buccal mucosa was mounted in the cell (diffusional area 1.1 cm^2) with the mucosal side facing the donor compartment. Artificial saliva and phosphate buffer (pH 6.8 ± 0.2) was used as donor and receiver medium respectively. Saturated solution of Carvedilol in artificial saliva was used in donor compartment. 2 ml samples were collected from receiver compartment at 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hrs and fresh medium was added to the receiver chamber. Carvedilol was quantified by UV spectrophotometry (1601 Shimadzu). All the measurements were made in triplicate and expressed as mean ± RSD. The steady-state flux was calculated from the slope of the linear region of the cumulative amount of Carvedilol permeated versus time plot.

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4.3 CARVEDILOL CORE IN CUP TABLETS (CCT):

4.3.1 Formulation: (Desai and Pramodkumar, 2004; Liabot et al., 2002; Nafee et al., 2004)

4.3.1.1 Design and Fabrication of a Special Punch:

A new punch (10 mm, flat-faced) was fabricated by making a protrusion having 6 mm diameter in available 10-mm flat faced punch as shown in Figure 4.1. However, the lower punch (10 mm) remained flat faced.

4.3.1.2 Preparation of core in cup tablets:

Direct compression technique in two stages was employed for the formulation. The preparation process of core in cup tablets is shown by schematic representation (Fig 4.2). The method mainly involved 3 steps:

(1) Formation of core, (6 mm diameter).

(2) Formation of buccal adhesive cup (10 mm diameter and having 6 mm cavity), and

(3) Formation of core in cup tablet (Compressing core tablet in cup).

The composition of core and cup are presented in Table 4.2 and 4.3 respectively. The tablet punching machine (Jaguar, Mumbai), equipped with 6-mm flat-faced punches and dies, was used for preparing the core tablet. Then, buccal adhesive cups were prepared by using the same punching machine equipped with special upper punch and lower flat faced punch. Finally, buccal adhesive cups were placed in 10-mm die cavity and core tablet was inserted into the cup and then compressed using 10-mm flat-faced upper and lower punches to obtain core in cup tablets. Various formulations were prepared by varying the ratio of Carbopol 934P and HPMC K4M in core tablet to choose the effective formulation and the composition are shown in table 4.2.

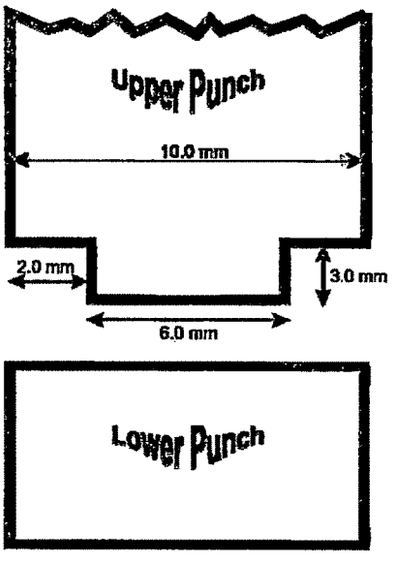

Fig 4.1 – Fabricated 10 mm flat faced punch by making a 2-mm protrusion on the upper punch.

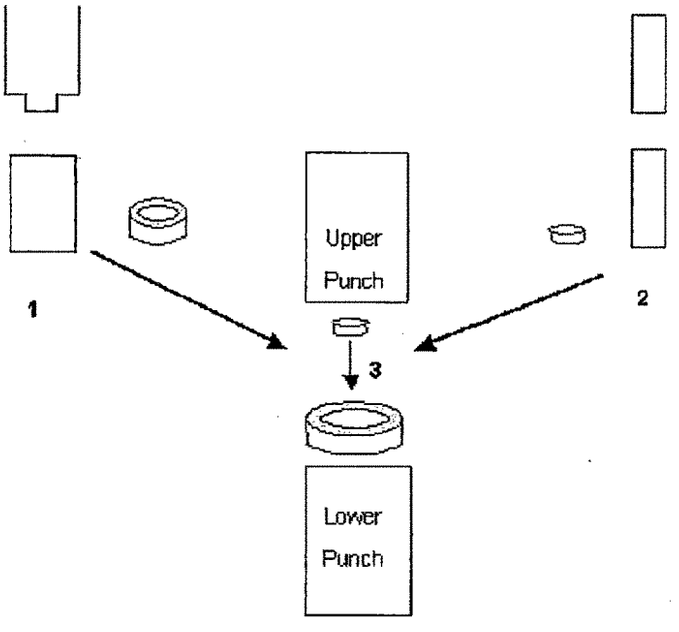

Fig 4.2 Schematic representation of process of core in cup tablets (Desai and Pramodkumar, 2004).

Table 4.2 Composition of Carvedilol core tablets

Composition (%)		FORMULATION CODE									
		CCT 1	CCT 2	CCT 3	CCT 4	CCT 5	CCT 6	CCT 7	CCT 8	CCT 9	CCT 10	CCT 11
Carvedilol		7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82
Carbopol 934P: HPMC	Ratio	0:10	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	10:0
	%	29.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00	29.00
Lactose (Tablettose 100)		59.18	59.18	59.18	59.18	59.18	59.18	59.18	59.18	59.18	59.18	59.18
Talc		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mg. Stearate		2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00

Weight of mucoadhesive layer - 80 mg.

Weight of protective layer - 320 mg.

Total weight of tablet (400 mg) = Weight of mucoadhesive layer + Weight of protective layer.

Table 4.3 Compositions for Cup (F_{cup})

Composition (%)	Qty (%)
Ethyl cellulose	27
Hydroxy Propyl Cellulose	17
Lactose (Tablettose 100)	52
Talc	2
Mg. stearate	2

4.3.2 Characterization:

4.3.2.1 Diameter and thickness:

The diameter and thickness of the formulated tablets were measured using Vernier caliper, Mitutoyo-Japan.

4.3.2.2 Hardness:

Hardness of the tablets was tested using a Monsanto hardness tester in kg/cm^2 (Campbell electronics, Mumbai).

4.3.2.3 Weight: The weights of the tablets were checked by digital weight balance, Afcoset-USA.

4.3.2.4 Friability:

It was determined by using Roche friabilator (Campbell electronics, Mumbai). The tablets were carefully dedusted prior to testing. Accurately weighed 10 tablets were placed in the friabilator which was rotated for 100 revolutions, and tablets were removed. Any loose dust from the tablets was removed and tablets were accurately weighed. A maximum weight loss of not more than 1% of the weight of the tablets being tested was considered acceptable (USP XXVI, 2003).

4.3.2.5 Assay:

The formulated bilayered tablet was dissolved in 100 mL isotonic phosphate buffer (pH 6.8 ± 0.2): methanol (9:1). The solution was filtered through 0.45μ filter to remove any undissolved components. The resulted solution was analyzed spectrophotometrically at 242 nm by UV spectrophotometer (Shimadzu 1601) $n = 5$ (Dortunc et al., 1998).

4.3.2.6 Surface pH

The micro environmental pH of the formulation was measured so as to determine its effect on buccal mucosa. The formulation was first allowed to get wet by adding 0.5 ml distilled water to its formulation surface. The surface pH was then recorded by bringing a combined glass electrode near the surface of the formulation and after allowing it to equilibrate for 1 min. (pH meter, DP-505, Digital instruments) (Shojaei et al., 2000: Govender et al., 2005).

4.3.2.7 Swelling

Tablets were weighed, before and after wetting with artificial saliva (Oliviera et al., 2005). The tablet was placed in 10 ml beaker; 10 ml of artificial saliva was added onto its surface using micropipette allowing it to swell. The system was incubated in dessicator at room temperature. The wetted formulation was removed at specified time intervals; the surface was gently dried using blotting paper and reweighed. The swelling index of wet formulation was determined as follows (Dortunc et al., 1998),

Swelling index (%) = (Wh-Wd)/Wd x 100

Where, Wh = Weight of hydrated formulation

Wd = Weight of formulation (dried).

4.3.2.8 *In vitro* Mucoadhesive force

Tensile strength tester (Instron 1121, UK) was used for measuring the *in vitro* mucoadhesive strength of the formulation.

Sheep mucosae, obtained from a slaughterhouse, was deprived of the connective tissue with surgical scissors and stored at −20 °C in deep freezer before testing. Before use for the experiment, the buccal mucosa was thrawed at ambient temperature. This sheep mucosa was then fixed onto the sample holder, facing the formulation, using cyanoacrylate glue and hydrated with 100µl of phosphate buffer (pH 6.8). The transfer arm was then moved until contact between the sample holder and the transfer arm was established. A preload of 44g was applied in order to allow the formation of the mucoadhesive bond. After a 3 min rest, the preload was removed and the movable carriage was moved forward at a constant speed (4 mm/min) till the two surfaces separated completely. The maximum detachment force was recorded. The mean of three observations were taken (Dortunc et al., 1998; Sandri et al., 2004).

4.3.2.9 *In vitro* diffusion:

In vitro drug diffusion was assessed by means of a Franz diffusion cell with a 20-mm diameter orifice (3.14 cm^2 area). It consisted of a receptor chamber, thermostated at 37^{0}C by means of a water jacket, and of a donor chamber, equipped with a cover lid. The two chambers were separated by a pretreated sheep buccal mucosa. The tablet was placed on the mucosa with the core surface facing the mucosa. 10 ml of artificial saliva was added in the donor chamber, over the tablet, to simulate buccal environment, whereas 20 ml phosphate buffer (pH 6.8 ± 0.2) was used as acceptor phase. At intervals of 1 hr., 1 ml of the acceptor phase was withdrawn and replaced with fresh buffer (Rossi et al., 2003; Sandri et al., 2004). Drug release was assayed by spectrophotometric detection as described earlier.

4.3.2.10 *In vitro* dissolution

The *in vitro* dissolution study of the core in cup tablets was carried out in a USP XXIV dissolution automated apparatus – type I (Electrolab, India). The dissolution medium was phosphate buffer (200 ml) containing 2% sodium lauryl sulphate, maintained at 37 ± 0.5 ^{0}C. The tablet was fixed to the bottom of the vessel by a double sided tape and dissolution was carried out at 50 rpm. Filtered aliquots were collected at intervals of 1, 2, 3, 4, 5, 6, 7 and 8 h. They were compensated with an equal volume of dissolution medium maintained at the same temperature. The concentration of drug released in the medium was assayed

spectrophotometrically at 242 nm. The experiment was carried out in triplicate (Narendra et al., 2005).

The release parameters and mechanism of release of Carvedilol from the core in cup tablets were investigated by fitting the data to Zero order, First order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas models using the following equations (Costa and Lobo, 2001).

Zero order - $Q_t = Q_0 + K_0 t$

First order - $\ln Q_t = \ln Q_0 + K_1 t$

Higuchi - $Q_t = K_H \sqrt{t}$

Hixson-Crowell - $Q_0^{1/3} - Q_t^{1/3} = K_s t$

Korsmeyer Peppas - $Q_t/Q = K_k t^n$

Where, Q_t is the amount of drug dissolved in time t, Q_O is the initial amount of drug in the solution (most times, $Q_O = 0$), t is the time, n is release exponent and K_0, K_1, K_H, K_s and K_k are the Zero order, First order, Higuchi, Hixson-Crowell and Korsmeyer-Peppas constants respectively.

4.3.2.11 Pharmacokinetic Study

Male white rabbits weighing 2.0-2.5 kg were used for the studies after an acclimatization period of one week (6 rabbits / group). Animal Ethical Committee Guidelines were observed during the studies. Rabbits were anesthetized by diazepam (5 mg/kg; i. m.) and ketamine (40mg/kg; i. p.). The core in cup tablet was applied directly to the buccal pouch of the rabbits after 10 min post anesthesia. Conventional marketed tablets (6.25 mg) were administered orally to one group to compare pharmacokinetic parameters after oral and buccal administration. Naive rabbits were used as a control for the experiment. At an interval of 1 h, up to 8 hrs, 0.5-1.0 ml of blood was withdrawn via marginal ear vein using a 26 gauge needle. The blood was centrifuged at 8000 rpm, 10 min at T- 15^{0}C (Sigma centrifuge- 3K30, Germany) and plasma was collected. Protein separation from the plasma was done by adding equivalent amount of methanol and centrifuging at 10000 rpm for 10 min at T - 15^{0}C. Then protein free plasma was collected and analyzed by High Performance Liquid Chromatography (HPLC, Dionex with Cromeolen) (Hokama et al., 1999; Gavini et al., 2006).

4.3.2.11.1 Analysis of blood sample

0.2 ml of above protein-free plasma was mixed with 50 µl propranolol acting as internal standard and 20 µl was injected through syringe filter into an isocratic HPLC with a UV-

visible detector (UVD 170U). The column employed was C18 (4.6 x 100 mm, 3.5 μm), Waters, Ireland. The chromatograph consisted of a high performance chromatographic system with Chromeleon software. The mobile phase consisted of methanol–50 mM KH_2PO_4, pH 2.5 (60:40, v/v) and the flow-rate was adjusted to 1.0 ml/min. Measurements were made at an excitation wavelength of 242 nm and emission wavelength of 344 nm (Hokama et al., 1999).

Area under the curve (AUC) of the plasma drug concentration vs. time was determined with the trapezoidal rule method, C_{max} and T_{max} were recorded. The pharmacokinetic data was compared with that obtained from the conventional oral tablets (Miyazaki et al., 1995). Statistical analyses were completed using ANOVA.

4.3.2.12 Histological study of buccal mucosa:

4.3.2.12.1 Light microscopy: (Veuillez et al., 2002)

At the end of the diffusion experiment, the buccal mucosa was collected. It was repeatedly washed with phosphate buffer (pH 6.8 ± 0.2). Small portion of the tissue was fixed in 10% buffered formalin solution and dehydrated. Sections were taken by microtome at 4 μm perpendicular to the epithelial surface. They were stained with hematoxylin eosin (HE) and examined by light microscopy at 10X (Olympus) to evaluate any histological changes, in the epithelium and the adjacent connective tissue. Control buccal mucosa was also treated and examined similarly.

4.3.2.12.2 Scanning electron microscopy of buccal mucosa: (Kitano et al., 1998)

At the end of the diffusion experiment, the buccal mucosa was collected and washed repeatedly with phosphate buffer pH 6.8 (± 0.2). Then it was treated with 2% glutaraldeheyde for 2 hr. After being soaked in saccharose buffer solution for 24 hr, the mucosa was again treated with 1% osmic acid solution for 1 hr. The mucosa was then dehydrated by repeated washing using a graded series of acetone solutions (60-100%). Thereafter it was completely dried at room temp for 24 hrs and then examined using scanning electron microscope at 5kV voltage by securing it on aluminum stab (SEM- JEOL, JSM 5610 LV).

4.3.2.12.3 *In vivo* acceptability Testing:

Treatment group: Ten healthy male volunteers, weighing approximately 55 to 60 kg and 25 to 28 years old, participated in the study. A written consent was obtained from the volunteers.

Study: Placebo core in cup tablets were evaluated for *in vivo* acceptability. The tablet was placed on the buccal mucosa between cheek and gingiva, gently pressed onto the mucosa for 30 sec. If it was not well adhered, its surface was moistened with phosphate buffer to facilitate adhesion. The volunteers were allowed no access to water and food during experiment. The bioadhesion was then checked by observing the adhesion of the tablet on the volunteer's cheek up to 6 h. Dimensional integrity of the tablet was checked after the study by removing the tablet from the buccal mucosa. (Nafee et al., 2003; Peh and Wong, 1999). The in vivo acceptability of dosage form was determined by volunteer's response on parameters like irritation, comfort, dryness of mouth, salivary secretion, heaviness of tablet at application site and dislodgement of system during study.

4.3.2.13 Pharmacodynamic studies:

4.3.2.13.1 Study protocol:

1. Induction of hypertension.
2. Treatment with conventional oral formulation (Tablets: 6.25 mg)
3. Treatment with buccoadhesive core in cup tablets.
4. Comparison of conventional and buccal formulation in terms of lowering of blood pressure.

4.3.2.13.2 Fructose model: (Vogel et al., 2002)

4.3.2.13.3 Rationale:

Increase in dietary carbohydrate intake can raise blood pressure in experimental animals. This model is based on the basis of above hypothesis and it includes regular administration of fructose until rat develops hypertension and the period generally varies from 4 to 6 weeks. Administration of 40% fructose solution developed hypertension in 4 weeks of time which can be detected by measuring systolic blood pressure and pulse rate by tail cuff method described here. (Dai and McNeil, 1995) Groups of 12 male wistar rats weighing 210-250 gm were used. They were housed 6 animals per group on a 12 hr light-12 hr day cycle and allowed free access to standard diet and water.40% fructose solution (5ml) was administered to the group three times a day for 4 weeks. Body weight of each rat was recorded every week during treatment. Using the tail cuff method (Sakima et al., 1998; Lee et al., 2002), systolic blood pressure and pulse rate was measured using LE 5002 Storage Pressure Meter (Letica LSI) before and every week during the treatment.

4.3.2.13.4 Procedure for tail cuff method: (Lee et al., 2002)

A cuff was fitted around the rat's tail and was inflated and deflated on a cycle. In the inflation stage, the device recorded the oscillations of the vascular wall produced by blood flow pulsation. The cuff was then deflated. When the occlusive pressure drops to a value equal to the systolic arterial pressure (AP), the oscillation reflects deformation of the vascular wall. The diastolic pressure was recorded with a sudden decrease in pulsatile sound. The mean AP (MAP) and heart rate (HR) were recorded with this method. The animals were placed in an incubator at 37°C for about 10 min before the AP recording. The animal was then placed into a restrainer. In order to stabilize the animal, it was confined in a restrainer during the measurement. Box temperature was kept at 37°C with temperature control system attached to it. The MAP was monitored every 2 min, 3–4 times, until a stable reading was obtained. The tail cuff method is shown in Fig 4.3.

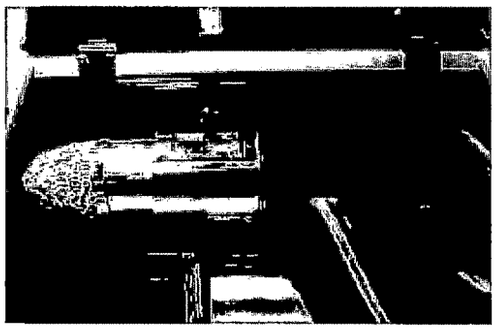

Fig 4.3 Tail cuff method showing restrained rat in restrainer.

Experimental animals were grouped as under,

Group I- Control

Group II - Treatment with oral conventional formulation for 2 w.

Group III – Treatment with buccal Carvedilol core in cup tablet for 2 w.

- Treatment with conventional marketed oral tablet – 6.25 mg strength.

After induction of hypertension, oral tablet was administered to rats (group II). Blood pressure was measured at an interval of 1hr up to 6 hr.

- Treatment with developed buccal core in cup tablet

After induction of hypertension, rats were anaesthetized by urethane for the ease of application of buccal dosage form. After application of buccal tablet, blood pressure was measured at an interval of 1hr up to 6 hr by tail cuff method described previously. After application with both oral and buccal tablet, the lowering of blood pressure was compared statistically.

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4.4 CARVEDILOL BILAYER PATCHES (CBP):

4.4.1 Formulation of bilayer patches:

Solvent casting method was employed for the formulation of bilayered patches consisting of two layers viz. medicated layer and backing layer. Hydroxypropylmethyl cellulose (HPMC K4M) and Carbopol 934P were used in different ratios for the formulation of medicated layer and hydroxypropyl cellulose (HPC) and ethyl cellulose (EC) were employed for backing layer.

Table 4.4 shows the composition of the medicated patch. For medicated layer, weighed amount of HPMC K4M and Carbopol 934P were dispersed in 10 ml of solvent system [methanol: dichloromethane (3:2)] under continuous stirring using a mechanical stirrer till a gel was formed. Thereafter, the drug solution (Carvedilol + 2 ml of above solvent system) was added to it under continuous stirring. Propylene glycol was gradually added as a plasticizer, 10 % v/v to solution and stirring continued for 60 min. The resultant gel was left for 2-3 hrs till a clear, bubble-free gel was obtained. The gel was then casted onto a glass petri dish and allowed to dry in an oven maintained at 40 °C till a peelable film was formed.

Table 4.5 shows the composition of the backing layer. For backing layer, weighed quantity of HPC and EC was dispersed in acetone. Propylene glycol (6.90 %) was added as a plasticizer and the system was stirred continuously for 30 min using mechanical stirrer. The resulted solution was casted on the dried medicated patch and allowed to dry for 24 h at room temperature.

The dried bilayer film was carefully removed from the petri dish and cut into patches of 14 mm diameter. The samples were packed in aluminum foil and stored at 15-20^{0}C. (Nafee et al., 2003; Cui and Mumper, 2002; Peh and Wong, 1999).

Table 4.4 Compositions for mucoadhesive layer of Carvedilol bilayer patches

Composition (%)		FORMULATION CODE										
		CBP 1	CBP 2	CBP 3	CBP 4	CBP 5	CBP 6	CBP 7	CBP 8	CBP 9	CBP 10	CBP 11
Carvedilol		7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82	7.82
Carbopol 934P: HPMC K4M	Ratio	0:10	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	10:0
	%	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0
Propylene glycol (%)		10.00	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methanol: dichloromethane	Ratio	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2
	%	53.18	53.18	53.18	53.18	53.18	53.18	53.18	53.18	53.18	53.18	53.18

Table 4.5 Compositions for protective layer of Carvedilol bilayer patches

Excipients	Qty (%)
Ethyl cellulose	36.55
Hydroxy Propyl Cellulose	29.30
Propylene glycol	06.90
Acetone	27.25

4.4.2 Characterization:

The Carvedilol bilayer patches were evaluated for the following parameters as per method followed for the core in cup tablets.

4.4.2.1 Diameter and thickness

4.4.2.2 Average weight

4.4.2.3 Assay

4.4.2.4 Mechanical properties

4.4.2.4.1 Tensile strength, Elongation at break, Elastic modulus and strain:

Mechanical properties of the patches were evaluated using tensile strength tester, (INSTRON 1121, UK) equipped with 2 kg load cell. The patch to be evaluated was held

between two clamps positioned at a distance of 2 cm. Uniform sized patches (40 x 10 mm) were taken for the study. To prevent damage to the patch from clamps, a cardboard was attached on the surface of the clamp. During experiment, the patch was pulled by the top clamp at a rate of 2.0 mm/s to a distance of 1 cm. The force and elongation at break were measured when the patch broke. The tensile strength, elongation at break, elastic modulus and strain was calculated using following equations (Peh and Wong, 1999),

$$\text{Tensile Strength}(\text{kg mm}^{-2}) = \frac{\text{Force at break (kg)}}{\text{Initial cross sectional area of the sample (mm}^2\text{)}}$$

$$\text{Elongation at break}(\%\ \text{mm}^{-2}) = \frac{\text{Increase in length (mm)} \times 100}{\text{Original length (mm)} \times \text{Cross sectional area (mm}^2\text{)}}$$

$$\textit{Elastic Modulus}(\text{kg mm}^{-2}) = \frac{\textit{Force at corresponding strain (kg)}}{\textit{Cross sectional area}(\text{mm}^{-2})} \times \frac{1}{\textit{Corresponding strain}}$$

$$\textit{Strain} = \frac{\textit{Tensile Strength}}{\textit{Elastic Modulus}}$$

Folding endurance test:

Folding endurance of the patches was determined by repeatedly folding and unfolding the patch at the same place till it broke or for 300 times, which is considered to be a satisfactory value to reveal good folding endurance properties. The number of times the patch could be folded at the same place without breaking gave the value of the folding endurance (Peh and Wong, 1999).

4.4.2.5 Surface pH

4.4.2.6 Swelling

4.4.2.7 *In vitro* mucoadhesive force

4.4.2.8 *In vitro* diffusion

4.4.2.9 *In vitro* dissolution study

4.4.2.10 Pharmacokinetic Study

4.4.2.11 Histological study of buccal mucosa

4.4.2.11.1 Light microscopy

4.4.2.11.2 Scanning electron microscopy of buccal mucosa

4.4.2.12 *In vivo* acceptability testing

4.4.2.13 Pharmacodynamic studies

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4.5 CARVEDILOL BILAYER TABLETS FORMULATED WITH MICROSPHERES (CTM):

4.5.1 Formulation of Carvedilol microspheres (CM): (Gavini, et al., 2002; Dhawan et al., 2004)

The microspheres were prepared by spray drying technique, with the drug to polymer weight ratios of 1:1, 1:1.5 and 1:2. Table 4.6 depicts the composition of microspheres prepared. Chitosan was dissolved in 2% acetic acid solution (50ml). Carvedilol was dissolved in 2 ml of methanol and added to it and the resultant solution was sprayed through the nozzle of a spray-dryer (Mini spray drier LSD-48, JISL Mumbai), equipped with a standard 0.7-mm nozzle. Lactose (5%) was added to solution. The process conditions were as follows: inlet air temperature, 98°C-100°C; outlet air temperature, 75°C-77°C; feed rate, approximately about 8 mL min. The spray-dried microspheres were harvested from the apparatus collector and kept under vacuum for 24 hours, at room temperature in vaccum dessicator.

Chitosan solution in acetic acid (2% w/v of total spraying solution, vol-50 ml) was sprayed through spray drier to obtain placebo microspheres (M4), prepared for comparison. The spray dried microspheres were stored at room temperature in tightly closed vials.

Table 4.6 Compositions of microspheres.

	CM1	CM2	CM3	P-CM
Drug: Polymer	1:1	1:1.5	1:2	0:1
Lactose	5%	5%	5%	5%

4.5.2 Characterization of Microspheres:

4.5.2.1 Flow properties:

4.5.2.1.1 Angle of repose: The fixed funnel method was used to find out angle of repose. In this method, a funnel that was secured with its tip at a 2 cm height (H) above a graph paper that was placed on a flat horizontal surface. Microspheres were carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. Thus, with the radius of the conical pile (R) was noted and angle of repose was calculated

$\tan \alpha = H/R$

Where α is the angle of repose (Taylor et al, 2000; Banker and Anderson, 1991).

4.5.2.1.2 Bulk density and tapped density:

The bulk density was determined as the quotient of weight to the volume of sample. The tapped density was determined as the quotient of weight to the volume of sample after manually tapping a measuring cylinder filled with microspheres for 100 times from a height of 2 inches (Gohel and Jogani, 2002).

4.5.2.1.3 Compressibility index:

The Carr's index (percent compressibility) was calculated as one hundred times the ratio of the difference between the tapped density and bulk density to the tapped density (Gohel and Jogani, 2002).

$I_c = 100 X (Pt - Pb) / Pt$

Where I_c is car's index, Pb is bulk density and Pt is tapped density

4.5.2.1.4 Hausner's ratio: The Hausner's ratio was determined as the ratio of the tap to the bulk density (Sainchez et al., 1995; Iida et al., 2001; Sinha et al., 2005).

HR= Pt / Pb,

Where, Pt is tapped density and Pb is bulk density.

4.5.2.2 Particle size:

It was determined by Malvern Mastersizer (HYDRO 2000SM, Malvern Instruments Ltd., UK) which works on laser scattering principle. Diluted suspension of microspheres in distilled water was added to sample dispersion unit containing stirrer and stirred at 2000 rpm in order to reduce interparticle aggregation, and laser obscuration range was maintained between 10-20%. The average particle size was measured after performing the experiment in triplicate (Dhawan et al, 2004; Goraltchouk et al., 2006).

4.5.2.3 Uniformity index:

Uniformity Index (UI) was determined by the following formula:

$$UI = Dw/Dn$$

where Dw and Dn are weight average diameter and number average diameter, respectively, and are calculated as follows:

$$Dw = \Sigma NiDi^{-4}/\Sigma NiDi^{-3}, Dn = \Sigma NiDi/\Sigma Ni$$

Where, Ni is the number of particles with Di diameter. As per Shukla et al, values of UI ranging from 1.0 to 1.1 and 1.1 to 1.2 indicate monodisperse and nearly monodisperse

particles. Values higher than 1.2 have been regarded as indicative of particles with broad particle size distribution (Shukla et al., 2002).

4.5.2.4 Encapsulation efficiency:

Microspheres equivalent to 10 mg of the drug, were triturated in mortar, dispersed in 25 ml of water: methanol (4:1) and then bath sonicated. The dispersion was filtered, and after suitable dilution it was measured spectrophotometrically. The drug content was calculated as a percentage with respect to the theoretical amount of drug (Gavini et al., 2002).

4.5.2.5 Scanning Electron Microscopy (SEM):

The shape and surface characteristics of spray dried microspheres were studied by SEM (JEOL, JSM 5610 LV). Samples of microspheres were placed on a double-sided tape, previously secured on aluminum stubs and then analyzed at 20 kV acceleration voltage under an argon atmosphere (Gavini et al., 2002; Kockisch et al., 2003).

4.5.2.6 FT-IR Spectroscopy:

FT-IR spectra of microspheres were recorded by using FT-IR Shimadzu 8300.

4.5.2.7 XRD: Powder X-ray diffraction pattern of Carvedilol was evaluated to analyse the crystallanity of the molecule by X-ray diffractometer.

4.5.3 Formulation of Carvedilol bilayer tablets with microspheres (CTM): (Liabot et al., 2002; Nafee et al., 2004)

The tablets were prepared by direct compression of 20 mg of Carvedilol microspheres and 80 mg of the listed excipients. Direct double compression technique was employed for the formulation. In this technique, first layer (drug layer containing microspheres) and then protective layer (backing layer) was formed. For first layer, microspheres along with listed excipients were loosely compressed and then second layer (protective layer) blend was placed on first intermediate layer and compressed to get bilayer tablet. Table 4.7 reports the composition of the tablets. Compositions for the protective layer are shown in Table 4.8. For protective layer a fixed combination of polymers and excipients were used for all the tablets.

Compression was performed in 2 steps:

4.5.3.1 Formation of first layer containing microspheres:

The physical blend of drug containing microspheres, Carbopol 934P, talc and Tablettose 100 was mixed for 15 minutes. Magnesium stearate was added to the above blend and mixed for 5 min. The mixed blend was slightly compressed on single station tablet compression

machine, Jaguar, Mumbai using 8 mm flat faced punches to obtain first layer. Weight of first layer was adjusted to 100mg.

4.5.3.2 Formation of backing layer:

Ethyl cellulose, hydroxypropyl cellulose, talc and Tablettose 100 were mixed thoroughly for 15 min. Magnesium stearate was added to the above blend and mixed for 5 min. Weighed quantity (100 mg) of this blend was poured on first layer (Compressed in first step) and compressed on single station compression machine. The bilayer tablet was visually observed for any separation of the layers. The total weight of each tablet was 200 mg.

Table 4.7 Composition of the tablets prepared from drug-loaded microspheres

Composition (%)	Formulation Code			
	CTM1	CTM2	CTM3	P-CTM
Microspheres (Drug:Polymer)	12.50 (1:1)	15.63 (1:1.5)	18.75 (1:2)	6.25 (0:1)
Carbopol 934P	40.00	40.00	40.00	40.00
Tablettose 100	43.50	40.37	37.25	49.75
Talc	2.00	2.00	2.00	2.00
Mg. Stearate	2.00	2.00	2.00	2.00

Weight of mucoadhesive layer - 100 mg.

Total weight of tablets - 200 mg.

Table 4.8 Compositions for protective layer

Composition (%)	%
Ethyl cellulose	27
Hydroxy Propyl Cellulose	17
Tablettose	52
Talc	2
Magnesium stearate	2

4.5.4 Characterization of Bilayer tablet with microspheres:

The Carvedilol bilayer tablets were then evaluated for the following parameters as per method followed.

4.5.4.1 Diameter and thickness

4.5.4.2 Hardness

4.5.4.3 Average weight

4.5.4.4 Friability

4.5.4.5 Assay

4.5.4.6 Surface pH

4.5.4.7 Swelling

4.5.4.8 *In vitro* mucoadhesive force

4.5.4.9 *In vitro* diffusion:

4.5.4.10 *In vitro* dissolution study

4.5.4.11 Pharmacokinetic Study

4.5.4.12 Histological study of buccal mucosa:

4.5.4.12.1 Light microscopy:

4.5.4.12.2 Scanning electron microscopy of buccal mucosa

4.5.4.13 *In vivo* acceptability testing:

4.5.4.14 Pharmacodynamic studies:

4.5.5 References:

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