5.0 EXPERIMENTAL (PRAVASTATIN SODIUM)

5.1 MATERIALS:

Pravastatin sodium was kindly supplied by Cipla Ltd, Mumbai, India.

Sodium glycocholate and sodium lauryl sulphate were obtained from Loba chemie, Mumbai. Heparin was gifted from Wuhan zhiyuan medical technology Co., Ltd, China. Cholesterol and peanut oil were obtained from Sigma chemical Co.

Carbopol 934P, HPMC K4M, Hydroxypropyl cellulose (Klucel HXF), Ethyl cellulose (14 cps), Chitosan, Tablettose 100, Magnesium stearate, talc, 1-Octanol, potassium dihydrogen phosphate, potassium bromide, propylene glycol, potassium dihydrogen phosphate, methanol, sodium phosphate, sodium chloride, potassium cyanide, potassium phosphate, potassium chloride, sodium carbonate, osmic acid, glutaraldehyde, formalin, sodium citrate, sucrose, fructose, hematoxylin eosin, diazepam and ketamine were used. Suppliers for above materials were same as that of Carvedilol.

5.2 Preformulation studies:

5.2.1 Determination of n-octanol: buffer partition coefficient:

The method is same as that followed for Carvedilol.

5.2.2 Compatibility studies using FT-IR spectroscopy:

Infrared spectra of Pravastatin, physical mixtures of the Pravastatin sodium and polymers such as Carbopol 934P, HPMC K4M were obtained using FT-IR 8300 (Shimadzu). The method is same as that followed for Carvedilol.

5.2.3 Scanning electron microscopy (SEM):

Shape and surface characteristics of plain drug were studied by scanning electron microscopy (JEOL, JSM 5610 LV). The samples were placed on double-sided tape that had previously been secured on aluminium stubs and then analyzed at 15 kV acceleration voltage, under argon atmosphere (Gavini et al., 2006).

5.2.4 *In vitro* permeation studies:

The method is same as that followed for Carvedilol.

5.2.5 Effect of permeation enhancers on the permeability of Pravastatin:

The following permeation enhancers were used.

Sodium glycocholate

Sodium lauryl sulphate

Solution of sodium glycocholate (100 mM) and solution of sodium lauryl sulphate (100 mM) in phosphate buffer with Pravastatin sodium solution was used as donor compartment media. (Shojaei and Li, 1997; Shojaei, 1998; Hoogstraate et al., 1996). The permeation enhancer effect was studied by the method same as that used for in vitro permeation studies of Carvedilol.

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5.3 PRAVASTATIN SODIUM CORE IN CUP TABLETS (PCT):

5.3.1 Preparation of core in cup tablets:

The composition of core and cup are presented in Table 4.9 and 4.10 respectively. Formulation process was same as that described in Carvedilol core in cup tablets

Table 4.9 Composition for mucoadhesive layer (Core)

Composition (%)		FORMULATION CODE										
		PCT 1	PCT 2	PCT 3	PCT 4	PCT 5	PCT 6	PCT 7	PCT 8	PCT 9	PCT 10	PCT 11
Pravastatin sodium		12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
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
	%	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Tablettose 100		48.5	48.5	48.5	48.5	48.5	48.5	48.5	48.5	48.5	48.5	48.5
Talc		2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mg. Stearate		2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

* Qty in mg

Weight of mucoadhesive layer (core tablet)-80 mg.

Table 4.10 Composition for Cup

Excipients	Qty (%)
Ethyl cellulose	27.00
Hydroxy Propyl Cellulose	17.00
Tablettose	52.00
Talc	2.00
Mg. Stearate	2.00
Total	**100.00**

Weight of cup tablet: 320.00 mg

Final weight of core in cup tablet: 400 mg.

5.3.2 Characterization:

The core in cup tablets were evaluated for following parameters as described in Carvedilol core in cup tablets.

5.3.2.1 Diameter and thickness:

5.3.2.2 Hardness:

5.3.2.3 Weight:

5.3.2.4 Friability:

5.3.2.5 Assay:

The formulated bilayered tablet was dissolved in 100 mL isotonic phosphate buffer (pH 6.8 ± 0.2). The solution was filtered through 0.45µ filter to remove any undissolved components. The resultant solution was analyzed spectrophotometrically at 239 nm by UV spectrophotometer (Shimadzu 1601) $n = 5$ (Dortunc et al., 1998).

5.3.2.6 Surface pH:

5.3.2.7 Swelling study:

5.3.2.8 *In vitro* mucoadhesive force:

5.3.2.9 *In vitro* diffusion:

5.3.2.10 *In vitro* dissolution study

The *in vitro* dissolution study of the bilayer tablets was carried out in a USP XXIV dissolution automated apparatus – type I (Electrolab, India). The dissolution medium was 200 mL phosphate buffer, 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 239 nm. The experiment was carried out in triplicate (Narendra et al., 2005).

The release parameters and mechanism of release of Pravastatin sodium from the buccal 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.

5.3.2.11 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 15 min post anesthesia. Conventional marketed tablets (10.0 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).

5.3.2.11.1 Analysis of blood sample

0.2 ml of above protein-free plasma was mixed with 50 µl Lovastatin 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 YMC packed (4.6 x 150 mm, 5 µm) Waters, Ireland. The chromatograph consisted of a high performance chromatographic system with Chromeleon software. The mobile phase consisted of acetonitrile: ammonium acetate (0.01 M), 2:1 and the flow-rate was adjusted to 1ml/min Measurements were made at an excitation wavelength of 239 nm (Otter and Mignat, 1998; Clarke, 2005).

Area under the curve (AUC) of the plasma drug concentration vs. time was determined with the trapezoidal rule method and C_{max}, 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.

5.3.2.12 Histological study of buccal mucosa

5.3.2.12.1 Light microscopy

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

5.3.2.13 *In vivo* acceptability testing:

5.3.2.14 Pharmacodynamic studies:

5.3.2.14.1 Study protocol:

- Induction of hyperlipidemia.
- Treatment with conventional oral tablets and buccal core in cup tablets.
- Comparison of conventional and buccal formulation in terms of lowering of hyperlipidemic parameters.

5.3.2.14.2 Induction of atherosclerosis or hyperlipidemia by cholesterol fed diet:

- **Animals:**

Male white rabbits (18 Nos.; 2.0 to 3.0 kg) were housed at an animal facility accredited by the Food and drugs administration, Vadodara, Gujarat. Experiments were conducted in accordance with the guidelines of the animal ethical committee.

- **Experimental:**

Cholesterol-fed rabbit model is notable for rapid development of atherosclerosis or hyperlipidemia and low maintenance cost. Rabbits were fed standard rabbit chow supplemented with 1.0 % in weight of cholesterol (USP grade, anhydrous, Sigma Chemical Co) dissolved in 6% peanut oil for 8 weeks (Nakayama et al., 1983; Sakamoto et al., 1987). The atherogenic diet was prepared by dissolving the cholesterol in the peanut oil and thoroughly coating the pellets of rabbit chow with this mixture. All animals received approximately 125 g cholesterol feed/day; water was provided ad libitum.

It is reported that, under these conditions, rabbits rapidly become hyperlipidemic (serum cholesterol >1000 mg/dL). At the conclusion of the cholesterol screen, animals were phlebotomized through the marginal ear vein, and blood sample (5 ml) was collected in heparin-containing tubes. Blood samples were analyzed for LDL, VLDL, HDL and triglycerides (Kolodgie et al., 1996).

After development of hyperlipidemia in rabbits, they were divided into group of 6 as follows.

Group 1- Treatment with oral conventional tablet for 4 weeks.

Group 2- Treatment with buccal core in cup tablet for 4 weeks.

Group 3- Control (Untreated).

2] Treatment of atherosclerosis or hyperlipidemia by conventional oral tablet.

Group 1 was treated with oral conventional tablet. The tablet was given in solution form so that it can be swallowed by rabbit easily. Frequency of administration of oral tablets was once a day. The blood sample was collected at an interval of 2 weeks up to 4 weeks.

5.3.2.14.3 Treatment of atherosclerosis or hyperlipidemia by buccal core in cup tablets.

Group 2 was treated with buccal core in cup tablet. 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 15 min post anesthesia. Frequency of application of buccal core in cup tablets was once a day. The blood sample was collected at an interval of 2 weeks up to 4 weeks.

5.3.2.14.4 Comparison of conventional and buccal formulation in terms of lowering of hyperlipidemic parameters.

After administration of both oral and buccal core in cup tablets, the data was compared and analyzed. The significant differences were determined by one way ANOVA test.

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5.4 PRAVASTATIN SODIUM BILAYER PATCHES (PBP):

5.4.1 Formulation of bilayer patches:

Solvent casting method was employed for the formulation of bilayer 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 4.11 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: water (3:2)] under continuous stirring using a mechanical stirrer till a gel was formed. Thereafter, the drug solution (Pravastatin sodium + 2 ml of above solvent system) was added to it under continuous stirring. Propylene glycol was gradually added as a plasticizer, 10 % to formulation weight 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 4.12 shows the composition of the backing layer. For backing layer, weighed quantity of HPC and EC was dispersed in acetone. Propylene glycol (5 %) 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 4.11 Compositions for mucoadhesive layer of Pravastatin sodium bilayer patches

Composition (%)		FORMULATION CODE										
		PBP 1	PBP 2	PBP 3	PBP 4	PBP 5	PBP 6	PBP 7	PBP 8	PBP 9	PBP 10	PBP 11
Pravastatin sodium		12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
CP: HPMC	Ratio	0:10	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1	10:0
	%	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
Propylene glycol (%)		10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Methanol: Water	Ratio	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2	3:2
	%	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5	42.5

Table 4.12 Compositions for protective layer of Pravastatin sodium bilayer patches

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

5.4.2 Characterization:

The Pravastatin sodium bilayer patches were then evaluated for the following parameters as per method followed for the Carvedilol bilayer patches.

5.4.2.1 Diameter and thickness

5.4.2.2 Average weight

5.4.2.3 Assay

5.4.2.4 Mechanical properties

5.4.2.5 Surface pH

5.4.2.6 Swelling

5.4.2.7 *In vitro* mucoadhesive force

5.4.2.8 *In vitro* diffusion

5.4.2.9 *In vitro* dissolution study

5.4.2.10 Pharmacokinetic study

5.4.2.11 Histological study of buccal mucosa

5.4.2.11.1 Light microscopy

5.4.2.11.2 Scanning electron microscopy of buccal mucosa

5.4.2.12 *In vivo* acceptability testing:

5.4.2.13 Pharmacodynamic studies

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