7.0 RESULTS AND DISCUSSION (CARVEDILOL CORE IN CUP TABLETS -CCT)

7.1 Formulation of core in cup tablets

It was done in three steps:

7.1.1 Formulation of core tablets (CT, 6 mm diameter):

Core tablets were loosely compressed on 8 station D-tooling machine. The obtained physicochemical parameters are shown in Table 7.1.

Table 7.1 Physicochemical parameters of core tablets

Parameters	CT1	CT2	CT 3	CT4	CT5	CT6	CT 7	CT8	CT 9	CT10	CT11
Diameter (mm)	6.01 ± 0.13	6.01 ± 0.12	6.01 ± 0.11	6.01 ± 0.09	6.01 ± 0.14	6.01 ± 0.10	6.01 ± 0.13	6.01 ± 0.11	6.01 ± 0.08	6.02 ± 0.11	6.02 ± 0.11
Thickness (mm)	3.02 ± 0.04	3.01 ± 0.04	3.01 ± 0.05	3.01 ± 0.05	3.02 ± 0.04	3.02 ± 0.03	3.02 ± 0.03	3.01 ± 0.05	3.01 ± 0.05	3.01 ± 0.04	3.02 ± 0.05
Hardness (Kg/cm^2)	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.0 ± 0.50
Average weight (mg)	81.20 ± 1.56	81.38 ± 1.50	81.66 ± 2.03	80.12 ± 1.44	80.22 ± 1.26	81.68 ± 1.78	80.55 ± 1.54	79.88 ± 1.82	79.90 ± 1.32	80.33 ± 1.27	79.98 ± 1.59
Assay (%)	98.30 ± 1.56	98.38 ± 2.42	101.26 ± 1.03	99.22 ± 1.94	98.87 ± 2.29	99.88 ± 1.28	101.15 ± 1.98	97.98 ± 1.53	101.90 ± 1.82	99.31 ± 1.87	99.98 ± 0.99

± R.S.D. (n=3)

Diameter of different batches of tablets ranged from 6.01 ± 0.13 (CT1) to 6.02 ± 0.11 (CT11) mm. Thickness values were found in between 3.01 ± 0.04 (CT2) to 3.02 ± 0.05 (CT11) mm. Hardness values were in between 2.0 ± 0.50 (CT11) to 2.5 ± 0.50 (CT1) kg/cm^2. Average weight of tablets was within acceptable limits (<5% deviation) and weight variation was within acceptable limits (<7.5 % deviation). Assay values for different batches ranged from 97.98 ± 1.53 (CT8) to 101.90 ± 1.82 (CT9) %. All the physicochemical characteristics were within acceptable limits.

7.1.2 Formation of buccal adhesive cup

Table 7.2 shows physical parameters of cup tablets. Outer and inner diameter of tablets was found to be 10.01 ± 0.23 mm and 6.01 ± 0.23 mm respectively. Obtained thickness was 4.60 ± 0.04 mm. Hardness value was 2.5 ± 0.50 kg/cm^2. Average weight was found within acceptable limits (<5% deviation).

Table 7.2 Physical parameters of cup tablets

Parameters	Cup
Outer Diameter (mm)	10.01 ± 0.23
Inner Diameter (mm)	6.01 ± 0.23
Thickness (mm)	4.60 ± 0.04
Hardness (Kg/cm^2)	2.5 ± 0.50
Average weight (mg)	321.45 ± 1.69

± R.S.D. (n=3)

7.1.3 Formation of core in cup tablets (Compressing core tablets in cup).

Table 7.3 exhibits physicochemical parameters of core in cup tablets. Diameter of different batches of tablets ranged from 10.01 ± 0.23 (CCT1) to 10.02 ± 0.10 (CCT11) mm. Thickness were found in between 4.45 ± 0.05 (CCT4) to 4.60 ± 0.05 (CCT11) mm. Hardness were in between 3.5 ± 0.50 (CCT1) to 4.5 ± 0.50 (CCT7) Kg/cm^2. Average weight was within acceptable limits (<5% deviation). Assay values of different batches were from 97.98 ± 1.53 (CCT8) to 101.90 ± 1.82 (CCT9) %. Thus all parameters were found within acceptable limits.

Table 7.3 Physicochemical parameters of core in cup tablets.

Parameters	CCT1	CCT2	CCT3	CCT4	CCT5	CCT6	CCT7	CCT8	CCT9	CCT10	CCT11
Diameter (mm)	10.01 ± 0.23	10.01 ± 0.09	10.01 ± 0.20	10.01 ± 0.08	10.01 ± 0.09	10.01 ± 0.10	10.01 ± 0.06	10.01 ± 0.13	10.02 ± 0.09	10.01 ± 0.09	10.02 ± 0.10
Thickness (mm)	4.60 ± 0.04	4.56 ± 0.04	4.51 ± 0.05	4.45 ± 0.05	4.58 ± 0.04	4.59 ± 0.03	4.58 ± 0.03	4.60 ± 0.05	4.60 ± 0.05	4.56 ± 0.04	4.60 ± 0.05
Hardness (Kg/cm^2)	3.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.0 ± 0.50	4.0 ± 0.50	4.0 ± 0.50	3.5 ± 0.50
Average weight (mg)	400.20 ± 1.70	400.38 ± 1.98	401.66 ± 2.01	401.22 ± 1.96	402.32 ± 1.67	400.68 ± 1.56	400.55 ± 1.74	401.88 ± 1.68	402.90 ± 1.15	401.33 ± 1.32	402.98 ± 1.95
Friability (%)	0.09	0.08	0.09	0.07	0.08	0.09	0.09	0.09	0.09	0.09	0.1
Assay (%)	98.30 ± 1.56	98.38 ± 2.42	101.26 ± 1.03	99.22 ± 1.94	98.87 ± 2.29	99.88 ± 1.28	101.15 ± 1.98	97.98 ± 1.53	101.90 ± 1.82	99.31 ± 1.87	99.98 ± 0.99

± R.S.D. (n=3)

7.2 Surface pH:

The surface pH of the tablets was determined in order to confirm that the Carvedilol core in cup tablets would not cause irritation to the buccal mucosa due to possible extremes in pH (Govender et al., 2005). It is a known fact that due to wide variety of excipients available for formulation design there are chances of extremes in pH. A low and higher pH would be expected to damage the contacting mucosal surface, and this has been reported in an in vivo study involving human volunteers (Tiwari et al., 1999). High proportion of Carbopol 934P in the mixtures may give strong acid characteristics to the matrix, which could produce some side effects in the mucosa (Liabot et al., 2004).

Table 7.4 shows that the surface pH of the buccoadhesive tablets remained fairly constant at a pH of approximately 5.85 ± 0.26 to 6.22 ± 0.27.

Formulation which contains only Carbopol 934P as a polymer has shown slightly less surface pH values (5.85 ± 0.26). This study confirmed that the surface pH of the buccoadhesive tablets was near the neutral conditions of saliva and hence would not alter the pH of the buccal fluids and cause no damage or alteration to the buccal mucosa due to altered pH conditions.

Table 7.4 Surface pH of CCT1 to CCT11.

Formulation Code	Surface pH
CCT1	6.21 ± 0.15
CCT2	6.12 ± 0.19
CCT3	6.13 ± 0.13
CCT4	6.19± 1.68
CCT5	6.20 ± 0.13
CCT6	6.22 ± 0.27
CCT7	6.15 ± 0.20
CCT8	6.09 ± 0.17
CCT9	6.05 ± 0.19
CCT10	6.01 ± 0.13
CCT11	5.85 ± 0.26

± R.S.D. (n=3)

7.3 Swelling:

Swelling is the prerequisite for the mucoadhesive dosage form to adhere to the buccal mucosa. Adequate or satisfactory swelling values of the formulation can be decided on the basis of satisfactory mucoadhesion (>35 x 10^3 dyne cm^{-2}) (Adel et al.., 2004) because primarily mucoadhesion depends upon swelling (Valenta, 2005). The % swelling of the core in cup buccoadhesive tablets in phosphate buffered saline pH 6.8 was investigated. It can be seen from swelling data shown in Table 7.5, Fig 7.1 and 7.2, that the Carvedilol buccoadhesive tablets displayed 3.78 ± 0.31 to 5.36 ± 0.39 % swelling. In general, maximum % swelling was achieved after 2 to 4 hr of study. Formulation with HPMC K4M and Carbopol 934P alone showed a comparatively higher swelling as compared to all other formulations i.e. 5.28 ± 0.28 and 5.36 ± 0.39 swelling after 8 hr by CCT1 and CCT11 respectively. Formulations with combination of HPMC and Carbopol 934P showed 3.78 to 4.72 % swelling but it was low as compared to formulations where HPMC K4M and Carbopol 934P were used alone. Comparatively least swelling was observed in CCT9 (Carbopol 934P: HPMC K4M, 9:1) i.e. 3.78 ± 0.31 % which may be due to less uptake of water.

It was observed in CCT1 and CCT11, where HPMC K4M and Carbopol 934P were used alone that there was formation of highly porous structure at the end of study. Ugwoke M. I et al., reported that formation of highly porous structure may loosen adhesive bonds with mucosa and results in weaker adhesion (Ugwoke et al., 2005). In CCT11 where only Carbopol 934P was present, different swelling pattern was seen as compared to other formulations i.e. sharp rise in swelling index after 4th hr of study. This may be due to its ionization constant. At pH 6.8, Carbopol 934P will get ionized, which will loosen the polymer integrity/matrix and result in high swelling.

Table 7.5 Swelling studies of CCT1 to CCT11.

Formulation Code	% Swelling			
	2 Hr	4 Hr	6 Hr	8 Hr
CCT1	1.89 ± 0.14	2.98 ± 0.21	4.01 ± 0.18	5.28 ± 0.28
CCT2	1.48 ± 0.18	2.54 ± 0.19	3.80 ± 0.16	4.72 ± 0.21
CCT3	1.41 ± 0.15	2.41 ± 0.18	3.68 ± 0.21	4.68 ± 0.24
CCT4	1.35 ± 0.18	2.37± 0.23	3.54 ± 0.24	4.53 ± 0.29
CCT5	1.27 ± 0.17	2.29 ± 0.24	3.42 ± 0.21	4.41 ± 0.34
CCT6	1.21 ± 0.13	2.21 ± 0.28	3.32 ± 0.28	4.37 ± 0.37
CCT7	1.10 ± 0.12	2.15 ± 0.29	3.21 ± 0.24	4.09 ± 0.39
CCT8	1.04 ± 0.18	2.06 ± 0.25	3.11 ± 0.24	4.01 ± 0.38
CCT9	0.98 ± 0.18	1.91 ± 0.12	3.02 ± 0.29	3.78 ± 0.31
CCT10	0.85 ± 0.14	1.79 ± 0.23	2.91 ± 0.31	4.31 ± 0.21
CCT11	0.78 ± 0.16	1.51 ± 0.14	3.47 ± 0.37	5.36 ± 0.39

± R.S.D. (n=3)

Fig 7.1 – Swelling Profile for CCT1 to CCT6.

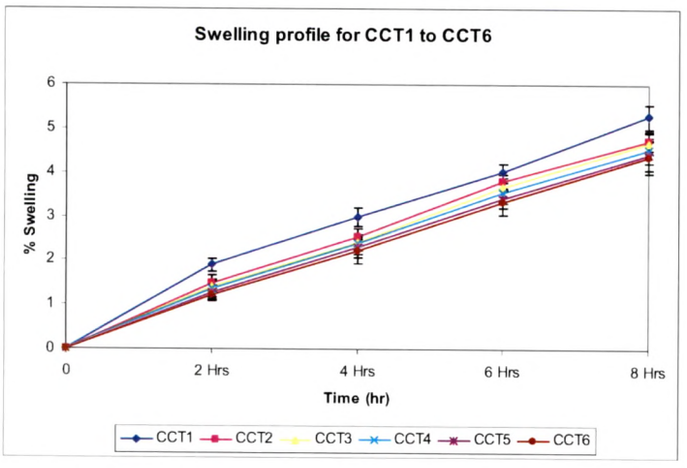
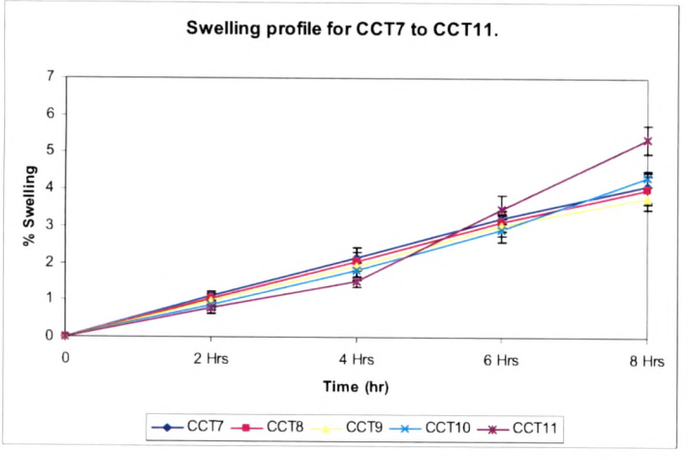


Fig 7.2 – Swelling Profile for CCT7 to CCT11.



7.4 In vitro Mucoadhesive force:

Mucoadhesion is the first and foremost important and significant prerequisite for the mucoadhesive drug delivery to adhere to mucosa. In this study, sheep buccal mucosa was used as biological membrane to investigate the effect of different polymeric combinations used in formulation on mucoadhesive force. Table 7.6 and Fig 7.3 show in vitro mucoadhesive force for CCT1 to CCT11.

Buccal core in cup tablets containing Carbopol 934P and HPMC K4M at the ratio of 6:4 (CCT7) exhibited comparatively highest mucoadhesive force (50 $\pm$ 2.45 x 10^3 dyne cm^{-2}) with the buccal mucosa when compared with other formulations. However, the formulations

CCT3 to CCT9 exhibited adequate mucoadhesive force in the range of 40 ± 2.89 to 50 ± 2.45 x 10^3 dyne cm^{-2} with buccal mucosa.

Mucoadhesive force is dependent on many parameters, including the % swelling, pH of medium and degree of ionization of polymer. Moreover, each of the polymers under consideration is known to exhibit optimum mucoadhesive strength at a well-defined state of swelling. Consequently, a change in any of these variables may yield different mucoadhesive strengths (Kockisch et al., 2003).

Formulation CCT11 which contains only Carbopol 934P showed weak mucoadhesive force (27 ± 2.36 x 10^3 dyne cm^{-2}) which may be because mucoadhesive force of Carbopol 934P is dependent on the pH of surrounding medium. The pH of the buffer solution used in the present study was 6.8, which presumably could have decreased the mucoadhesive force because of the change in the ionization property of carboxylic groups present in Carbopol 934P [pKa of Carbopol 934P is 6.5 (Shojaei and Li, 1997)]. Desai K. G. H. et al., also found weak mucoadhesive force for formulation where only Carbopol 934P was used in the tablets (Desai and Pramodkumar, 2004).

On the contrary, other researchers have reported maximum mucoadhesive force for tablets containing Carbopol alone but they have used different grades of Carbopol e.g. Carbopol 980. Carbopol 980 is having the highest molecular mass, high degree of cross-linking and found to adhere to the mucosa for the longest time period among the polyacrylates (Grabovac et al., 2005, Agarwal and Mishra, 1999).

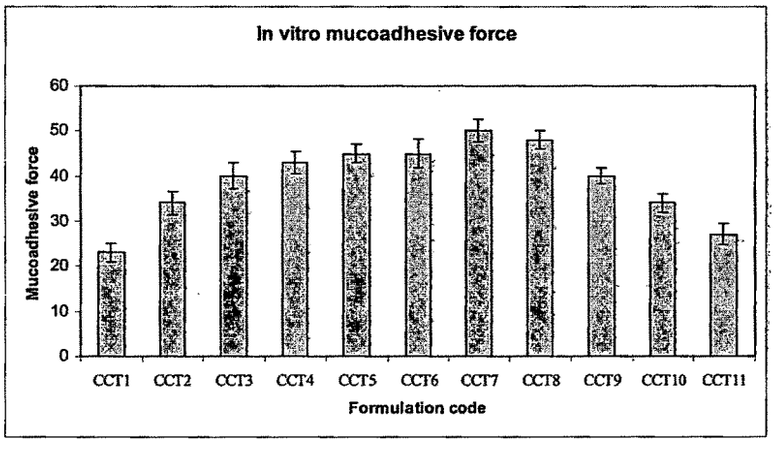
Swelling affects the mucoadhesive force of formulation as it was seen in CCT11 (Valenta C., 2005). Reason behind less mucoadhesive force of formulation CCT11 was its high % swelling (5.36 ± 0.39 %) which might affect its mucoadhesive force (27 ± 2.36 x 10^3 dyne cm^{-2}). Formulation CCT1 where only HPMC was used also showed weak mucoadhesive force (23 ± 2.13 x 10^3 dyne cm^{-2}) might be because of high swelling of polymer (5.28 ± 0.28 %) which weakened its mucoadhesive force. CCT7 exhibited comparatively highest mucoadhesion (50 ± 2.45 x 10^3 dyne cm^{-2}) and displayed 4.09 ± 0.39 % of swelling.

Table 7.6 In vitro mucoadhesive force for CCT1 to CCT11.

Formulation Code	In vitro mucoadhesive force (x 10^3 dyne cm^{-2})
CCT1	23 ± 2.13
CCT2	34 ± 2.56
CCT3	40 ± 2.89
CCT4	43 ± 2.47
CCT5	45 ±1.98
CCT6	45 ± 3.21
CCT7	50 ± 2.45
CCT8	48 ± 2.11
CCT9	40 ± 1.87
CCT10	34 ± 2.11
CCT11	27 ± 2.36

± R.S.D. (n=3)

Fig 7.3 In vitro mucoadhesive force for CCT1 to CCT11.



7.5 *In vitro* diffusion:

To study the *in vitro* diffusion of the prepared formulations, Franz diffusion cell was used. Table 7.7 and Fig 7.4 show the *in vitro* diffusion for CCT1 to CCT11 studied through sheep buccal mucosa at 8^{th} hr and pure drug diffusion for 2 hr.

Formulations containing HPMC K4M (CCT1) and Carbopol 934P (CCT11) alone showed highest diffusion (84.98 ± 2.89 and 84.12 ± 2.23 %) respectively as compared to other prepared formulations. The combination of Carbopol 934P and HPMC K4M (CCT2 to CCT10) showed less diffusion of drug as compared to formulations where Carbopol 934P

and HPMC K4M were used alone. It implies that combination of polymers have better control over drug diffusion than individual polymers because combination of polymers imparts better matrix structure to tablets which will decrease the diffusion of Carvedilol. Sustained release of Carvedilol can be expected from combination of Carbopol 934P and HPMC K4M than with individual polymers. Formulations (CCT2 to CCT10) with combination of polymers at different ratios showed 70.97 ± 4.01 to 84.00 ± 3.56 % diffusion.

Pure drug diffusion has shown 93.02 ± 2.93 % in 2 hr. Carvedilol being a BCS – class II drug having a lipophilic property has itself showed a higher permeation and diffusion because of its higher flux (6.09 ± 0.43 x 10^{-6} µg cm^{-2} min^{-1}) at pH 6.8 as shown by preformulation studies.

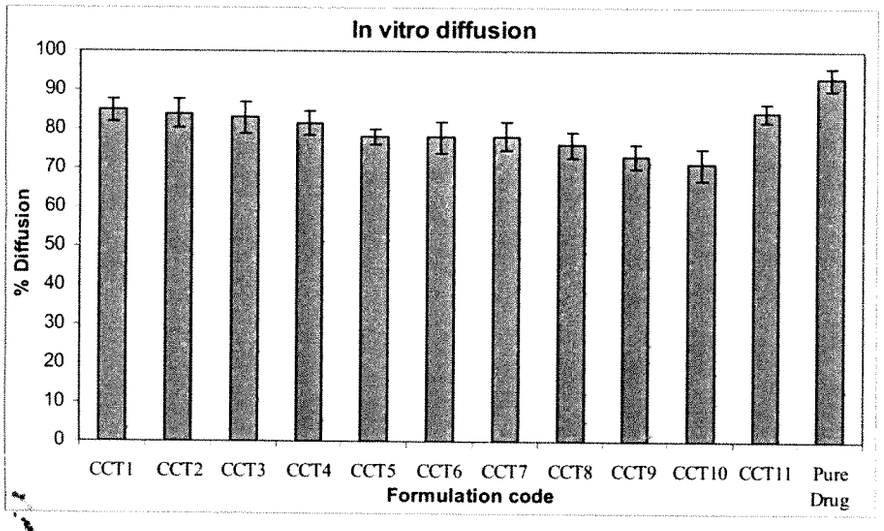
If the drug has to diffuse through the matrix, the polymeric chains must first arrange (relax) to allow the diffusion process. In this way, the chain mobility is decisive for drug transfer kinetic, so diffusion rate increases with increase in swelling rate of polymeric chains (Siepmann et al., 1999). This is reflected in current study as CCT1 and CCT11 shows 5.28 ± 0.28 % and 5.36 ± 0.39 % swelling exhibited 84.98 ± 2.89% and 84.12 ± 2.23 % diffusion respectively while CCT9 shows less % swelling (3.78 ± 0.31) resulted in slightly lower diffusion (73.00 ± 2.99 %)

Table 7.7 In vitro diffusion for CCT1 to CCT11 and pure drug at 8th hr.

Formulation Code	In vitro diffusion (%)
CCT1	84.98 ± 2.89
CCT2	84.00 ± 3.56
CCT3	83.00 ± 4.00
CCT4	81.56 ± 3.01
CCT5	78.01 ± 2.00
CCT6	77.96 ± 4.00
CCT7	78.23 ± 3.56
CCT8	75.98 ± 3.12
CCT9	73.00 ± 2.99
CCT10	70.97 ± 4.01
CCT11	84.12 ± 2.23
*Pure Drug	93.02 ± 2.93

± R.S.D., (n=3), * = Study conducted for 2hr

Fig 7.4 In vitro diffusion for CCT1 to CCT11 and pure drug



7.6 In vitro drug dissolution:

In vitro dissolution of pure drug and formulations (CCT1 to CCT11) is shown in Fig 7.5, 7.6 and 7.7. Table 7.8a and Table 7.8b shows % in vitro dissolution. Maximum Carvedilol dissolution was observed in CCT1; it released 88.00 ± 3.00 % in 6 hr and 100 ± 3.14 % of the drug in 7 h. The fastest rate of drug dissolution was exhibited by formulations containing HPMC K4M alone (CCT1), which could be attributed to its high swelling (5.28 ± 0.28 %), as greater swelling of the matrices leads to faster diffusion of the drug and results in faster dissolution of drug (Agarwal and Mishra, 1999).

Formulation (CCT11) containing Carbopol 934P alone also showed high dissolution of drug, It released 87.44 ± 3.11 % in 6 hr and 100 ± 2.60 % in 7 hrs also due to its high swelling (5.36 ± 0.39 %). During this study it was observed that formulation CCT11 did not maintain its integrity and tended to collapse. The possible reason behind it is that there might be ionization of Carbopol 934P at experimental pH 6.8, which is higher than its ionization constant (pKa) 6.0. This ionization process will lead to development of negative charges at the polymer surface and due to this; polymer state will change into an extended structure allowing water molecules to penetrate into it, leading to higher swelling. Desai et al. found high cumulative dissolution for the formulation containing only Carbopol 934P as a polymer as compared to other formulations where combination of Carbopol 934P and HPMC K4M

was used (Desai K. G. H., 2004). Formulations (CCT2 to CCT10) where combinations of polymers were used extended the drug dissolution up to 8th hr. This may be because combination of two polymers imparts better matrix characteristics to the tablets than individual polymer. Strong matrix integrity will inhibit the entry of dissolution media and delay the release of drug. Singh et. al. reported that combination of Carbopol and HPMC fairly regulated the Metoprolol tartarate release up to 10 hr (Singh and Ahuja, 2002).

To investigate the kinetics of Carvedilol dissolution from bilayered buccal tablets, the dissolution data was applied to zero order, first order, Higuchi (suited for the modeling of drug release from a homogeneous planar matrix, assuming that the matrix does not dissolve), Hixson-Crowell (models drug release from systems with dissolution-rate limitations) and Korsmeyer Peppas (diffusion and polymer relaxation phenomena or anomalous transport) models and best fit was determined (Kockisch et al., 2005). The values of r^2, K and n are listed in Table 7.9. It is known that, if the values of n are in between 0 – 0.5, then it follows fickian diffusion and if n values lies in between 0.5 -1.0, it supports non-fickian diffusion pattern (Dortunc et al., 1998, Costa and Lobo, 2001).

The results indicate that the dissolution mechanism changed with the type and amount of polymer incorporated in the formulation and this can be reflected by the observed values of release exponent. CCT1, which contains only HPMC K4M, n value was 0.72; indicating non-fickian release i.e. drug dissolution is the combination of diffusion and erosion. CCT11 where only HPMC is present showed n value as 0.74, followed non-fickian release.

When concentration of Carbopol 934P was gradually increased in the formulation, n values were found to increase from 0.72 to 1.02. Formulations CCT1 to CCT5, CCT7 and CCT11 showed n values below 1.00 where it follows non-fickian pattern indicates diffusion is the dominant release mechanism. If the n values greater than 1.00 then it follows non-fickian release with super case II transport (Ritger and Peppas, 1987, Jug and Becirevic-Lacan, 2004). Therefore formulations CCT6, CCT8 to CCT10 follows nonfickian diffusion pattern with super case II transport i.e. dissolution is the combination of diffusion and minor contribution of chain relaxation (Singh B. and Ahuja N., 2002). Dissolution pattern followed nearly zero order pattern. Formulation CCT7 showed best fit as it showed $r^2 = 0.995$ for zero order.

Table 7.8a – In vitro dissolution of pure drug, CCT1 to CCT5.

Formulation Code	Pure Drug	CCT1	CCT2	CCT3	CCT4	CCT5
Time (hr)	% Carvedilol dissolution					
1	89.23±2.23	19.01 ±2.01	16.00±2.01	14.89±2.11	13.00±1.88	12.24±2.89
2	100±3.45	31.00 ±3.0	24.88±2.23	21.00±2.56	19.13±3.15	17.09±3.02
3	-	49.00±3.0	45.98±2.89	43.03±3.14	37.89±3.17	35.00±3.11
4	-	69.89±3.15	59.01±3.12	54.97±3.01	51.00±3.47	47.89±3.65
5	-	76.01±3.56	73.00±2.24	71.88±1.87	54.99±3.16	68.95±3.42
6	-	88.00±3.00	81.99±2.12	80.11±2.15	81.00±3.18	82.00±3.13
7	-	100.00±3.14	92.33±3.40	91.12±3.15	96.00±3.5	92.23±2.85
8	-	-	99.23±3.12	98.89±3.84	99.15±3.45	98.12±3.84

Table 7.8b – In vitro dissolution of CCT6 to CCT11.

Formulation Code	CCT6	CCT7	CCT8	CCT9	CCT10	CCT11
Time (hr)	% Carvedilol dissolution					
1	11.00±2.89	10.02±1.87	10.89±3.12	11.00±3.00	12.25±3.11	18.09±2.56
2	20.21±2.35	24.99±2.00	17.67±3.14	19.78±3.47	15.36±2.60	30.89±3.00
3	34.98±3.00	39.00±3.47	31.00±3.00	28.00±3.15	28.69±2.36	48.23±3.15
4	44.87±3.11	47.02±3.84	41.10±3.01	41.01±3.00	42.23±2.45	59.87±1.87
5	65.09±3.14	59.98±3.12	61.93±1.87	63.98±3.11	68.01±2.69	75.34±2.00
6	80.11±2.56	69.00±2.56	78.00±2.00	68.96±2.56	77.12±2.56	87.44±3.11
7	89.63±2.85	82.12±2.85	98.11±3.71	97.00±2.00	98.12±1.99	100±2.60
8	98.00±3.1	92.25±3.01	98.12±3.84	98.47±3.00	99.81±2.85	-

Fig 7.5 In vitro drug dissolution of pure drug, CCT1, CCT2, CCT3 and CCT4.

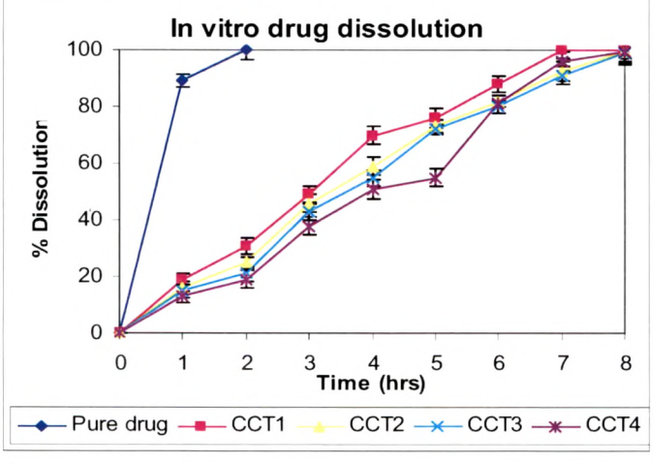


Fig 7.6 In vitro drug dissolution of pure drug, CCT5, CCT6, CCT7 and CCT8.

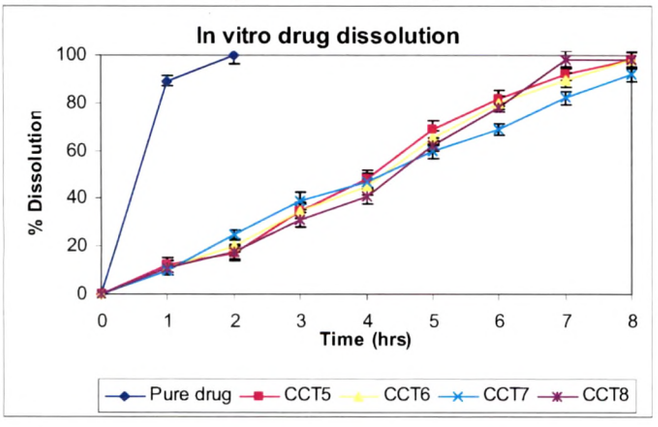


Fig 7.7 In vitro drug dissolution of pure drug, CCT9, CCT10 and CCT11.

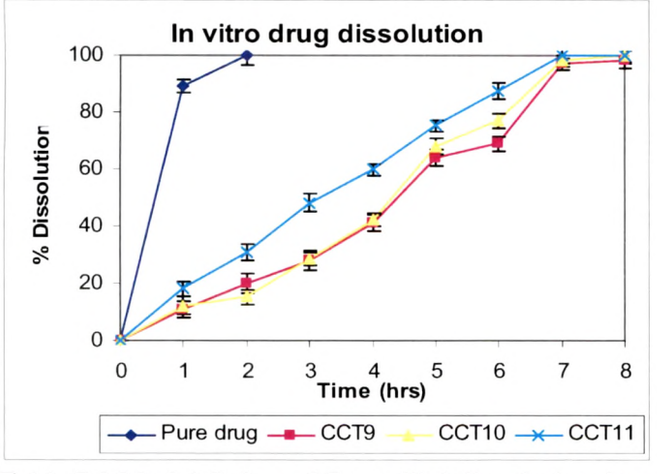


Table 7.9 Model fitting of Carvedilol dissolution from core in cup buccal tablets

F. Code	K_0	r^2	K_1	r^2	K_H	r^2	K_S	r^2	K_k	r^2	n
CCT1	12.32	0.961	0.29	0.860	48.56	0.983	0.28	0.912	0.85	0.985	0.72
CCT2	12.40	0.981	0.16	0.941	48.54	0.989	0.30	0.929	0.92	0.985	0.81
CCT3	12.69	0.982	0.23	0.819	49.49	0.983	0.31	0.934	0.98	0.974	0.86
CCT4	13.01	0.987	0.21	0.858	50.52	0.978	0.33	0.947	1.05	0.979	0.93
CCT5	13.28	0.985	0.21	0.884	51.37	0.970	0.34	0.951	1.09	0.972	0.97
CCT6	13.22	0.989	0.19	0.863	51.02	0.969	0.35	0.956	1.13	0.976	1.01
CCT7	**11.47**	**0.995**	**0.13**	**0.903**	**44.59**	**0.989**	**0.31**	**0.934**	**1.03**	**0.990**	**0.99**
CCT8	13.98	0.977	0.25	0.804	53.56	0.943	0.36	0.973	1.14	0.981	1.02
CCT9	13.62	0.972	0.24	0.788	52.03	0.934	0.35	0.976	1.11	0.980	1.01
CCT10	14.25	0.972	0.34	0.754	54.60	0.938	0.37	0.964	1.14	0.952	1.02
CCT11	12.52	0.978	0.15	0.942	49.02	0.986	0.28	0.935	0.86	0.994	0.74

F. Code: Formulation code

7.7 Pharmacokinetic Study

The plasma concentration profile for buccal core in cup tablets in rabbits were measured and shown in Table 7.10 Plasma concentration profile is shown in Fig 7.8. The plasma concentration profile for buccal core in cup tablets showed sustained release of Carvedilol, as evident by observed t_{max} and plasma concentration profile of oral and buccal formulations. The t_{max} was observed to be 4.0 hr for buccal core in cup tablets as compared to 1.00 hr for oral tablets. After the administration of Carvedilol oral conventional tablets, t_{max} was observed 1.00 hr indicated a rapid absorption. t_{max} of buccal core in cup tablets was 4.00 hr shows slow absorption but it was sustained which was seen by plasma concentration profile. The C_{max} values observed were also higher (64.14 ± 7.36 ng/ml) for core in cup tablets than oral tablets (58.25 ± 4.26 ng/ml).

The AUC values (297.53 ± 8.20 ng/ml/hr) after buccal administration of core in cup tablets was significantly higher than that of oral administration (152.22 ± 8.43 ng/ml/hr) which indicates increase in bioavailability of the buccal formulations. Administration of Carvedilol by core in cup tablets through buccal route to rabbits, showed about 1.95 fold increase in bioavailability compared with the conventional tablets by oral administration. The one way ANOVA test showed statistically significant differences ($P < 0.01$) between the AUC of oral conventional tablets and buccal core in cup tablets.

The absorption from the buccal core in cup tablets in the initial phase appeared to be slightly slow i.e. 14.12 ± 3.8 ng/ml concentration at 1st hr. This is may be due to low availability of Carvedilol for initial absorption through buccal mucosa due to matrix structure of tablets. The fast buccal absorption in the latter phase might be explained by swelling of tablets and release of Carvedilol.

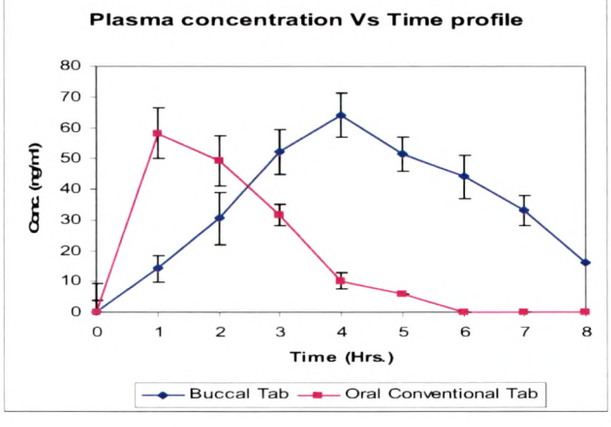
The results obtained in these studies prove the feasibility and significance of administering Carvedilol through the buccal route as a useful alternative to the oral route for avoiding pre-systemic metabolism and improving bioavailability.

Table 7.10 Plasma concentration of Carvedilol following oral administration of conventional tablets and core in cup tablets.

Time (Hr)	Plasma concentration (ng/ml)	
	Oral Tablets	Core in cup Tablets
1	58.25 ± 4.26	14.12± 4.38
2	49.26 ± 8.22	30.54 ±4.26
3	31.55 ± 8.28	52.14 ± 8.52
4	10.11 ± 3.50	64.14 ± 7.36
5	6.07 ± 2.60	51.56 ± 7.01
6	B.LoQ	44.11 ± 5.52
7	B.LoQ	33.00 ±7.10
8	B.LoQ	15.87 ± 4.83
t_{max} (Hr)	**1.0 ± 0.2**	**4.00 ± 1.00**
C_{max} (ng/ml)	**58.25 ± 4.26**	**64.14 ± 7.36**
AUC (ng/ml/hr)	**152.22 ± 8.43**	**297.53 ± 8.20**

± RSD, (n=6), B.LoQ-Below limit of quantitation

Fig 7.8 Plasma concentration Vs Time profile for Carvedilol oral conventional tablets and buccal core in cup tablets.



7.8 Histological studies of buccal mucosa:

7.8.1 Light microscopy of buccal mucosa:

Buccal mucosal sections were stained with hematoxylin eosin (HE) and examined by light microscopy (Olympus) to evaluate any histological changes in the epithelium and the adjacent connective tissues due to buccal administration of the prepared tablets. Control buccal mucosa was also treated similarly and examined. Fig 7.9 and 7.10 shows section of control buccal mucosa and section of sample mucosa.

Section examined by light microscopy reveals three distinct layers of maturation of the oral mucosa based on various regions of the oral cavity. Control buccal mucosa shows all the three distinctive layers of the oral mucosa, the epithelium, basement membrane, and connective tissues.

It was also seen that there was a clear separation of the epithelium from the connective tissue and that the lowermost layer contained cells that were cuboidal, indicative of basal cells. The undulated appearance of this lower layer was indicative of the junction between the epithelium and lamina propria.

Sample mucosa appeared to be slightly different when compared with control mucosa. Sections showed little modification in the epithelial layer. As the permeability barrier of the buccal mucosa has been attributed to the upper one-third of the epithelium (Squier, 1973), slight disruption of the superficial cells due to formulation may have resulted in increased permeability of Carvedilol. The epithelium and basal membrane, the principal components of the permeation barrier, appeared same as control at the end of the study.

It is clear from the observations of the sections examined by light microscopy that the buccal formulation provoked no major alteration in the barrier function of the mucosa.

Fig 7.9 Section of control buccal mucosa.

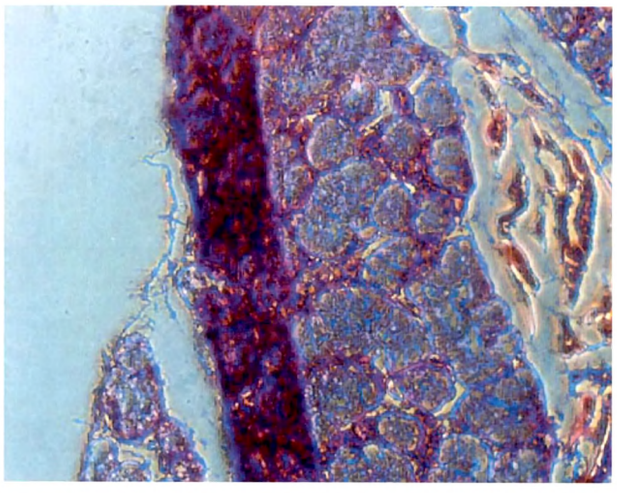
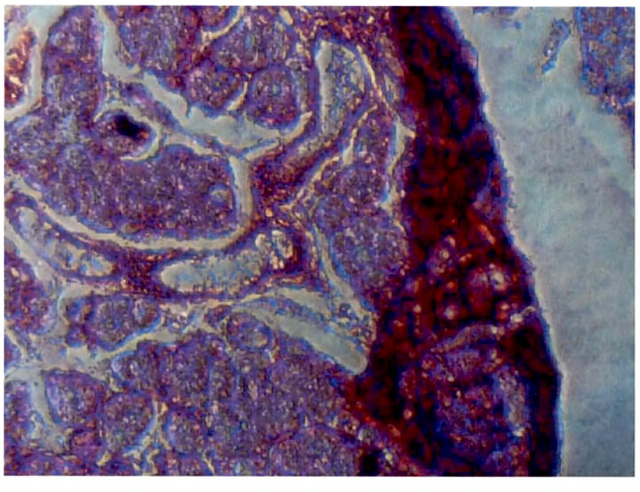


Fig 7.10 Section of sample mucosa.



7.8.2 Scanning Electron Microscopy of buccal mucosa:

At the end of the diffusion experiment, the buccal mucosa was collected, washed and treated as per the protocol given in experimental part. Fig 7.11 and 7.12 shows SEM of control buccal mucosa and SEM of sample buccal mucosa respectively.

SEM of the control buccal mucosa showed the presence of the superficial cells of the epithelium which represents the major absorption site in the oral cavity. It also shows that stratified squamous cells have intact cell junctions with microridges. These observations are in accordance with Attia and coworkers (Attia et al., 2004) who reported that the control

buccal mucosa showed presence of the superficial cells of the epithelium and stratified squamous cells with microridges or micropillee.

Sample buccal mucosa showed that the squamous cells are normal and to some extent similar to those of the control. But, slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue. In fact, diffusion of Carvedilol through the oral mucosal membrane resulted in a highly permeable tissue and that can be seen from the shrinkage of squamous cells in sample mucosa. **From available literature it can be expected that these slight changes may be reversible and will not affect overall structure, surface and function of the buccal mucosa** (Attia et al., 2004).

Fig. 7.11 SEM of Control Buccal Mucosa:

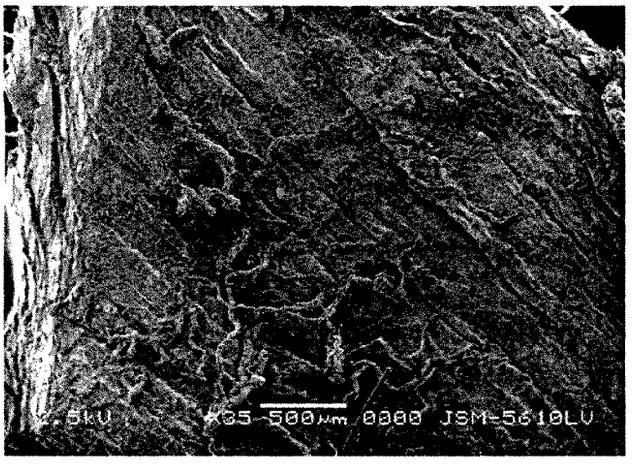
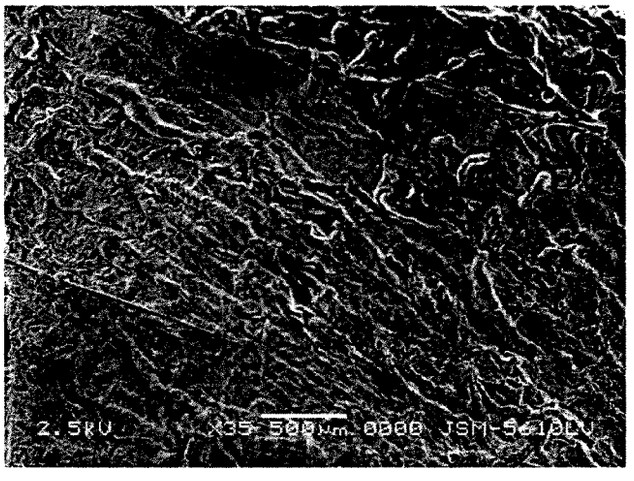


Fig. 7.12 SEM of Sample Buccal Mucosa:



7.9 *In vivo* acceptability testing:

This study documented the response of human volunteers to some of the parameters like comfort ability, irritation, taste, dryness of mouth, salivary secretion, heaviness of tab at the application site and dislodgement of system, associated with the *in vivo* feasibility and acceptability of the core-in-cup tablets in the oral cavity. The response of volunteers to each subjective parameter was calculated, and obtained results are presented in Table 7.11.

Volunteer's response in irritation criteria showed that 80% had no complaint while 20% volunteers expressed slight irritation. In comfort testing, 70% and 30% volunteers reported comfortable and slightly comfortable levels respectively. None of the volunteers reported moderately uncomfortable and severely uncomfortable levels.

Dryness of mouth was not experienced by any of the volunteers. 10% of volunteers did not experience salivary secretion and 90% reported slight salivary secretion. When volunteers were asked to express their views on heaviness of tablets at the application site, 90 % experienced no heaviness while 10% reported slight heaviness. None of the volunteers reported dislodgement of the system during the study for 6 hr.

Based on above results, it can be concluded that the core in cup tablets would be comfortable and acceptable by the patients and would be retained in the buccal cavity long enough for the complete drug release to occur.

Table 7.11 Evaluation criteria and results for *in vivo* acceptability study.

Criteria	Volunteer's response (%)
Irritation	
None	80
Slight (Tolerated)	20
Moderate	-
Severe (Not tolerated)	-
Comfort	
Very comfortable	-
Comfortable	70
Slightly uncomfortable	30
Moderately uncomfortable	-
Severely uncomfortable	-
Dryness of mouth	
None (Not experienced)	100
Slight (Tolerated)	-
Moderate	-
Severe (Not tolerated)	-
Salivary Secretion	
None (Not experienced)	10
Slight (Tolerated)	90
Moderate (Feeling of discomfort)	-
Severe	-
Heaviness of tablets at the application site	
None (Not experienced)	90
Slight (Tolerated)	10
Moderate (Feeling of discomfort)	-
Severe (Not tolerated)	-
Dislodgement of the system during study (upto 6 hr)	
No	100
Yes	-

7.10 Pharmacodynamic studies:

These studies were divided into 2 stages,

Stage 1: Development of hypertension for 6 weeks.

Stage 2: Treatment with oral conventional tablets and buccal core in cup tablets.

7.10.1 Development of fructose induced hypertension:

Hypertension shows elevation of arterial pressure, heart rate, body weight and triglyceride levels. Parameters like mean arterial pressure (MAP), heart rate (HR/min), body weight (g) and triglyceride levels (mg/dl) were measured at start and at every week during the study. At the end of 6 weeks of fructose administration, the MAP of treatment group was 149 ± 08 mm Hg, as compared to 122 ± 08 mm Hg in the rats before the fructose intake and MAP of control group was observed to be 122 ± 08 mm Hg. Control group maintained their MAP, HR/minute, body weight and triglyceride level without major changes. After 6 weeks, HR/minute and body weight in treatment group were found to be 410 ± 19 and 260 ± 24 gm as compared to 354 ± 25 and 218 ± 28 gm for the control group respectively. Triglycerides level was also increased in treatment group (210 ± 26 mg/dl) as compared to control (92 ± 26 mg/dl) at the end of 6 weeks.

Thus fructose induced hypertension was developed in rats after 6 weeks. These hypertensive rats were then used for studying effect of Carvedilol when administered in the form of conventional oral as well as buccal core in cup tablets.

Table 7.12 Hypertensive parameters for Control (without fructose intake) group.

Parameters	At start	During study					
		1W	2W	3W	4W	5W	6W
MAP (mm Hg)	121 ± 07	122 ± 09	122 ± 07	124 ± 09	124 ± 10	122 ± 08	122 ± 08
HR/min	354 ± 27	355 ± 26	357 ± 26	356 ± 19	359 ± 26	358 ± 17	354 ± 25
Body Weight (gm)	214 ± 18	215 ± 17	215 ± 19	214 ± 23	218 ± 15	218 ± 24	218 ± 28
Triglyceride (mg/dl)	87 ± 19	NM	NM	NM	NM	NM	92 ± 26

± RSD, NM – Not measured. (n = 6)

Fig 7.13 Graphical representation of hypertensive parameters for Control (without fructose intake) group.

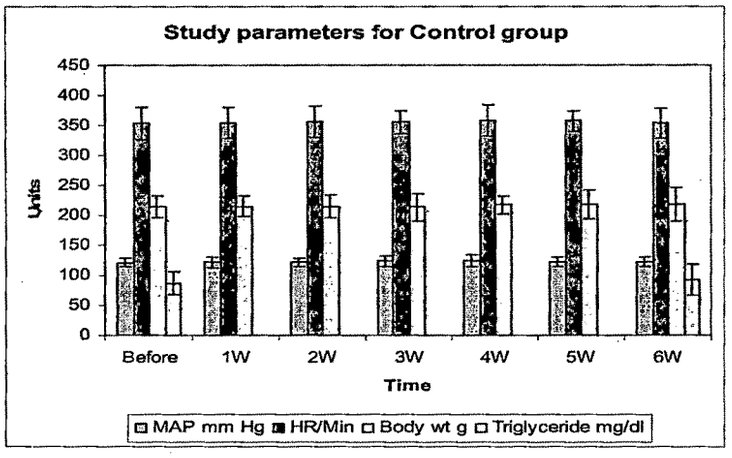
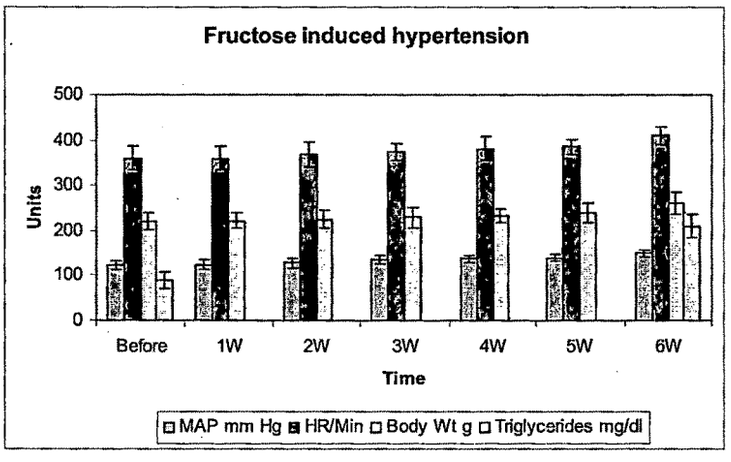


Table 7.14 Hypertensive parameters for treatment (fructose intake) group

Parameters	At start	During study					
		1W	2W	3W	4W	5W	6W
MAP (mm Hg)	122 ± 09	124 ± 10	128 ± 10	134 ± 09	137 ± 08	139 ± 08	149 ± 08
HR/min	360 ±27	360 ± 26	368 ± 28	374 ± 19	381 ± 26	385 ± 17	410 ± 19
Body Weight (gm)	220 ±18	221 ± 17	225 ± 19	230 ± 23	233 ± 15	239 ± 21	260 ± 24
Triglyceride (mg/dl)	90 ± 18	NM	NM	NM	NM	NM	210 ± 26

± RSD, NM – Not measured. (n = 6)

Fig 7.14 Graphical representation of hypertensive parameters for test (fructose intake) group.



7.10.2 Treatment of hypertension with oral conventional tablets and buccal core in cup tablets.

After developing hypertension in rats, they were treated with oral conventional and buccal core-in-cup tablets and observations are recorded in Table 7.15 and 7.16. Table 7.17 gives % reduction in hypertensive parameters. Fig 7.15, 7.16, 7.17 and 7.18 shows comparative evaluation of MAP, heart rate, triglycerides and body weight respectively after administration of oral conventional and buccal core in cup tablets.

When the hypertensive group was treated with oral conventional tablets for 2 weeks, slight reduction of hypertension was found (Table 7.15). At the end of 2 weeks, observed MAP, HR and body weight were 136 ± 09 mmHg, 389 ± 29 /min and 235 ± 21 gm respectively. Triglyceride levels were found to be 139 ± 16 mg/dl. When second hypertensive group of rats was treated with core in cup buccal tablets, considerable reduction of hypertension was found. The values obtained for MAP, HR and body weight were 117 ± 11 mmHg, 363 ± 28 /min and 211 ± 19 gm respectively. Triglyceride levels were found to be 98 ± 14 mg/dl. The one way ANOVA test (Table 7.18) showed statistically significant differences ($P < 0.001$) between the results of oral conventional tablets and buccal core in cup tablets. Statistical significance between oral conventional tablets and core in cup tablets was same for 1st week. In 2nd week oral conventional tablets show significance only for triglycerides while core in cup tablets show significance for all parameters except HR.

Hypertensive parameters were also compared in terms of percent reduction by administering oral conventional and buccal core in cup tablets. At the end of 2 weeks, reduction in MAP (mm Hg) was found 8.72 and 21.47% by oral conventional and buccal core in cup tablets respectively. Reduction in HR/min was found to be 5.12 and 11.46 % while 9.61% and 18.84% reduction was found in body Weight (gm) by oral conventional and buccal core in cup tablets respectively. Similarly triglycerides levels were found to be reduced up to 33.80 and 53.33 % by oral conventional and buccal core in cup tablets respectively. This clearly indicated that buccal core in cup tablets provided better antihypertensive treatment as compared to oral conventional tablets.

Buccal core in cup tablets decreased the BP significantly as compared to oral conventional tablets and the effect continued for 8-10 hours as seen by plasma concentration profile by

pharmacokinetic studies. This clearly indicates that the buccal core in cup tablets release the drug gradually over a period of time, which results in prolonged control of hypertension.

Oral Carvedilol is rapidly and extensively absorbed following oral administration, but has absolute bioavailability of approximately 25% to 35% due to a significant degree of first-pass metabolism. In studies that compared the acute haemodynamic effects of oral Carvedilol to baseline measurements in patients with congestive heart failure, there was significant reduction in mean arterial pressure, and heart rate (Weir and Darjie, 2005). The current study revealed increase in therapeutic activity of Carvedilol when it was administered through buccal route which bypasses the first pass metabolism, and hence resulted in increased bioavailability (as evidenced by pharmacokinetic studies). Buccal core in cup tablets being sustained release dosage form showed optimized treatment by a sustained control over a blood pressure.

Thus, the results showed that buccal core-in-cup tablets is more effective in the treatment for hypertension when compared with oral conventional tablets.

Table 7.15 Treatment with oral conventional tablets

Conventional Tablets			
Parameters	Before Treatment (Initial)	After Treatment	
		1W	2W
MAP (mm Hg)	149 ± 08	142 ± 08	136 ± 09
HR/min	410 ± 19	395 ± 19	389 ± 29
Body Weight (gm)	260 ± 24	249 ± 17	235 ± 21
Triglyceride (mg/dl)	210 ± 26	181 ± 16	139 ± 16

(± R. S. D.) (n=6).

Table 7.16 Treatment with buccal core in cup tablets

Core in cup tab			
Parameters	Before Treatment (Initial)	After Treatment	
		1W	2W
MAP (mm Hg)	149 ± 08	140 ± 09	117 ± 11
HR/min	410 ± 19	383 ± 26	363 ± 28
Body Weight (gm)	260 ± 24	239 ± 17	211 ± 19
Triglyceride (mg/dl)	210 ± 26	161 ± 18	98 ± 14

(± R. S. D.) (n=6)

Table 7.17 - % Reduction in Hypertensive Parameters

% Reduction in Hypertensive Parameters				
Parameters	Oral conventional tablets		Buccal core in cup tablets	
	1W	2W	1W	2W
MAP (mm Hg)	4.69	**8.72**	6.04	**21.47**
HR/min	3.65	**5.12**	6.58	**11.46**
Body Weight (gm)	4.23	**9.61**	8.07	**18.84**
Triglyceride (mg/dl)	13.80	**33.80**	23.33	**53.33**

Table 7.18 Statistical significance at $p<0.001$ between oral conventional tablets and core in cup tablets.

Statistical significance(p<0.001)				
Parameters	Oral conventional tablets		buccal core in cup tablets	
	1W	2W	1W	2W
MAP (mm Hg)	NS	NS	NS	S
HR/min	NS	NS	NS	NS
Body Weight (gm)	NS	NS	NS	S
Triglycerides (mg/dl)	NS	S	NS	S

NS: Not significant, S= Significant, n=6.

Fig 7.15 Comparative evaluation of MAP after administration of oral conventional and buccal core in cup tablets.

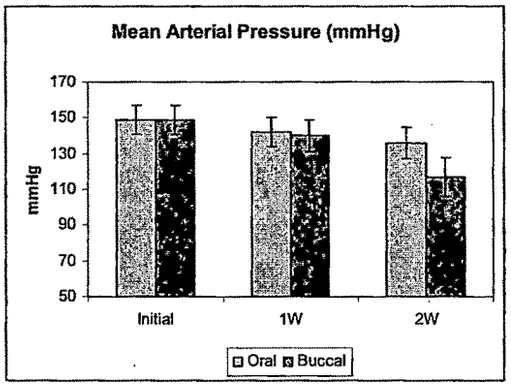


Fig 7.16 Comparative evaluation of heart rate after administration of oral and buccal core in cup tablets.

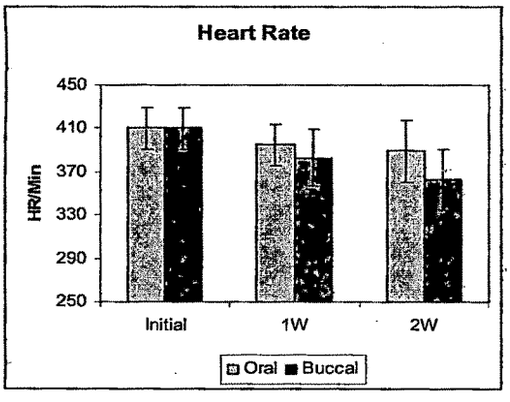


Fig 7.17 Comparative evaluation of triglycerides after administration of oral and buccal core in cup tablets.

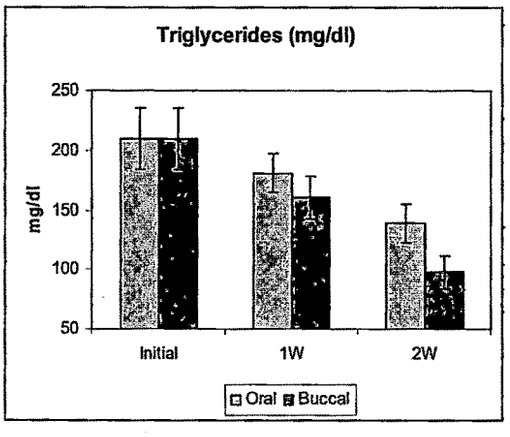
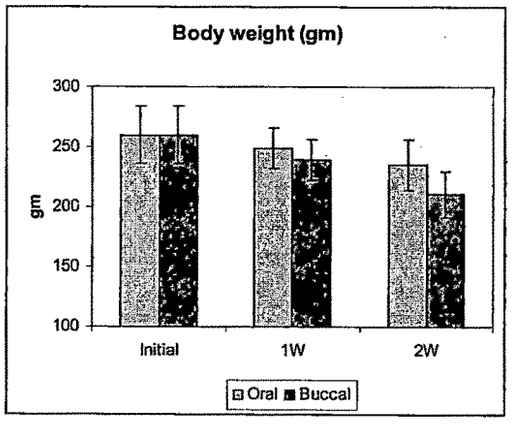


Fig 7.18 Comparative evaluation of body weight after administration of oral and buccal core in cup tablets.



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8.0 RESULTS AND DISCUSSION (CARVEDILOL BILAYER BUCCAL PATCHES-CBP)

8.1 Formulation of Carvedilol bilayer patches

Carvedilol bilayer buccal patches were prepared by solvent casting method which found to be a simple and reliable method for the preparation of the same. Preparation of Carvedilol bilayer buccal patches was divided into two parts,

8.1.1 Preparation of medicated layer:

The process of solvent casting was followed as mentioned in experimental part. Optimization of formulation composition was carried out by varying the ratios of HPMC K4M and Carbopol 934P to get the desired patch characteristics. Once the polymer composition was finalized, propylene glycol as a plasticizer was also investigated in the concentration of 5 to 15 % and optimized in the final formulation. Physicochemical parameters such as diameter, thickness, average weight and assay values are given in Table 8.1. It was found that all the parameters were within acceptable limits.

Table 8.1 Physiochemical parameters of the medicated layer.

Parameters	CBP1	CBP2	CBP3	CBP4	CBP5	CBP6	CBP7	CBP8	CBP9	CBP10	CBP11
Diameter (mm)	14.01 ± 0.13	14.01 ± 0.11	14.01 ± 0.10	14.00 ± 0.09	14.01 ± 0.14	14.02 ± 0.10	14.02 ± 0.13	14.01 ± 0.11	14.01 ± 0.09	14.01 ± 0.13	14.01 ± 0.04
Thickness (mm)	1.02 ± 0.05	1.01 ± 0.04	1.01 ± 0.06	1.01 ± 0.04	1.02 ± 0.05	1.02 ± 0.04	1.02 ± 0.04	1.01 ± 0.06	1.01 ± 0.06	1.01 ± 0.06	1.02 ± 0.04
Average weight (mg)	90.19 ± 1.96	97.38 ± 1.90	91.66 ± 2.13	92.12 ± 0.98	92.22 ± 1.29	91.56 ± 1.66	90.55 ± 1.04	85.88 ± 1.72	89.10 ± 1.02	90.33 ± 1.37	97.98 ± 1.49
Assay (%)	99.12 ± 1.44	99.18 ± 2.12	103.26 ± 1.23	97.22 ± 1.44	97.88 ± 2.24	98.88 ± 1.38	103.15 ± 1.68	98.98 ± 1.33	102.90 ± 1.12	97.31 ± 1.84	98.98 ± 0.99

± R.S.D., (n=3)

Optimization of propylene glycol % in the formulation was evaluated by adding 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 % of propylene glycol. From 2.5 to 7.5 % of propylene glycol, it was found that the patch was brittle and gets cracked. This may create problem at the time of application of patch to the buccal mucosa. When 10% of propylene glycol was used in formulation it was found to give good plasticity to the patch and removal of patch was easy. Patch with 12.5 and 15 % of propylene glycol was very difficult to remove from the casting petry plate because it lacks enough plasticity. Therefore 10% propylene glycol as a plasticizer was selected for further studies.

Table 8.2 Optimization of propylene glycol in formulation:

% Propylene glycol	Patch characteristics
2.5	Patch was very brittle in nature and gets cracked.
5.0	Patch was brittle in nature.
7.5	Patch was somewhat brittle.
10.0	Easy to remove from petry dish.
12.5	Patch was difficult to remove from petry dish.
15.0	Patch was very difficult to remove from the petry dish.

8.1.2 Preparation of backing layer:

The backing layer helps to prevent the washout of drug in to saliva, thus enhancing the adhesion time and reducing drug loss into the oral cavity. The solvent casting method was employed for formulation of backing layer and found to be simple and reliable. The composition for the same is shown in experimental part. After preparation of backing layer it was casted onto already dried medicated layer. Then the whole system was allowed to dry for 24 hr. Special care was taken to prevent the rapid evaporation of solvent from the patches as it will leave dry rough spots on patch. Physical parameters such as thickness, diameter and average weight of the bilayer patches were studied and listed in Table 8.3. As expected final thickness was slightly increased e.g. For medicated layer of CBP1 it was 1.02 ± 0.05 mm and for bilayer patch it was observed to be 1.71 ± 0.10 mm. similarly average weight increased from 90.00 mg to 140.00 mg for the bilayer patches.

Table 8.3 Physical parameters of the bilayer patches

Parameter	CBP1	CBP2	CBP3	CBP4	CBP5	CBP6	CBP7	CBP8	CBP9	CBP10	CBP11
Diameter (mm)	14.20 ± 0.14	14.11 ± 0.12	14.11 ± 0.10	14.01 ± 0.19	13.98 ± 0.16	14.12 ± 0.09	13.91 ± 0.12	14.12 ± 0.09	14.11 ± 0.10	14.11 ± 0.10	14.10 ± 0.08
Thickness (mm)	1.71 ± 0.10	1.79 ± 0.14	1.81 ± 0.10	1.81 ± 0.09	1.76 ± 0.06	1.73 ± 0.11	1.71 ± 0.10	1.70 ± 0.08	1.74 ± 0.08	1.79 ± 0.08	1.75 ± 0.10
Average weight (mg)	135.11 ± 2.11	137.28 ± 1.98	139.67 ± 2.18	133.19 ± 2.02	139.22 ± 1.99	141.56 ± 1.69	141.50 ± 1.74	145.89 ± 2.01	139.11 ± 2.02	140.36 ± 2.07	137.99 ± 1.61

± R.S.D., (n=3)

8.2 Evaluation of Carvedilol bilayer patches

8.2.1 Mechanical properties of Carvedilol bilayer buccal patches:

The mechanical properties exhibits the strength and elasticity of the patch, reflected by the parameters, tensile strength (TS), elastic modulus (EM), elongation at break (E/B), folding endurance (FE) and strain (SN). A soft and weak polymer is characterized by a low TS, EM, FE , SN and E/B; a hard and brittle polymer is defined by a moderate TS, high EM, low FE, low SN and low E/B; a soft and tough polymer is characterized by a moderate TS, low EM, high E/B, FE and SN values; whereas a hard and tough polymer is characterized by a high TS, EM and E/B (Aulton et al., 1981).

Another parameter, Strain (SN) has been used as an indicator of the overall mechanical quality of the film (Peh and Wong., 1999). A high SN value indicates that the film is strong and elastic. Hence, it is suggested by Rowe, R.C., et al that a suitable buccal film should have relatively high TS, E/B and Strain but a low EM (Rowe, 1983). Table 8.4, Fig 8.1 to 8.5 shows mechanical properties of Carvedilol bilayer buccal patches. For patches formulated with higher quantity of Carbopol 934P, increase in Carbopol 934P content was found to initially increase and then reduce the TS and EM. It also increase FE, E/B and SN significantly, indicative of a weaker, more elastic, flexible and softer film. A reverse pattern was seen in the HPMC films indicating different mechanical properties of Carbopol 934P and HPMC K4M. There was no significant decrease in TS when the Carbopol 934P content was increased from 50% to 70%.

The greater elasticity exhibited by films containing higher Carbopol 934P content could be related to its conformation and configuration, which is highly crosslinked (Peh and Wong.,

1999). When Carbopol 934P was increased from 30% to 50%, there was not much increase in the E/B but when it was further increased to 60%, a significant increase in the E/B value was observed, statistically higher than those with 30% Carbopol 934P content.

As for the parameter SN, an increase in the mean value was seen when the Carbopol 934P content was increased to 60% although there was no significant difference between films of 30% and 50% Carbopol 934P. These results indicated that Carbopol 934P generally reduced the strength while increased the softness, elasticity and flexibility of HPMC patches when both the polymers were used simultaneously. FE values increased with increase in Carbopol 934P content in the formulation and exhibited best FE values at Carbopol 934P: HPMC ratio of 6:4. FE values were found to decrease when there was excess amount of Carbopol 934P in the formulation.

Mechanical properties of CBP7 were found to be suitable as it demonstrated relatively high TS (7.92 ± 0.34 $kgmm^{-2}$), high E/B (137.36 ± 7.49 % mm^{-2}) and high Strain (2.01 ± 0.34 kg) but a low EM (3.94 ± 0.11 $kgmm^{-2}$) indicating that the patch had both strength as well as elasticity.

Table 8.4 Mechanical properties of Carvedilol bilayer buccal patches

Formulation code	Tensile Strength ($kgmm^{-2}$)	Elastic modulus ($kgmm^{-2}$)	Elongation at break (% mm^{-2})	Strain (kg)	Folding endurance (no of folds)
CBP1	3.01 ± 0.36	2.36 ± 0.08	42.12 ± 2.56	1.27 ± 0.36	210 ± 21
CBP2	4.12 ± 0.58	5.63 ± 0.10	62.45 ± 2.36	0.73 ± 0.28	224 ± 19
CBP3	7.12 ± 0.87	5.92 ± 0.09	110.26 ± 7.12	1.2 ± 0.29	228 ± 21
CBP4	6.87 ± 0.36	5.98 ± 0.16	114.56 ± 5.63	1.14 ±0.31	239 ± 24
CBP5	5.62 ± 0.69	4.56 ± 0.10	115.23 ± 4.98	1.23 ± 0.35	256 ± 31
CBP6	5.52 ± 0.36	5.69 ± 0.08	117.36 ± 5.32	0.97 ± 0.39	281 ± 26
CBP7	7.92 ± 0.34	3.94 ± 0.11	137.36 ± 7.49	2.01 ± 0.34	360 ± 24
CBP8	6.45 ± 0.56	3.32 ± 0.09	112.23 ± 4.63	1.94 ± 0.29	316 ± 24
CBP9	6.01 ± 0.38	3.65 ± 0.10	98.23 ± 2.89	1.65 ± 0.31	310 ± 18
CBP10	4.23 ± 0..39	2.13 ± 0.12	74.23 ± 2.11	1.98 ± 0.39	281 ± 26
CBP11	3.88 ± 0.41	2.05 ± 0.14	69.12 ± 1.92	1.89 ± 0.31	218 ± 19

± R.S.D., (n=3)

Fig 8.1 Tensile strength for CBP1 to CBP11.

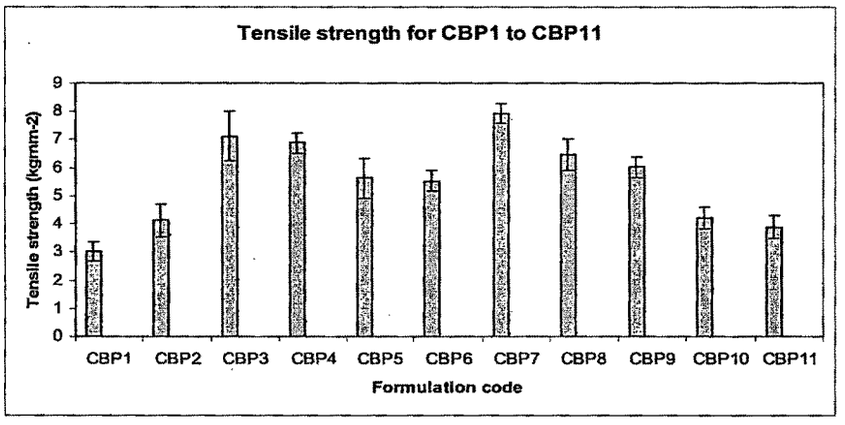


Fig 8.2 Elastic modulus for CBP1 to CBP11.

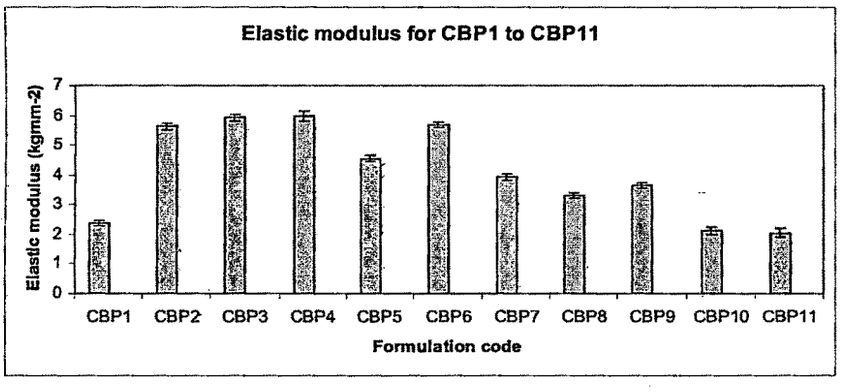


Fig 8.3 Elongation at break for CBP1 to CBP11.

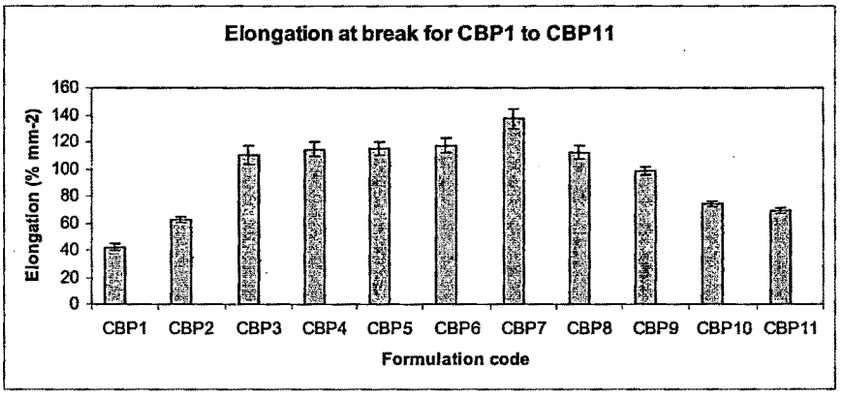


Fig 8.4 Folding endurance for CBP1 to CBP11.

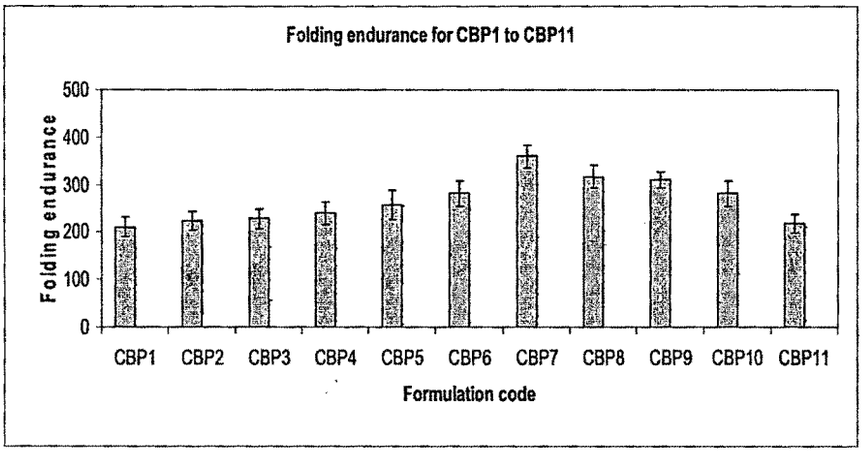
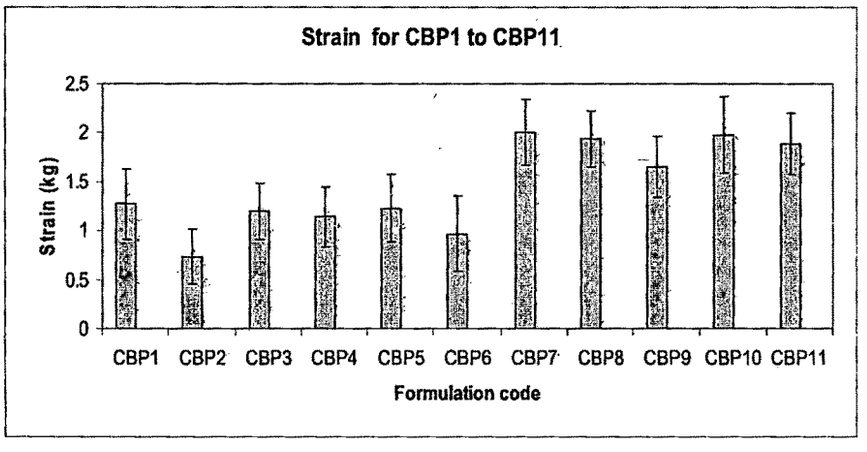


Fig 8.5 Strain for CBP1 to CBP11.



8.2.2 Surface pH:

A low and higher pH would be expected to damage the contacting mucosal surface, and this has been reported in an in vivo study involving human volunteers (Tiwari et al., 1999). High proportion of Carbopol 934P in the mixtures may impart strong acid characteristics to the matrix, which could produce some side effects in the mucosa (Llabot et al., 2004).

Table 8.5 shows that the surface pH of the bilayer buccal patches remained fairly constant at a pH of approximately 5.65–6.45. Therefore, this study confirmed that the surface pH of the buccoadhesive patches was near the neutral conditions of saliva and hence would not alter the pH of the buccal fluids and cause no damage or alteration to the buccal mucosa due to altered pH conditions.

Table 8.5 Surface pH of CBP1 to CBP11

Formulation Code	Surface pH
CBP1	6.10 ± 0.21
CBP2	6.18 ± 0.17
CBP3	6.21 ± 0.19
CBP4	6.31 ± 1.98
CBP5	6.41 ± 0.19
CBP6	6.45 ± 0.21
CBP7	6.01 ± 0.25
CBP8	5.90 ± 0.11
CBP9	5.81 ± 0.12
CBP10	5.80 ± 0.20
CBP11	5.65 ± 0.11

± R.S.D. (n=3)

8.2.3 Swelling:

Swelling is the prerequisite for the mucoadhesive dosage form to adhere to the buccal mucosa. Polymer swelling is a significant factor which permits a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or electrostatic interaction between the polymer and the mucous network and in turn results in satisfactory mucoadhesion (Miller et al., 2005).

The swelling behavior of the Carvedilol bilayer buccal and placebo patches in phosphate buffered saline (pH 6.8 ± 0.2) were investigated. Table 8.6 and 8.7 represents % swelling of Carvedilol patches and placebo patches respectively. Fig 8.6 and 8.7 shows % swelling with time for CBP1 to CBP6 and CBP7 to CBP11 of Carvedilol patches respectively. Fig 8.8 and 8.9 shows % swelling with time for P-CBP1 to P-CBP6 and P-CBP7 to P-CBP11 of placebo patches respectively.

It was seen from data that Carvedilol patches displayed 3.30 to 5.11 % of swelling. Formulation with HPMC K4M alone (CBP1) showed highest swelling i.e. 5.11 ± 0.31 % swelling after 8 hrs. Formulation with Carbopol 934P alone (CBP11) showed 5.10 ± 0.28 % swelling. A maximum degree of swelling was achieved after 2 to 4 hr of exposure to the

phosphate buffer saline. At the end of study, when matrix structure was physically evaluated, it was found to be very porous in nature.

In placebo patches 3.16 to 4.88% swelling was observed. Here also, formulation with HPMC K4M alone (CBP1) showed highest swelling i.e. 4.88 ± 0.36% swelling after 8 hrs. Formulation with Carbopol 934P alone showed 4.80 ± 0.20 % swelling.

When swelling behavior of Carvedilol and placebo patches were compared, it was found that addition of drug to the patches increased their swelling. At the end of 8 hrs, it was found that Carvedilol patches (CBP1) showed 5.11 ± 0.31 % swelling while that of placebo patches (PCBP1) was 4.88 ± 0.36 %. This can be attributed to the fact that dispersed drug particles may have weakened cohesive forces between the polymer chains allowing each chain to hydrate freely and increased the swelling of medicated patches. Yong C.S. et al also found high % swelling for medicated patches than placebo patches (Yong et al., 2001).

Table 8.6 Swelling studies of Carvedilol patches.

Formulation Code	% Swelling			
	2 Hrs	4 Hrs	6 Hrs	8 Hrs
CBP1	1.76 ± 0.12	2.81 ± 0.18	4.09 ± 0.28	5.11 ± 0.31
CBP2	1.39 ± 0.09	2.49 ± 0.12	3.77 ± 0.19	4.69 ± 0.29
CBP3	1.45 ± 0.08	2.43 ± 0.22	3.58 ± 0.18	4.69 ± 0.34
CBP4	1.47 ± 0.10	2.41 ± 0.22	3.49 ± 0.21	4.57 ± 0.33
CBP5	1.37 ± 0.12	2.31 ± 0.18	3.39 ± 0.18	4.49 ± 0.38
CBP6	1.22 ± 0.11	2.27 ± 0.19	3.29 ± 0.23	4.47 ± 0.34
CBP7	1.09 ± 0.11	2.17 ± 0.20	3.19 ± 0.29	4.19 ± 0.35
CBP8	1.07 ± 0.09	2.09 ± 0.19	3.18 ± 0.23	4.04 ± 0.29
CBP9	0.89 ± 0.09	1.98 ± 0.17	3.10 ± 0.23	3.81 ± 0.39
CBP10	0.85 ± 0.09	1.81 ± 0.17	2.88 ± 0.19	3.30 ± 0.37
CBP11	0.88 ± 0.12	1.61 ± 0.12	2.61 ± 0.26	5.10 ± 0.28

± R.S.D., n=3

Table 8.7 Swelling studies of placebo patches.

Formulation Code	% Swelling			
	2 Hrs	4 Hrs	6 Hrs	8 Hrs
P-CBP1	1.56 ± 0.09	2.61 ± 0.16	3.89 ± 0.27	4.88 ± 0.36
P-CBP2	1.19 ± 0.12	2.14 ± 0.10	3.57 ± 0.18	4.41 ± 0.39
P-CBP3	1.25 ± 0.09	2.13 ± 0.21	3.50 ± 0.15	4.47 ± 0.31
P-CBP4	1.25 ± 0.10	2.21 ± 0.21	3.29 ± 0.19	4.41 ± 0.30
P-CBP5	1.26 ± 0.11	2.01 ± 0.19	3.10 ± 0.10	4.28 ± 0.28
P-CBP6	1.12 ± 0.08	2.20 ± 0.10	3.19 ± 0.29	4.27 ± 0.30
P-CBP7	1.01 ± 0.13	2.01 ± 0.20	3.01 ± 0.18	4.01 ± 0.31
P-CBP8	1.00 ± 0.09	1.98 ± 0.19	3.01 ± 0.29	3.91 ± 0.29
P-CBP9	0.79 ± 0.10	1.88 ± 0.14	3.01 ± 0.17	3.61 ± 0.31
P-CBP10	0.79 ± 0.10	1.61 ± 0.27	2.67 ± 0.11	3.16 ± 0.30
P-CBP11	0.78 ± 0.11	1.51 ± 0.13	2.52 ± 0.19	4.80 ± 0.20

± R.S.D., n=3

Fig 8.6 Swelling Profile for CBP1 to CBP6 of Carvedilol patches.

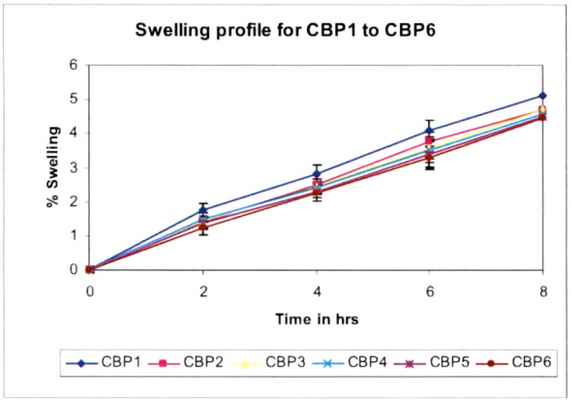


Fig 8.7 Swelling Profile for CBP7 to CBP11 of Carvedilol patches.

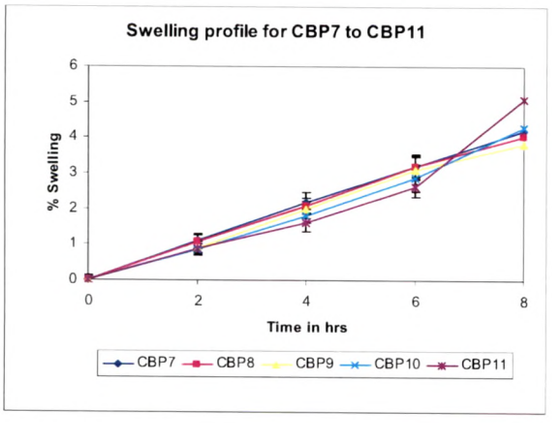


Fig 8.8 Swelling Profile for P-CBP1 to P-CBP6 of placebo patches.

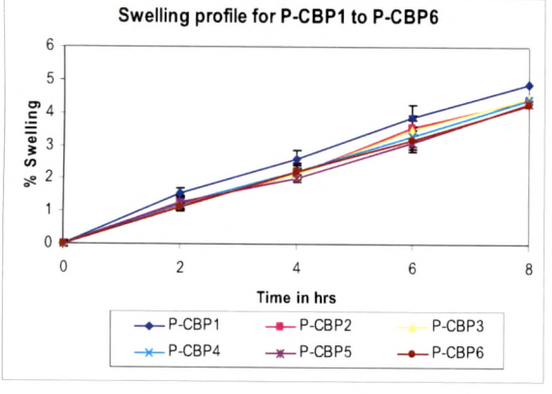
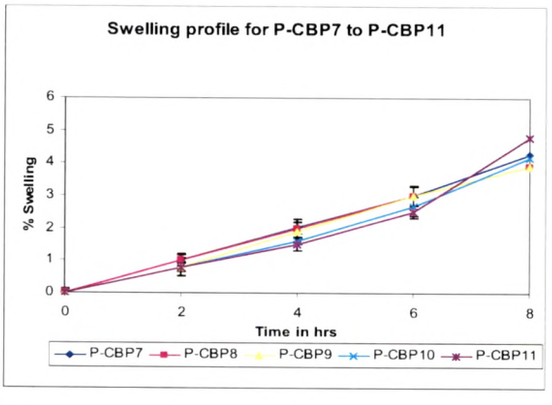


Fig 8.9 Swelling Profile for P-CBP7 to P-CBP11 of placebo patches.



8.2.4 In vitro Mucoadhesive force:

Mucoadhesion is the first and foremost significant prerequisite for the mucoadhesive drug delivery to adhere to mucosa. In current study, sheep buccal mucosa was used as biological membrane to investigate the mucoadhesive force of formulations. Table 8.8 and Fig 8.10 show in vitro mucoadhesive force for placebo and Carvedilol buccal patches.

All the formulations from CBP2 to CBP10 (40 ± 2.36 to 53 ± 2.13 x 10^3 dyne cm^{-2}) exhibited good mucoadhesion required by dosage form to adhere to buccal mucosa (Adel et al., 2004). However the patch containing Carbopol 934P and HPMC K4M at the ratio of 6:4 (CBP7) exhibited highest mucoadhesion (53 ± 2.13 x 10^3 dyne cm^{-2}) with buccal mucosa when compared with other ratios.

Formulation CBP11 which contains only Carbopol 934P showed weak adhesion (27 ± 1.98 x 10^3 dyne cm^{-2}) which may be because mucoadhesive force of Carbopol 934P is dependent on the pH of experimental medium. If pH of medium is more than ionization constant of Carbopol 934P (6.00) then Carbopol 934P will ionized and loose its integrity (Desai K.G.H., 2004). This will result in loss of hydrogen bonding with the mucus. The pH of the buffer solution used in the present study was 6.8 ± 0.2, which could have decreased the mucoadhesion because of the change in the ionization property of carboxylic groups present in Carbopol 934P (Shojaei and Li, 1997). On the contrary, Grabovac et al. reported maximum mucoadhesion for formulations containing Carbopol alone but they had used different grades of Carbopol e.g. Carbopol 980, displaying comparatively the high molecular mass and a high degree of cross-linking and adhere to the mucosa for the longest time period among the polyacrylates (Grabovac et al., 2005).

Swelling affects the mucoadhesive force (Valenta, 2005) of formulation as it was seen in CBP1. The formulation (CBP1) containing only HPMC showed less mucoadhesive force (23 ± 2.33 x 10^3 dyne cm^{-2}) may be because higher swelling (5.11 ± 0.31 %) of polymer which may have weakened its adhesive property.

Effect of presence of drug in the Carvedilol and placebo patches on the mucoadhesive force of the formulation was also studied. In CBP1 mucoadhesive force of 23 ± 2.33 x 10^3 dyne cm^{-2} was obtained while that of placebo was 29 ± 2.13 x 10^3 dyne cm^{-2}. CBP11 shows 27 ± 1.98 x 10^3 dyne cm^{-2} of mucoadhesive force while placebo patch shows 32 ± 1.88 x 10^3 dyne cm^{-2}. Average difference of 5 x 10^3 dyne cm^{-2} mucoadhesive force was obtained between Carvedilol and placebo patches. The dispersed Carvedilol particles may have weakened

cohesive forces between the polymer chains allowing each chain to hydrate freely and increased the swelling of Carvedilol patches (Yong et al., 2001).This implies that the addition of Carvedilol was found to decrease mucoadhesive force because of increase swelling of patches as compared to placebo patches.

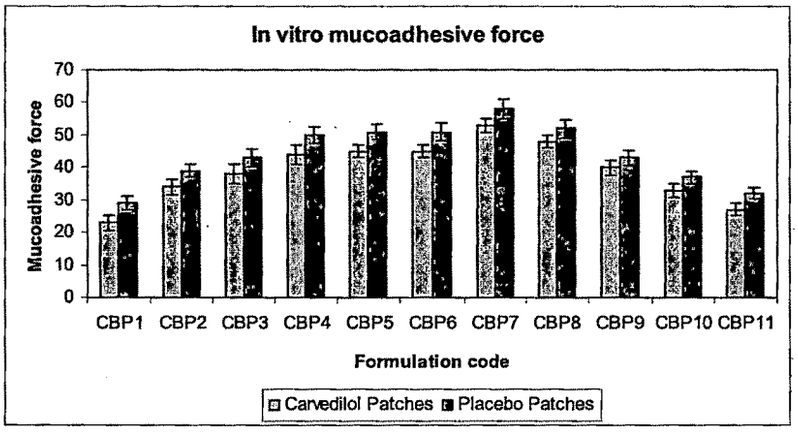
CBP7 (Carbopol 934P: HPMC; 6:4) shows highest mucoadhesive force of 53 ± 2.13 x 10^3 dyne cm^{-2}.

Table 8.8 In vitro mucoadhesion force for CBP1 to CBP11.

Formulation Code	Mucoadhesive force (10^3 dyne cm^{-2})		Formulation Code (Placebo)
	Carvedilol Patches	Placebo Patches	
CBP1	23 ± 2.33	29 ± 2.13	P-CBP1
CBP2	34 ± 2.47	39 ± 1.98	P-CBP2
CBP3	38 ± 3.01	43 ± 2.69	P-CBP3
CBP4	44 ± 2.85	50 ± 2.68	P-CBP4
CBP5	45 ± 1.98	51 ± 2.15	P-CBP5
CBP6	45 ± 2.10	51 ± 2.69	P-CBP6
CBP7	53 ± 2.13	58 ± 3.01	P-CBP7
CBP8	48 ± 2.10	56 ± 2.78	P-CBP8
CBP9	40 ± 2.36	43 ± 2.45	P-CBP9
CBP10	33 ± 2.14	37 ± 2.01	P-CBP10
CBP11	27 ± 1.98	32 ± 1.88	P-CBP11

± R.S.D., n=3

Fig 8.10 In vitro mucoadhesive force for Carvedilol and placebo buccal patches.



8.2.5 *In vitro* diffusion:

To study the *in vitro* diffusion of the prepared Carvedilol bilayer buccal patches Franz diffusion cell was used. Table 8.9 and Fig 8.11 show the *in vitro* diffusion for CBP1 to CBP11 studied through sheep buccal mucosa up to 8 hr and pure drug diffusion for 2 hr.

Formulations containing HPMC K4M (CBP1) alone showed highest diffusion (85.02 ± 2.86 %). Formulation (CBP10) alone showed least diffusion (74.23 ± 3.56%). Diffusion of pure drug was 93.02 ± 2.93 in 2 hr. Carvedilol being a biopharmaceutical classification system – class II drug showed a higher permeation and diffusion because of its higher flux 6.09 ± 0.43 x 10^{-6} µg cm^{-2} min^{-1} at pH 6.8 as shown by preformulation studies.

The combination of polymers of Carbopol 934P and HPMC K4M (CBP2 to CBP10) has shown 74.23 ± 3.56 % to 84.25 ± 2.00 % diffusion of Carvedilol. Obtained diffusion values for formulation CBP2 to CBP10 shows that combination of polymers plays a part in sustaining diffusion of Carvedilol up to 8 hr. It also highlight that combination of polymers have control over sustaining drug diffusion than individual polymers because combination of polymers imparts better matrix structure to patches which may control Carvedilol diffusion.

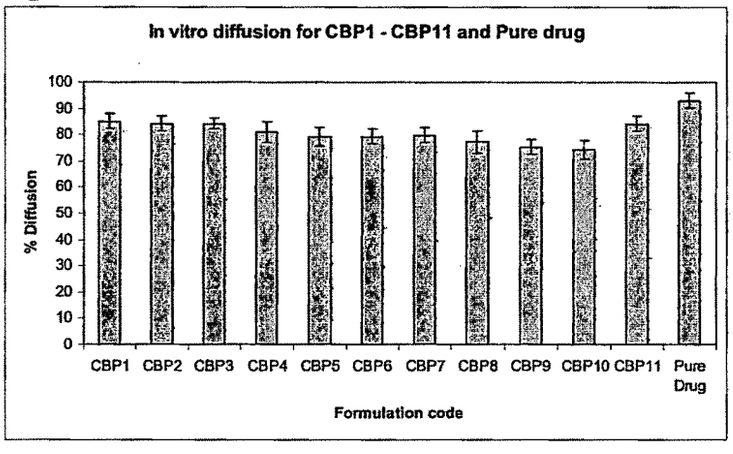
The in vitro diffusion values obtained for bilayer patches are slightly higher than core in cup tablets e.g. core in cup tablets (CCT10) showed 70.97 ± 4.01 % diffusion while bilayer patches (CBP10) showed 74.23 ± 3.56 % diffusion in 8 hr. This may be because of available surface area of formulation available for diffusion. The diameter of Core in cup tablets is 6 mm while that of bilayer patch is 14mm. Secondly, in bilayer patches, propylene glycol has been used as a plasticizer which might have acted as penetration enhancer for diffusion of Carvedilol. Aungst B.J. et al. used propylene glycol as a penetration enhancer for insulin and they found increased permeation of insulin with propylene glycol (Aungst and Rogers, 1989).

Table 8.9 In vitro diffusion for CBP1-CBP11 and Carvedilol.

Formulation code	In vitro Diffusion (%)
CBP1	85.02 ± 2.86
CBP2	84.22 ± 3.00
CBP3	84.25 ± 2.00
CBP4	81.01 ± 3.87
CBP5	79.36 ± 3.56
CBP6	79.23± 2.89
CBP7	79.86 ± 2.99
CBP8	77.23 ± 4.00
CBP9	75.36 ± 3.00
CBP10	74.23 ± 3.56
CBP11	84.23 ± 2.89
*Pure Drug	93.02 ± 2.93

± R.S.D., (n=3), *= Study conducted for 2hr

Fig 8.11 In vitro diffusion of Carvedilol, CBP1 to CBP11.



8.2.6 In vitro dissolution profile:

In vitro dissolution profile of the designed formulations is shown in Fig 8.12, 8.13 and 8.14. Table 8.10a and 8.10b shows % drug dissolution values for pure drug and CBP1 to CBP11. CBP1 released the drug at fastest rate with 95.12 ±3.1 % release in 6 hr and 100.00 ± 3.14 in

7 hr. This could be attributed to the high swelling (5.11 ± 0.31 %) of HPMC K4M as greater swelling of the matrices leads to faster dissolution of the drug (Agarwal and Mishra, 1999).

In formulation containing Carbopol 934P alone (CBP11), it was observed that there was formation of gel and collapsing of formulation at 6th hr of study. The possible reason behind it is that there might be ionization of Carbopol 934P at experimental pH (6.8) which is higher than its ionization constant (pKa) 6.0.

It was seen that formulations with combinations of polymers contributed significantly in extending Carvedilol dissolution. On this basis it can be concluded that combination of polymers imparts better matrix characteristics to the patches. Strong matrix integrity will inhibit the entry of dissolution media and delay the dissolution of drug. Singh et. al. reported that combination of Carbopol and HPMC fairly regulated the Metoprolol tartarate release up to 10 hr (Singh and Ahuja, 2002).

To investigate the kinetics of Carvedilol release from bilayered buccal tablets, the release data was applied to, zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer Peppas models and best fit was determined. The values of r^2, K and n are listed in Table 28.

All the formulations showed n values in between 0.5 to 1.0 i.e. 0.73 to 0.99 indicating that they followed nonfickian diffusion pattern. The results indicate that the release mechanism changed with the type and amount of polymer incorporated in the formulation and this can be reflected by the observed values of release exponent (n). When concentration of Carbopol 934P was gradually increased in the formulation, n values were found to increase. CBP2 to CBP10 where combination of polymers were used showed n value from 0.78 to 0.97 respectively. For CBP1, which contained only HPMC K4M, n value was 0.73 while that of CBP5 was 0.92 where HPMC K4M: Carbopol 934P; 4:6 was used. In CBP11 where only HPMC K4M is present showed n value as 0.73, followed non-fickian release. This shows that release pattern followed non-fickian release; implying diffusion is dominant release mechanism.

Singh B. and Ahuja N. formulated controlled release matrices of Diltiazem hydrochloride with Carbopol 934P and HPMC K4M and reported non-fickian release approaching nearly zero order (Singh and Ahuja, 2002). Ponchel G. et. al. formulated bioadhesive controlled

release systems for metronidazole by compressing HPMC and Carbomer 934 and reported that release behavior of the system was non-Fickian (Ponchel et al., 1987).

None of the formulations followed first order release and Hixson-Crowell kinetics as seen from its r^2 values shown in Table 8.11. For zero order, all the formulation showed $r^2 \approx 1.0$, this implies that they followed nearly zero order kinetics. Formulation CBP7 showed best fit as it showed $r^2 = 0.997$ for zero order implied drug release is by diffusion and erosion mechanism. Peppas model for CBP7 showed n = 0.96 implies non-fickian diffusion pattern.

Table 8.10a In vitro release of pure drug and formulations CBP1 to CBP5.

Formulation Code	Pure Drug	CBP1	CBP2	CBP3	CBP4	CBP5
Time (hr)	% Carvedilol dissolution					
1	89.23±2.23	19.89±2.36	17.00±2.06	14.00±2.14	12.99±2.06	13.00±2.14
2	100±3.45	32.00±3.00	27.12±2.64	23.11±2.56	21.00±2.87	18.97±2.15
3	-	50.56±2.45	47.00±2.89	46.00±3.01	38.91±2.14	37.00±2.87
4	-	62.32±2.36	60.89±3.01	55.89±2.36	54.00±3.14	51.08±3.12
5	-	77.15±3.02	75.00±2.45	73.00±2.45	56.00±3.69	71.10±2.45
6	-	95.12±3.1	86.99±2.06	88.96±3.00	83.11±3.00	84.95±2.45
7	-	100.00±3.14	94.12±2.45	92.36±3.01	92.06±2.98	99.00±2.14
8	-	-	99.12±2.89	99.29±3.84	99.26±3.45	99.03±3.01

± R.S.D. (n=3)

Table 8.10b In vitro release of formulations CBP6 to CBP11.

Formulation Code	CBP6	CBP7	CBP8	CBP9	CBP10	CBP11
Time (hr)	% Carvedilol dissolution					
1	11.39±2.15	12.02±2.15	12.36±2.65	11.35±3.00	13.35±3.25	19.36±2.56
2	18.00±2.65	19.00±2.17	17.88±2.36	20.14±3.47	17.39±2.6	30.01±3.00
3	36.04±2.98	35.50±2.56	38.09±2.65	30.11±3.11	29.12±2.36	51.25±3.55
4	45.00±3.14	46.13±3.25	51.00±3.12	41.11±3.00	43.36±2.45	59.98±1.87
5	67.04±2.19	59.87±3.25	57.01±2.69	64.99±3.11	70.00±2.69	78.23±2.00
6	82.00±2.89	71.98±2.15	79.89±2.00	71.00±3.44	79.04±2.56	89.36±3.11
7	91.25±2.45	85.23±2.65	98.23±2.82	97.14±2.00	99.36±1.99	100.00±2.60
8	97.88±3.89	94.56±3.12	98.26±3.25	98.96±2.89	99.87±2.85	-

± R.S.D. (n=3)

Fig 8.12 In vitro dissolution of pure drug, CBP1, CBP2, CBP3 and CBP4.

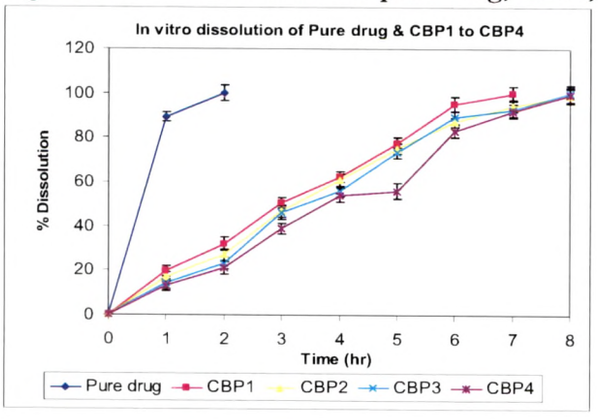


Fig 8.13 In vitro dissolution of pure drug, CBP5, CBP6, CBP7 and CBP8.

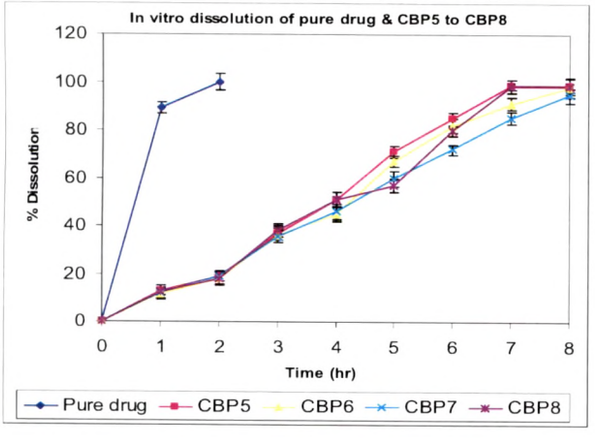


Fig 8.14 In vitro dissolution of pure drug, CBP9, CBP10 and CBP11.

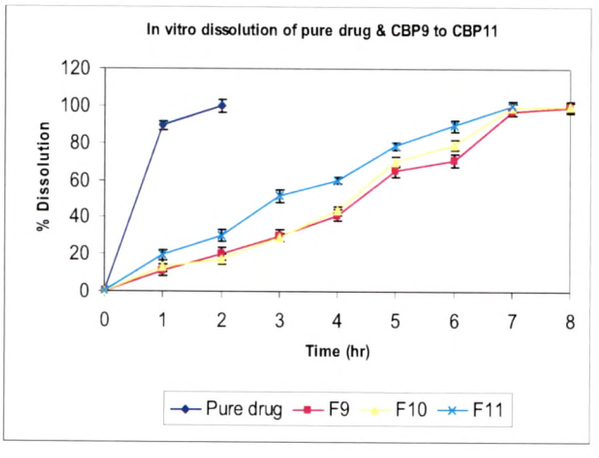


Table 8.11 Model fitting of Carvedilol dissolution from bilayer buccal patches

Formulation Code	Zero Order		First Order		Higuchi		Hixson Crowell		Korsmeyer Peppas		
	K_0 (h^{-1})	r^2	K_1 (h^{-1})	R^2	K_H ($mg/h^{1/2}$)	r^2	K_S (h^{-3})	r^2	K_t (h^{-n})	r^2	n
CBP1	13.71	0.983	0.16	0.962	50.75	0.986	0.32	0.943	0.88	0.989	0.73
CBP2	12.42	0.975	0.15	0.950	48.77	0.980	0.29	0.925	0.90	0.987	0.78
CBP3	13.00	0.972	0.16	0.905	50.93	0.980	0.32	0.922	1.00	0.982	0.86
CBP4	13.02	0.981	0.16	0.869	50.30	0.962	0.32	0.953	1.02	0.983	0.91
CBP5	13.71	0.981	0.26	0.727	53.14	0.969	0.34	0.953	1.06	0.986	0.92
CBP6	13.47	0.984	0.13	0.905	52.07	0.967	0.35	0.954	1.11	0.982	0.99
CBP7	**12.28**	**0.997**	**0.12**	**0.927**	**47.37**	**0.975**	**0.32**	**0.966**	**1.04**	**0.989**	**0.96**
CBP8	13.50	0.976	0.25	0.804	52.03	0.954	0.34	0.954	1.08	0.974	0.96
CBP9	13.62	0.975	0.26	0.785	52.13	0.940	0.35	0.975	1.09	0.984	0.99
CBP10	14.18	0.970	0.38	0.756	54.38	0.937	0.36	0.964	1.09	0.954	0.97
CBP11	13.48	0.991	0.17	0.932	50.95	0.983	0.32	0.959	0.87	0.989	0.73

8.2.7 Pharmacokinetic Study

8.2.7.1 Selection of optimized formulation for pharmacokinetic study:

On the basis of *in vitro* parameters such as mechanical properties, mucoadhesive force, diffusion and in vitro dissolution, it was concluded that CBP7 (Carbopol 934P: HPMC, 6:4) has excellent mechanical properties, mucoadhesion (53 ± 2.13 x 10^3 dyne cm^{-2}), diffusion (79.86 ± 2.99 %) and 94.56±3.12 % dissolution in 8hrs. On the above basis, CBP7 was finalized to be used for pharmacokinetic studies, histological examination, in-vivo patient acceptability studies on human volunteers and pharmacodynamic studies.

8.2.7.2 Comparison of plasma profile of oral and buccal bilayer patches.

The pharmacokinetic parameters for oral conventional tablets and buccal bilayer patches in rabbits were studied and shown in Table 8.12. Plasma concentration profile is shown in Fig 8.15. The plasma concentration Vs time profile for buccal bilayer patches showed sustained release of Carvedilol, as indicated by high t_{max} of 4.0 hr for tablets as compared to 1.00 hr for oral administration and as seen from graphical representation of plasma concentration Vs time profile. The C_{max} values observed were also higher (69.18 ± 6.69 ng/ml) for Carvedilol bilayer buccal patches than oral tablets (58.25 ± 4.26 ng/ml) indicating greater absorption. The AUC values after buccal administration of buccal patches (319.44 ± 6.65 ng/ml/hr)

were significantly higher than that of oral administration (152.22 ± 8.43 ng/ml/hr) which revealed increase in bioavailability. Carvedilol bilayer buccal patches showed about 2.09 fold increase in bioavailability compared with the conventional tablets by oral administration. The one way ANOVA test showed statistically significant differences ($P < 0.01$) between the AUC of oral conventional tablets and buccal bilayer patches.

Oral tablet was absorbed rapidly as seen by its tmax (1 hr) as compared to buccal patch (tmax: 4 hr). Its effect rapidly falls off as reflected by plasma concentration of 6.07 ± 2.60 ng/ml at 5th hr. On the other hand, absorption of Carvedilol from the bilayer buccal patch in the initial phase appeared to be slow for 1st hr, which may be due to lag time for the diffusion of drug but afterwards there was increase in absorption of Carvedilol (69.18 ± 6.69 ng/ml) because of faster diffusion of drug across buccal mucosa as seen by the prolonged plasma levels (16.11 ± 4.99 ng/ml) up to 8 hr.

When Carvedilol buccal patches and buccal core in cup tablets was compared for its pharmacokinetic efficacy, it was found that Carvedilol buccal patches (319.44 ± 6.65 ng/ml/hr) showed slightly high bioavailability than buccal core in cup tablets (297.53 ± 8.20 ng/ml/hr). This may be because greater surface area available for absorption of Carvedilol from the patch (14.00 mm) when compared with core in cup tablets (6.00 mm). Another reason behind higher bioavailability of bilayer patches could be permeation enhancing activity of propylene glycol used as a plasticizer in the formulation. Birudaraj R., et. al. reported 3% increase in flux of Buspirone due to Propylene glycol and in turn increase in bioavailability (Birudraj et al., 2005).

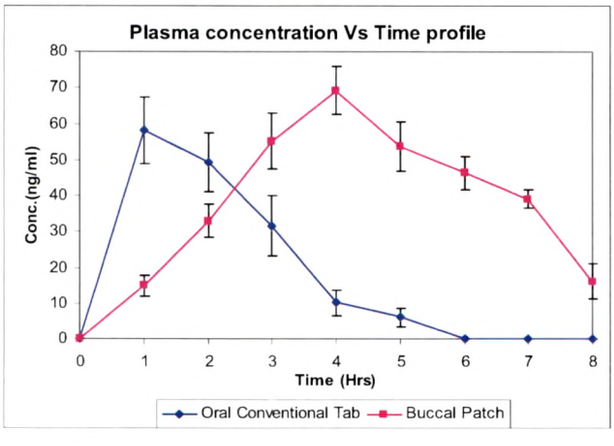
The results obtained in these studies prove the significance of administering Carvedilol through the buccal route as a bilayer patches for avoiding pre-systemic metabolism and improving bioavailability.

Table 8.12 Plasma concentration of Carvedilol (ng/ml) following administration of oral tablets and buccal patch.

Time (Hr)	Plasma concentration (ng/ml)	
	Oral Tablet	Buccal Patch
1	58.25 ± 4.26	14.98 ± 2.93
2	49.26 ± 8.22	32.98 ± 4.69
3	31.55 ± 8.28	55.18 ± 7.78
4	10.11 ± 3.50	69.18 ± 6.69
5	6.07 ± 2.60	53.59 ± 6.76
6	B.LoQ	46.38 ± 4.70
7	B.LoQ	39.11 ± 2.56
8	B.LoQ	16.11 ± 4.99
Tmax (Hr)	**1.0 ± 0.2**	**4.0 ± 1.0**
Cmax (ng/ml)	**58.25 ± 4.26**	**69.18 ± 6.69**
AUC (ng/ml/hr)	**152.22 ± 8.43**	**319.44 ± 6.65**

± R.S.D., n=6, B.LoQ-Below limit of quantitation.

Fig 8.15 Plasma concentration Vs Time profile for CBP7 and oral conventional tablets.



8.2.8 Histological study of buccal mucosa:

8.2.8.1 Light microscopy:

Buccal mucosal sections 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 tissues. Control buccal mucosa was also treated similarly and examined. Fig 8.16 and 8.17 shows the sections of control and sample buccal mucosa respectively.

Control buccal mucosa shows all the three distinctive layers of the oral mucosa, the epithelium, basement membrane, and connective tissues. Sections of sample mucosa showed little modification in the epithelial layer. Because the permeability barrier of the buccal mucosa has been attributed to the upper one-third of the epithelium (Squier, 1973), this slight disruption of the superficial cells due to formulation may have resulted in increased permeability of Carvedilol as observed during permeation studies. Permeation enhancing effect of propylene glycol might have caused certain disruption of cells of epithelium. Nicolazzo J. A., et al. concluded that permeation enhancing activity of buccal permeation enhancers are attributed to extracting intercellular lipids and interacting with epithelial protein domains which may caused certain disruption of epithelial cells (Nicolazzo et al., 2005).

It is clear from the results of the permeation experiments that no major alterations in the barrier function of the tissue had been provoked for 8 hr exposure of the tissue to the formulation.

Fig 8.16 Section of Control Buccal Mucosa.

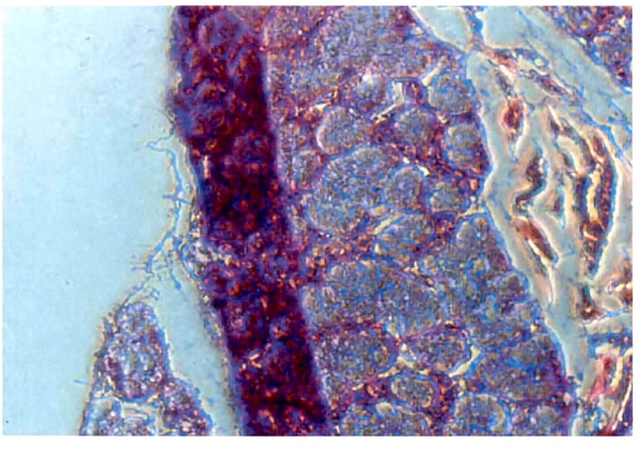
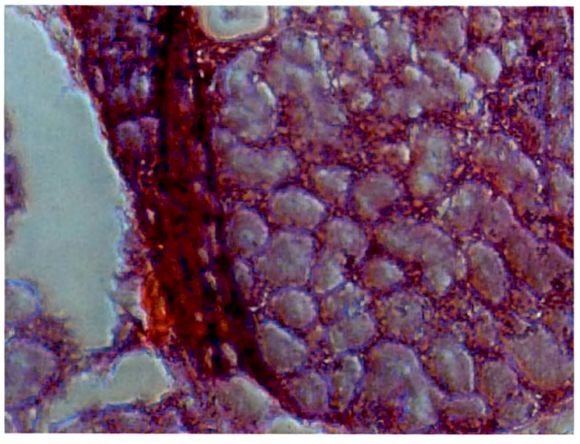


Fig 8.17 Section of Sample Buccal Mucosa.



8.2.8.2 Scanning electron microscopy of buccal mucosa:

At the end of the diffusion experiment, the buccal mucosa was collected, washed and treated as per the protocol given in experimental part. Fig 8.18 and 8.19 shows SEM of control buccal mucosa and sample buccal mucosa respectively.

SEM of the control buccal mucosa showed the presence of the superficial cells of the epithelium which represents the major absorption site in the oral cavity. It also shows that stratified squamous cells have intact cell junctions with microridges. These findings are in accordance with Attia and coworkers (Attia et al., 2004) who found the presence of the superficial cells of the epithelium and stratified squamous cells with microridges or micropillee in their control buccal samples.

SEM of sample buccal mucosa showed slight histological changes such as shrinkage of superficial cells in some parts of the tissue. Use of dichloromethane in the buccal patches might have contributed to the shrinkage of cells.

Fig 8.18 SEM of Control Buccal Mucosa

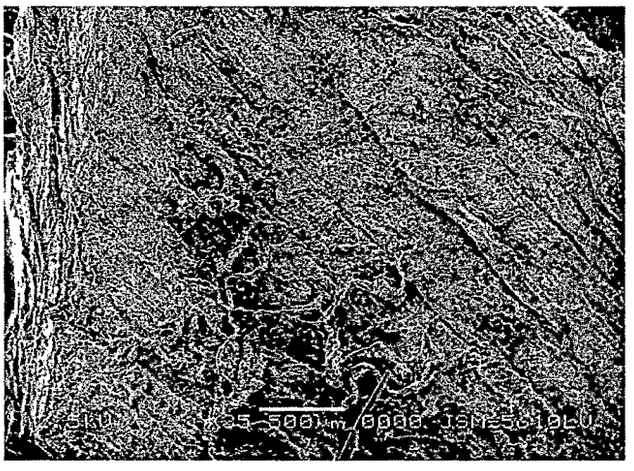
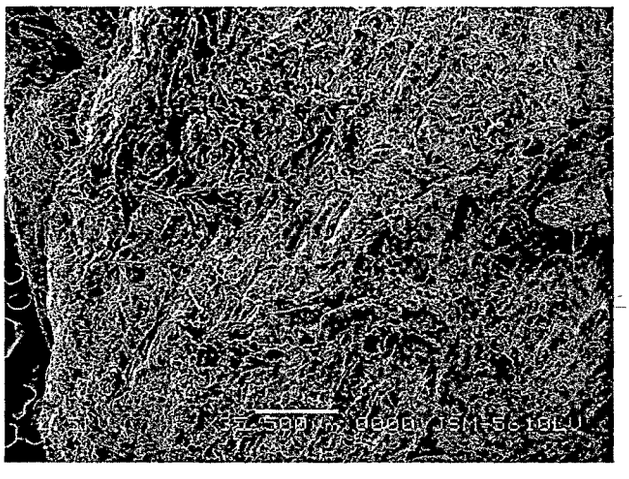


Fig 8.19 SEM of Sample Buccal Mucosa



8.2.9 *In vivo* acceptability testing:

The response of volunteers to each subjective parameter was calculated, and obtained results are presented in Table 8.13. Volunteer's response in irritation criteria showed that none of them have complaints. In comfort testing of 90% and 10% volunteers reported comfortable and slightly comfortable levels respectively. None of the volunteers reported moderately comfortable and severely uncomfortable levels.

Dryness of mouth was not experienced by any of the volunteers. 20% of volunteers have not experience salivary secretion and 80% of them reported slight salivary secretion. When volunteers were asked to express their views on heaviness of tablets at the application site, all

of them experienced no heaviness. None of the volunteers reported dislodgement of the system during the study up to 6 hr.

Based on above results, it can be concluded that the bilayer buccal patch would be comfortable and acceptable by the patients and would be retained in the human oral cavity long enough for the drug release to occur.

Table 8.13 Evaluation criteria and results for *in vivo* acceptability study.

Criteria	Volunteer's response %
Irritation	
None	100
Slight (Tolerated)	-
Moderate	-
Severe (Not tolerated)	-
Comfort	
Very comfortable	-
Comfortable	90
Slightly uncomfortable	10
Moderately uncomfortable	-
Severely uncomfortable	-
Dryness of mouth	
None (Not experienced)	100
Slight (Tolerated)	-
Moderate	-
Severe (Not tolerated)	-
Salivary Secretion	
None (Not experienced)	20
Slight (Tolerated)	80
Moderate (Feeling of discomfort)	
Severe	
Heaviness of patch at the application site	
None (Not experienced)	100
Slight (Tolerated)	
Moderate (Feeling of discomfort)	
Severe (Highly discomfort)	
Dislodgement of the system during study (up to 6 hr)	
No	100
Yes	-

8.2.10 Pharmacodynamic studies:

These studies were divided into 2 parts,

1. Development of hypertension in rats for 6 weeks.
2. Treatment with oral conventional tab and bilayer buccal patch (CBP7).

8.2.10.1 Development of fructose induced hypertension:

Development of hypertension was same as described in Carvedilol buccal core in cup tablets.

8.2.10.2 Treatment with oral conventional tablets and buccal bilayer patches.

After developing hypertension in rats, they were treated with oral conventional tablets and buccal bilayer patch and observations are recorded in Table 8.14 and 8.15. Table 8.16 gives % reduction in hypertensive parameters. Fig 8.20, 8.21, 8.22 and 8.23 shows comparative evaluation of MAP, heart rate, triglycerides and body weight respectively after administration of oral conventional and buccal bilayer patch.

When the hypertensive group was treated with oral conventional tablets for 2 weeks, slight reduction of hypertension was found (Table 8.14). At the end of 2 weeks, observed MAP, HR and body weight were 136 ± 09 mmHg, 389 ± 29 /min and 235 ± 21 gm respectively. Triglyceride levels were found to be 139 ± 16 mg/dl. When second hypertensive group of rats was treated with buccal bilayer patches, considerable reduction of hypertension was found. The values obtained for MAP, HR and body weight were 114 ± 13 mmHg, 351 ± 28/min and 210 ± 21 gm respectively. Triglyceride levels were found to be 91 ± 10 mg/dl. Table 8.17 shows statistical significance ($p<0.001$) between oral conventional tablets and bilayer buccal patches. When hypertension was treated with oral conventional tablets for 2 weeks, it does not show significant values except for triglycerides. When it was treated with bilayer buccal patches all values were significant except HR/min.

Hypertensive parameters were also compared in terms of percent reduction in values by administering oral conventional and buccal bilayer patches. At the end of 2 weeks, reduction in MAP (mm Hg) was found 8.72 and 23.48 % by oral conventional and buccal bilayer patches respectively. Reduction in HR/min was found 5.12 and 14.39 % by oral conventional and buccal bilayer patches respectively. 9.61% and 19.23% reduction was found in body Weight (gm) by oral conventional and buccal bilayer patches respectively. Reduction in triglycerides (mg/dl) was found 33.80 and 56.66 % by oral conventional and

buccal bilayer patches respectively. This clearly indicated that buccal bilayer patch provided good treatment as compared to oral conventional tablets.

Carvedilol buccal bilayer patches decreased the hypertension significantly as compared to oral conventional tablets. This clearly indicates that the buccal bilayer patches release the drug gradually over a period of time, which results in prolonged control of hypertension.

The current study revealed increase in activity of Carvedilol when it was administered through buccal route which bypasses the first pass metabolism, and hence resulted in increased bioavailability.

Table 8.14 Treatment with oral conventional tablets

Conventional Tablets			
	Before treatment (Initial)	After Treatment	
		1W	2W
MAP (mm Hg)	149 ± 08	142 ± 08	136 ± 09
HR/min	410 ± 19	395 ± 19	389 ± 29
Body Weight (gm)	260 ± 24	249 ± 17	235 ± 21
Triglyceride (mg/dl)	210 ± 26	181 ± 16	139 ± 16

(± R. S. D.) (n=6).

Table 8.15 Treatment with bilayer buccal patches

Bilayer buccal patches			
Parameters	Before treatment (Initial)	After Treatment	
		1W	2W
MAP (mm Hg)	149 ± 08	138 ± 10	114 ± 13
HR/min	410 ± 19	391 ± 26	351 ± 28
Body Weight (gm)	260 ± 24	238 ± 19	210 ± 21
Triglyceride (mg/dl)	210 ± 26	149 ± 12	91 ± 10

(± R. S. D.) (n=6).

Table 8.16 - % Reduction in Hypertensive Parameters

% Reduction in Hypertensive Parameters				
Parameters	Oral conventional tablets		Buccal bilayer patch	
	1W	2W	1W	2W
MAP (mm Hg)	4.69	**8.72**	6.04	**23.48**
HR/min	3.65	**5.12**	4.63	**14.39**
Body Weight (gm)	4.23	**9.61**	8.46	**19.23**
Triglyceride (mg/dl)	13.80	**33.80**	29.04	**56.66**

Table 8.17 Statistical significance at $p<0.001$ between oral conventional tablets and bilayer buccal patches.

Statistical significance($p<0.001$)				
Parameters	Oral conventional tablets		Bilayer buccal patches	
	1W	2W	1W	2W
MAP (mm Hg)	NS	NS	NS	S
HR/min	NS	NS	NS	NS
Body Weight (gm)	NS	NS	NS	S
Triglyceride (mg/dl)	NS	S	NS	S

NS: Not significant, S= Significant, n=6.

Fig 8.20 Comparative evaluation of MAP after administration of oral and buccal patches.

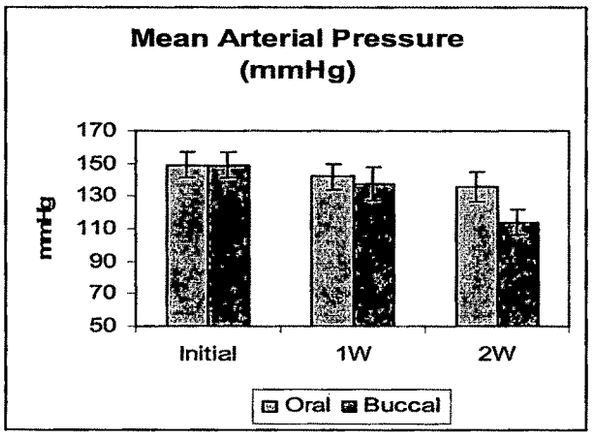


Fig 8.21 Comparative evaluation of heart rate after administration of oral and buccal patches.

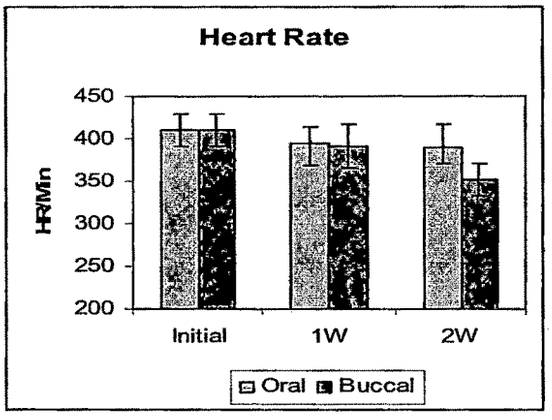


Fig 8.22 Comparative evaluation of triglycerides after administration of oral and buccal patches.

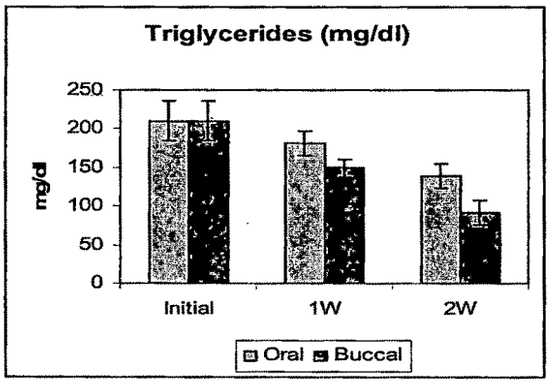
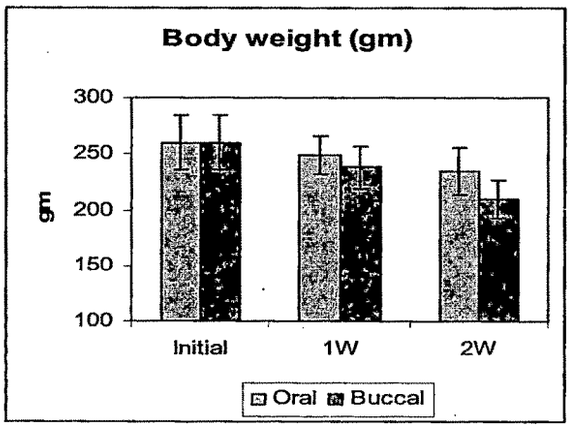


Fig 8.23 Comparative evaluation of body weight after administration of oral and buccal patches.



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9.0 RESULTS AND DISCUSSION (CARVEDILOL BILAYER TABLETS FORMULATED WITH MICROSPHERES-CTM):

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

The microspheres were prepared by spray drying technique, with the drug to polymer ratio of 1:1, 1:1.5 and 1:2. Spray-drying was selected as technique for the preparation of chitosan microspheres as it is a single step, rapid and simple method involving the preparation of drug and polymer solution and spraying it through spray drier. The placebo microspheres were also prepared with the same method without using Carvedilol.

9.2 Evaluation of Chitosan Microspheres:

Flow properties such as angle of repose, bulk and tapped density, compressibility index and Hausner's ratio were evaluated. The obtained results are shown in Table 9.1. All the values of angle of repose lie between $21.25^0 \pm 1.58$ (CM3) to $29.11^0 \pm 1.98$ (CM1). Carr's compressibility index is a parameter providing an indication of powder flowability (Wells and Aulton, 1988). Compressibility index values ranged from 14.7 ± 0.49 % (CM3) to 20.35 ± 0.71 % (P-CM) which indicates a fair compressibility of the microspheres. Best value for compressibility index (14.7 ± 0.49 %) was shown by CM3 in accordance with Carr's classification. According to Wells and Aulton, a Hausner's ratio value of less than 1.20 indicates good flowability of the powder, whereas a value of 1.5 or higher suggests poor flow. In current study, Hausner's ratio values lies between 1.17 (CM3) to 1.25 (P-CM), showing that the microspheres exhibited good flow properties. Microspheres containing higher proportion of chitosan (CM3) possessed best flow characteristics as they showed lowest angle of repose (21.25 ± 1.58^0), Car's index (14.7 ± 0.49 %) and Hausner's ratio (1.17).

Table 9.1 Flow properties of chitosan microspheres.

Formulation code →	CM1	CM2	CM3	P-CM
Drug: Polymer →	1:1	1:1.5	1:2	0:1
Angle of repose (^{0})	29.11 ± 1.98	24.36 ± 1.74	21.25 ± 1.58	26.23 ± 1.88
Bulk density (gm/ml)	0.618 ± 0.02	0.612 ± 0.03	0.609 ± 0.05	0.634 ± 0.02
Tapped density (gm/ml)	0.756 ± 0.03	0.748 ± 0.04	0.714 ± 0.05	0.796 ± 0.04
Compressibility index (%)	18.25 ± 0.56	18.18 ± 0.47	14.7 ± 0.49	20.35 ± 0.71
Hausner's ratio	1.22 ± 0.12	1.22 ± 0.17	1.17 ± 0.11	1.25 ± 0.19

± R.S.D., n=3

9.2.1 Particle size distribution, Clumping and Uniformity index:

Table 9.2 shows particle size distribution, clumping and uniformity index for CM1 to P-CM. Figure 9.1 shows particle size distribution for CM1 to P-CM. Particle size ranged from 5.10 ± 2.11 to 8.98 ± 2.98 μm. Increase in concentration of chitosan resulted in an increase mean particle size of chitosan microspheres. This increase may be because of the increase in viscosity of the droplets (due to the increase in concentration of chitosan solution). Jeyanthi et al reported that increase in mean particle size due to increased viscosity of the polymer solution for microspheres (Jeyanthi et al, 1997).

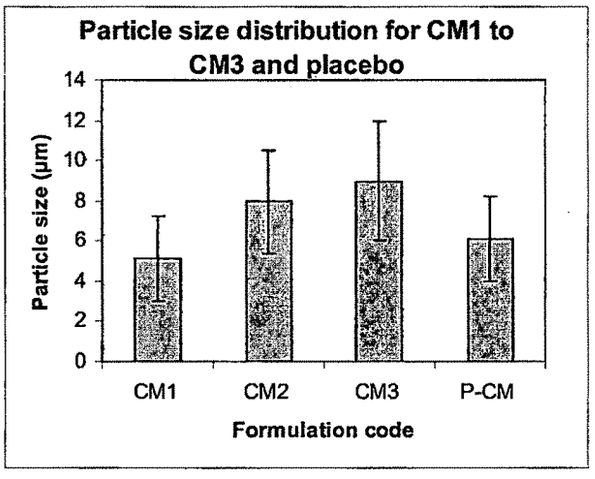
Dubey and Parikh reported that particle size uniformity index of microspheres is affected by concentration of chitosan in microspheres. They concluded that as concentration of chitosan was increased in the microspheres it shows broad particle distribution (Dubey and Parikh, 2004). However, in current studies no exact correlation could be established between uniformity index and concentration of chitosan. The values of particle size uniformity index for all the batches were in between 1.02 to 1.22 indicating that the microspheres have narrow particle size distribution. All the formulations showed particle size range from 5.10 ± 2.11 to 8.98 ± 2.98 μm and uniformity index between 1.02 ± 0.04 to 1.22 ± 0.02 indicates narrow particle size distribution. Low to moderate clumping was observed in all the formulations.

Table 9.2 Particle size, clumping and uniformity index for CM1 to P-CM.

Formulation code (Carvedilol: Chitosan)	Mean Particle Size (μm) d(90)	Clumping*	Uniformity index
CM1 (1:1)	5.10 ± 2.11	+	1.14 ± 0.02
CM2 (1:1.5)	7.95 ± 2.56	++	1.16 ± 0.03
CM3 (1:2)	8.98 ± 2.98	++	1.22 ± 0.02
P-CM (0:1)	6.11 ± 2.10	+	1.02 ± 0.04

*** (+++) very high; (++) high; (+) less.**
± R.S.D., n=3

Fig 9.1 Particle size distribution for CM1 to P-CM



9.2.2 SEM Study:

Fig 9.2, 9.3, 9.4 and 9.5 shows the SEM of CM1, CM2, CM3 and P-CM respectively. All the figures show that the microspheres were spherical. However, clumping of the microspheres was observed in batches where chitosan was used in high concentration e.g. in CM2 and CM3. Less clumping was observed in CM1 may be due to less chitosan concentration used in formulation. Placebo microspheres (P-CM) also showed less clumping where the chitosan concentration was same as that of CM1. Surface roughness was also increased with increased drug concentration i.e. drug content in microspheres was high in CM1 (47.5%) which shows high surface roughness. These results are in accordance with Miglani, who reported increase in surface roughness of microspheres with increased drug loading (Miglani, 2002). The porous nature of the microspheres can also be observed in all cases.

Particle size of microspheres can also be observed from SEM study. It was seen that particles was observed in between 5 to 10 µm and size of microspheres increased as chitosan concentration was increased from CM1 to CM3.

Fig 9.2 SEM for CM1

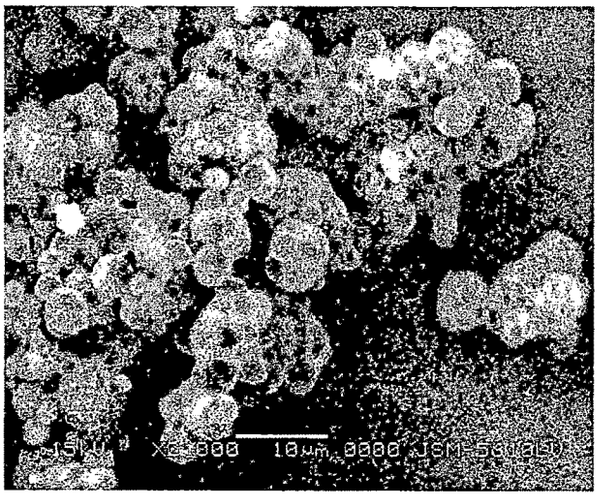


Fig 9.3 SEM for CM2

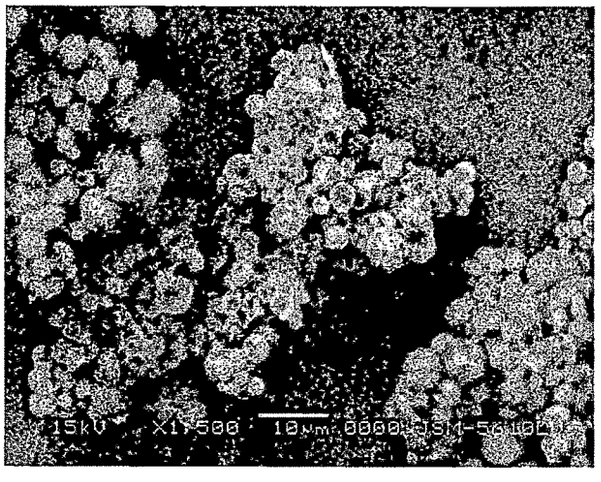


Fig 9.4 SEM for CM3

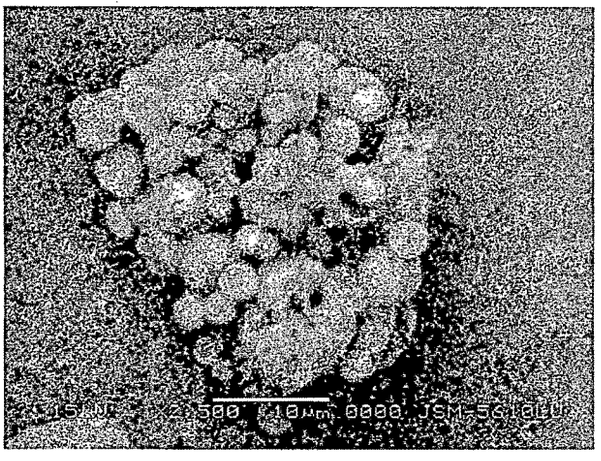
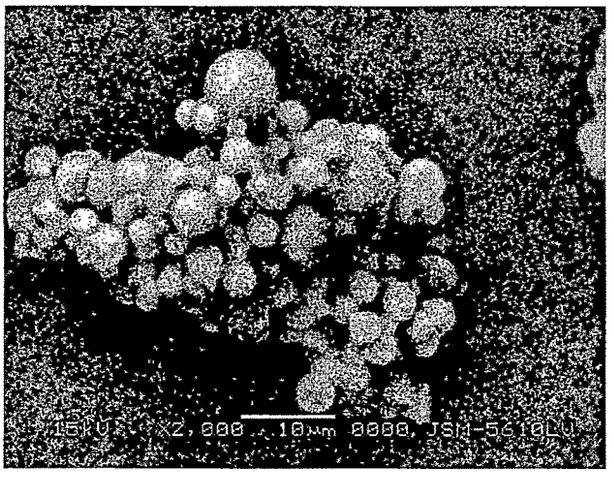


Fig 9.5 SEM for P-CM



9.2.3 Encapsulation efficiency:

Table 9.3 and Fig 9.6 show encapsulation efficiency for CM1 to CM3. Maximum encapsulation efficiency was shown by CM3 i.e. 90.32 ± 2.11%. Results show that as chitosan concentration increased in the microspheres, encapsulation efficiency was also found to increase. Chitosan concentration was increased from CM1 (47.5%) to CM2 (57.0%) and CM3 (63.33%), encapsulation efficiency was increased from CM1 (81.98 ± 2.32 %) to CM2 (87.63 ± 1.98 %) and CM3 (90.32 ± 2.11 %). These results are in accordance with Nishioka et al. who reported increased in encapsulation efficiency of Cisplatin with increase in

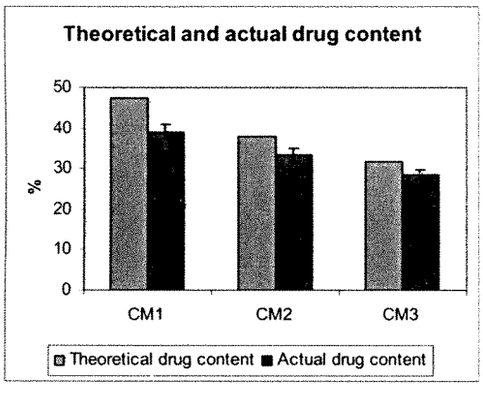
concentration of chitosan (Nishioka et al., 1990). It should be noted that though actual drug loading decreased the encapsulation was increased.

Table 9.3 Encapsulation efficiency for CM1 to CM3

Formulation Code	Theoretical drug content (%)	Actual drug content (%) ± R.S.D.	Encapsulation efficiency (%)± R.S.D.
CM1	47.5	39.94 ± 1.78	81.98 ± 2.32
CM2	38	33.29 ± 1.56	87.63 ± 1.98
CM3	31.66	28.59 ± 1.14	90.32 ± 2.11

± R.S.D., n=3

Fig 9.6 Theoretical and actual drug content.



9.2.4 Characterization of microspheres by FT-IR Spectra:

FT-IR spectrum of Carvedilol, physical mixture of Carvedilol and chitosan and Carvedilol microspheres were recorded and shown in Fig 9.7, 9.8 and 9.9 respectively. Spectra shows that the IR spectra of Carvedilol has the principal peaks at wave numbers 1591, 1502, 1444, 1348, 1251 and 1099 cm^{-1}. Reported FT-IR spectra of Chitosan show peak in the region of 3000 cm^{-1} to 3700 cm^{-1} of the spectrum, exhibits a band corresponding to the stretching of OH groups. Chitosan presents a broad band centered at 1076 cm^{-1} associated with the stretching of C-O. The band amide ($n_{C=O}$), characteristic of Chitosan with acetylated units is present in all the spectra as a shoulder at 1605 cm^{-1} (Taboada et al., 2003).

FT-IR spectrum of physical mixture of Carvedilol and chitosan shows all the principal peaks of Carvedilol. When FT-IR spectrum of Carvedilol microspheres was recorded it shows peaks at wave numbers 1348, 1251 cm^{-1}. There was absence of other principal peaks of Carvedilol 1591, 1502, 1444 and 1099 cm^{-1}.

This can be interpreted as Carvedilol was encapsulated with chitosan in microspheres. Formation of matrix of Carvedilol and chitosan resulted in absence of principal peaks of Carvedilol in IR spectra of Carvedilol microspheres.

Fig 9.7 FT-IR Spectra of Carvedilol.

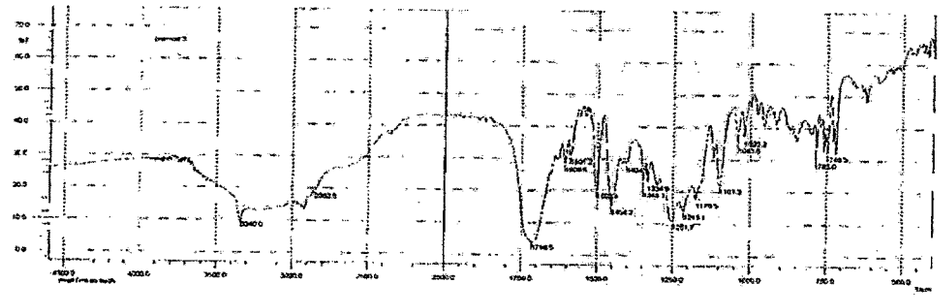


Fig 9.8 FT-IR Spectra of physical mixture of Carvedilol and Chitosan.

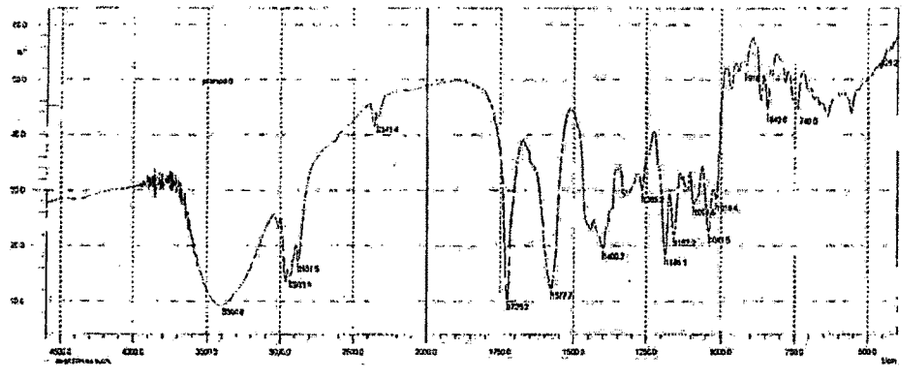
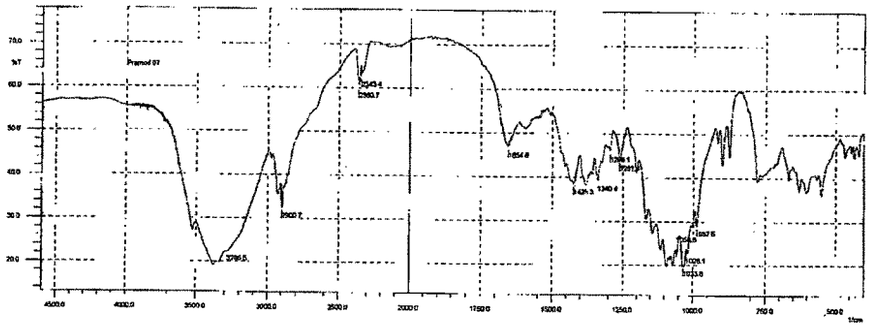


Fig 9.9 FT-IR Spectra of Carvedilol microspheres.



9.2.5 Characterization of microspheres by XRD study:

The XRD studies help to understand the nature of drug (crystalline or amorphous) (Palmieri et al. 2001). X-ray diffraction pattern of Carvedilol (Fig 9.10) and Carvedilol microspheres (Fig 9.11) were recorded and evaluated for presence or absence of crystallinity of the Carvedilol in microspheres. From XRD pattern of Carvedilol, it is evident that Carvedilol exhibited characteristic peaks at 2Θ of 14.79, 17.49, 18.41, 24.30, 26.17, 31.42, 34.19 and 41.84, indicating that it exists in crystalline form. Carvedilol microspheres showed peaks at 2Θ of 18.93 and 19.87 with high intensity. It can be concluded that XRD pattern of Carvedilol microspheres show presence of less crystalline regions in Carvedilol when formulated into microspheres. This may be due to molecular dispersion or encapsulation of Carvedilol into microspheres that resulted into formation of less crystalline structure. Desai K.G.H. reported less crystalline structure of Vitamin C when it was formulated into chitosan microspheres (Desai and Park, 2005).

Fig 9.10 XRD pattern of Carvedilol

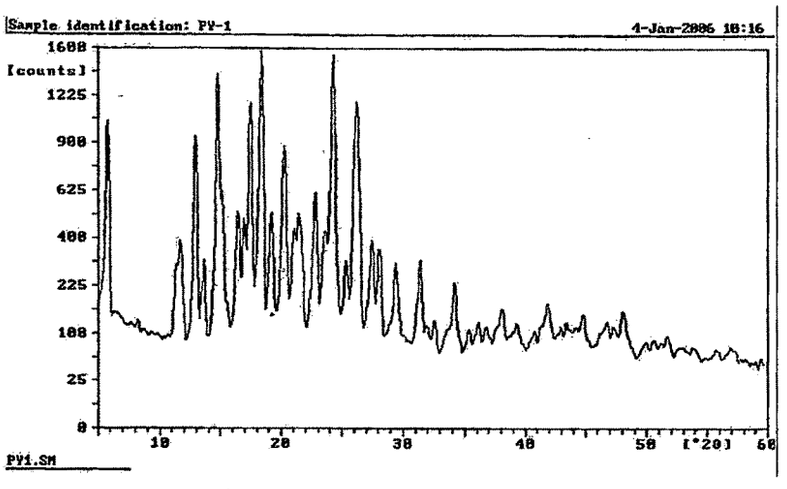
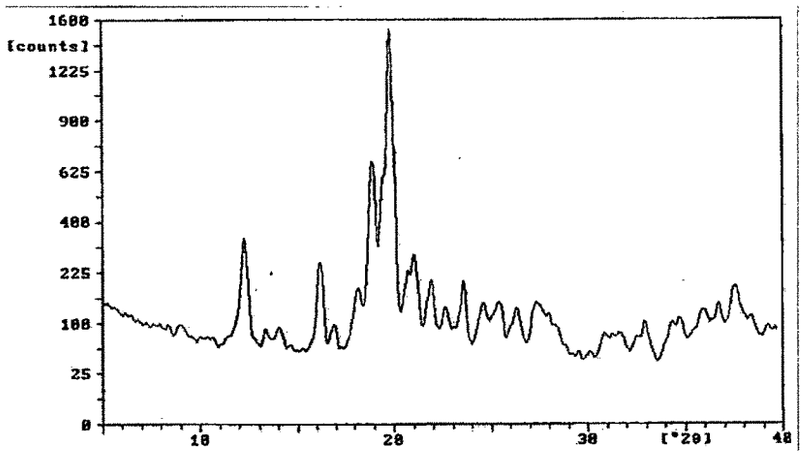


Fig 9.11 XRD pattern of Carvedilol microspheres



9.3 Bilayer tablets formulated with microspheres (CTM): (Liabot et al., 2002; Adel et al, 2004)

9.3.1 Physicochemical parameters of bilayer tablets with microspheres:

Table 9.4 shows physicochemical parameters of bilayer tablets formulated with microspheres. Diameter and thickness values ranged from 8.01 ± 0.05 (CTM1) to 8.02 ± 0.09 (P-CTM) mm and 2.91 ± 0.08 (CTM1) mm to 2.99 ± 0.09 (P-CTM) mm respectively. Hardness (2.5 ± 0.50 kg/cCM2) was observed to be same for all the formulations. Friability values were 0.19 ± 0.02 % (CTM2) to 0.31 ± 0.03 (P-CTM) % and found in acceptable limits (< 1%). Average weight of tablets was within acceptable limits (<5% deviation) and weight variation was within acceptable limits (<5 % deviation). Assay values were found to be 101.26 ± 1.03 %, 97.31 ± 2.12 % and 102.31 ± 1.12 % for CTM1, CTM2 and CTM3 respectively.

Table 9.4 Physicochemical parameters of bilayer tablets formulated with microspheres.

Parameters	Formulation Code			
	CTM1	CTM2	CTM3	P-CTM
Diameter (mm)	8.01 ± 0.05	8.01 ± 0.08	8.02 ± 0.09	8.01 ± 0.09
Thickness (mm)	2.91 ± 0.08	2.96 ± 0.07	2.92 ± 0.07	2.99 ± 0.09
Hardness (kg/cm^2)	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50
Friability (%)	0.21 ± 0.02	0.19 ± 0.02	0.29 ± 0.03	0.31 ± 0.03
Average Wt.(mg)	204.21 ± 1.98	205.38 ± 1.44	207.66 ± 3.19	202.12 ± 1.41
Assay (%)	101.26 ± 1.03	97.31 ± 2.12	102.31 ± 1.12	NA

± R.S.D., (n=3),

9.3.2 Surface pH:

Table 9.5 show the surface pH of the buccal bilayer tablets formulated with microspheres. It remained fairly constant at a pH of 5.13 ± 0.18 to 5.71 ± 0.17. Therefore, this study confirmed that the surface pH of the buccoadhesive tablets was within the neutral conditions of saliva and that no extremes in pH occurred throughout the test period.

Table 9.5 Surface pH of the bilayer tablets.

Formulation Code	Surface pH
CTM1	5.71 ± 0.17
CTM2	5.28 ± 0.14
CTM3	5.13 ± 0.18
P-CTM	5.55 ± 0.20

± R.S.D., (n=3)

9.3.3 Swelling:

The % swelling of the bilayer buccal tablets was investigated in phosphate buffered saline (pH 6.8 ± 0.2). Table 9.6 and Fig 9.12 show % swelling values of CTM1 to P-CTM. %

Swelling observed for CTM1, CTM2, CTM3 and P-CTM was 2.95 ± 0.29%, 3.08 ± 0.26%, 3.50 ± 0.30% and 2.88 ± 0.29 % respectively. It can be seen from data that the Carvedilol bilayer tablets displayed limited amount of swelling.

Chitosan, which was the main polymer used for the formulation of microspheres, has excellent swelling characteristics in acidic medium but in neutral or alkaline medium it shows less swelling. This pH-sensitive swelling behaviour is related to ionization degree of amino group on chitosan in different pH solutions (Prabaharan and Mano, 2005). Therefore in current study, this may be the reason behind less swelling of tablets formulated with chitosan microspheres. These results are in accordance with Govender et al. who reported less % swelling for chitosan microspheres at pH 6.8 (Govender et al., 2005).

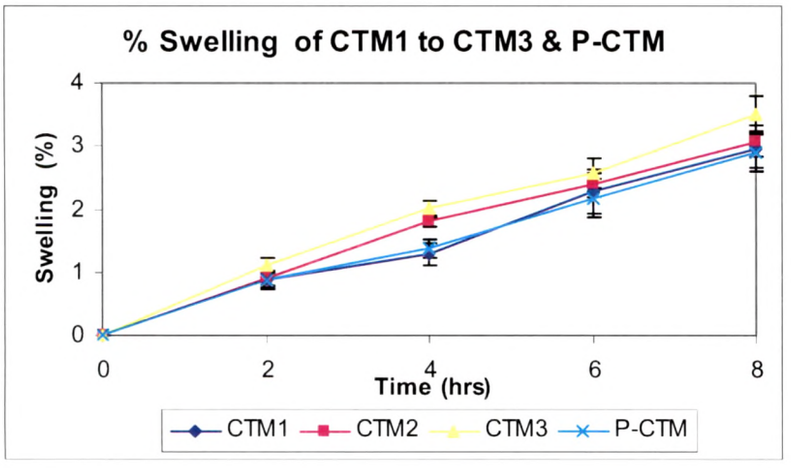
In current study, high molecular weight chitosan was used which may be also a reason behind less swelling of tablets formulated with microspheres. High molecular weight chitosan takes more time to swell than low and medium molecular weight chitosan (Genta et al., 1998). Increase in molecular weight of chitosan increases the viscocity of gel layer and inhibits further swelling of tablets. Genta et al. reported comparative fast % swelling for medium molecular weight chitosan than high molecular weight chitosan (Genta et al., 1998). Formulation with higher concentration of chitosan (CTM3) showed a higher swelling as compared to all other formulations i.e. 3.50 ± 0.30 % swelling after 8 hrs. As proportion of chitosan increased in the tablets, swelling values were found to increase. Swelling of Carvedilol microspheres (CTM1) and placebo microspheres (P-CTM) with same concentration of chitosan was statistically insignificant ($p<0.1$) indicating that the incorporation of drug did not significantly alter the swelling behavior of chitosan.

Table 9.6 Swelling studies of CTM1 to P-CTM

Formulation Code	% Swelling			
	2 Hr	4 Hr	6 Hr	8 Hr
CTM1	0.88 ± 0.13	1.28 ± 0.13	2.28 ± 0.23	2.95 ± 0.29
CTM2	0.90 ± 0.09	1.80 ± 0.09	2.39 ± 0.19	3.08 ± 0.26
CTM3	1.11 ± 0.17	2.01 ± 0.17	2.58 ± 0.34	3.50 ± 0.30
P-CTM	0.87 ± 0.15	1.37 ± 0.15	2.17 ± 0.31	2.88 ± 0.29

± R.S.D., (n=3)

Fig 9.12 Swelling profile with time.



9.3.4 In vitro Mucoadhesive force:

Table 9.7 and Fig 9.13 shows in vitro mucoadhesive force of CTM1 to P-CTM. All the batches show moderate to good (39 ± 1.98 to 50 ± 1.84 x 10^3 dyne cm^{-2}) in vitro mucoadhesive force. Highest mucoadhesive force (50 ± 1.84 x 10^3 dyne cm^{-2}) was shown by CTM1 which contains drug: polymer ratio of 1:1. As concentration of chitosan was increased mucoadhesive force was found to decrease. Thus, it can be concluded that as chitosan concentration increased, it showed a negative effect on mucoadhesion. At a higher chitosan concentration, coiling of the polymer molecules may have occurred, reducing the flexibility of the polymeric chains thereby reducing the mucoadhesive strength. At lower chitosan concentrations, the polymer structure of the chitosan may have been loosened and the polymer chains therefore had more space to extend within the mucin. Miller et al. concluded that increase in polymer concentration many times actually diminishes mucoadhesive force (Miller et al., 2005).

The moderate to good values of mucoadhesion of tablets formulated with chitosan microspheres may be due to the ability of microspheres to absorb water from the mucous layer and allow polymer penetration in to the mucin network.

It has been reported that swelling affects the mucoadhesive force of formulations (Valenta, 2005). But in spite of limited swelling behavior of tablets formulated with chitosan microspheres, they show moderate to good mucoadhesive force. This may be because mucoadhesive performance of chitosan is attributed mainly to its cationic nature. Cationic

materials display a mechanism of mucoadhesion in which not only hydrogen bonding but also salt-bridge effects involving the positively charged chitosan microparticles and the negatively charged mucus glycoproteins are of importance. Govender et al. also observed limited swelling of chitosan microspheres but satisfactory in vitro mucoadhesive force (Govender et al., 2005).

Sinha V.R et al. postulated that positively charged chitosan develop additional molecular forces by electrostatic interaction with negatively charged sugar moieties of the mucosal surface (Sinha et al., 2004).

In current study, high molecular weight chitosan was used which also might have resulted in satisfactory mucoadhesive force values. High molecular weight chitosan have sufficient chain flexibility to react with mucin (Dhawan et al., 2004). Presence of Carbopol in tablets was also a significant factor in improving the in vitro mucoadhesive force of tablets. Presence of drug does not affect the mucoadhesive force of formulation as it was seen by mucoadhesive force obtained by CTM1 ($50 \pm 1.84 \times 10^3$ dyne cm^{-2}) and P-CTM ($47 \pm 1.08 \times 10^3$ dyne cm^{-2}).

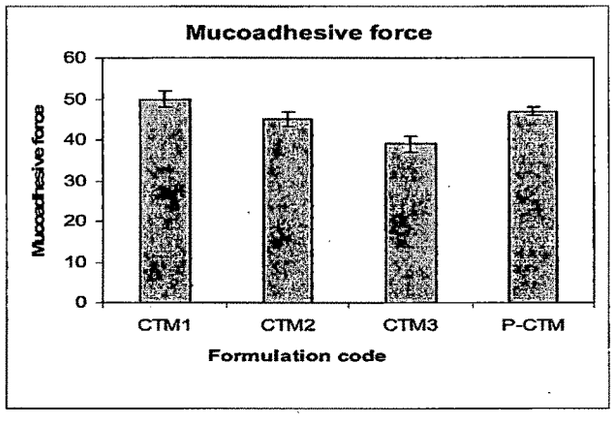
Thus, it was concluded that CTM1 showed better in vitro mucoadhesive force compared with other formulations.

Table 9.7 In vitro mucoadhesive force of CTM1 to P-CTM

Formulation Code	Mucoadhesive force (10^3 dyne cm^{-2})
CTM1	50 ± 1.84
CTM2	45 ± 1.78
CTM3	39 ± 1.98
P-CTM	47 ± 1.08

± R.S.D., (n=3)

Fig 9.13 In vitro mucoadhesive force of CTM1 to P-CTM.



9.3.5 *In vitro* diffusion:

In vitro diffusion through sheep buccal mucosa for 8 hr of the Carvedilol bilayer buccal tablets formulated with chitosan microspheres was studied as shown in Table 9.8 and Fig 9.14.

Formulation containing drug: polymer ratio of 1:1 (CTM1) showed highest diffusion (71.12 ± 2.59 %). For CTM2 and CTM3 with drug: polymer ratio of 1:1.5 and 1:2, the diffusion was found to be 68.25 ± 2.35 % and 64.58 ± 3.11 % respectively. Pure drug diffused 93.02 ± 2.93 % in 2 hr.

The low diffusion values in formulation with higher concentration of chitosan may be due to comparative less swelling of these formulations. Lesser or moderate swelling resulted into less amount of drug available for diffusion and showed less diffusion values.

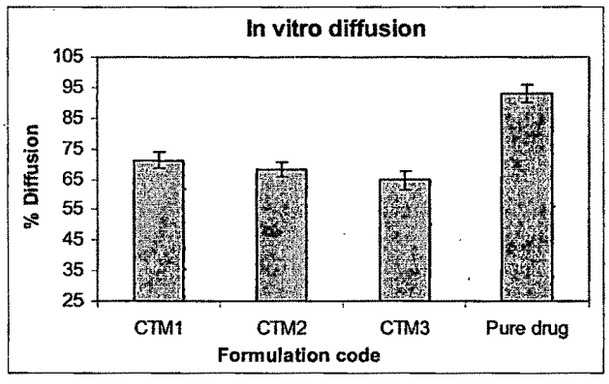
Permeation enhancing effect of chitosan by paracellular transport is well reported in previous studies (Bjork et al., 1995; Alpar et al., 2005). The mechanism of action of chitosan to improve diffusion was suggested to be a combination of improved mucoadhesion and widening of paracellular junctions in the membrane (Thanou et al., 2001). Paracellular transport may not be important for lipophilic drugs such as Carvedilol, for which the transcellular transport is the main pathway of permeation through buccal mucosa.

Table 9.8 In vitro diffusion of pure drug and CTM1 to CTM3.

Formulation code	In vitro diffusion (%)
CTM1	71.12 ± 2.59
CTM2	68.25 ± 2.35
CTM3	64.58 ± 3.11
*Pure Drug	93.02 ± 2.93

± R.S.D., (n=3), * = Study conducted for 2hr

Fig. 9.14 In vitro diffusion of pure drug and CTM1 to CTM3.



9.3.6 *In vitro* dissolution study

9.3.6.1 Chitosan microspheres:

In vitro dissolution of the chitosan microspheres was studied and is shown in Table 9.9 and Fig 9.15. In case of microspheres, maximum drug dissolution was observed in CM1 which released 92.34 ± 2.89 % of the drug in 8 hr. Other formulations CM2 and CM3 showed slow dissolution profile and released 89.19 ± 2.85 % and 81.58 ± 2.36 % of Carvedilol respectively.

It was found that as percentage of chitosan increased, the rate of dissolution of Carvedilol decreased. This may be the result of less swelling of microspheres which resulted in limited dissolution. Ko et al found the same phenomenon for the controlled release from chitosan microspheres (Ko et al., 2002).

Chitosan shows limited swelling behavior at pH 6.8 (Kockisch et al., 2003). Experimental pH used in this study was 6.8; less swelling of microspheres at this pH could be the reason behind the less dissolution of Carvedilol from chitosan microspheres. It may be assumed that initially outer layers of the microspheres may have hydrated to form a gel layer so that water penetration into the core of the particles is impeded, hindering the transport of the drug result in slow dissolution of drug. Kockisch S. et al. reported slow in vitro dissolution profile of triclosan at neutral experimental pH (Kockisch et al., 2005).

In current study, high molecular weight chitosan has been used which also might have delayed the release of Carvedilol. Shiraishi et al. investigated the effect of molecular weight

of chitosan on the release of indomethacin and observed that the release rate of indomethacin decreased with increasing molecular weight of chitosan (Shiraishi et al., 1993). Dissolution data was subjected to model fitting by zero order, first order, Higuchi and Hixson Crowell and Peppas. Table 9.10 shows the model fitting data for Carvedilol dissolution from chitosan microspheres.

From the results it was observed that all the formulations followed nearly zero order kinetics. Best fit was shown by CM1, as it shows $r^2 = 0.996$ for zero order. CM2 and CM3 show r^2 as 0.991.

When Peppas model was applied it showed n values (release exponent) between 0.83 and 0.98. It is known that, if the values of n are in between 0 – 0.5, then it follows fickian diffusion and if n values lies in between 0.5 -1.0, it supports non-fickian diffusion pattern (Dortunc et al., 1998, Costa and Lobo, 2001). Therefore all the formulations followed non-fickian diffusion mechanism. These results can be correlated with Sezer and Akbuga, who reported non fickian diffusion mechanism for the dissolution of piroxicam from chitosan microspheres (Sezer and Akbuga, 1995).

Table 9.9 In vitro dissolution of pure drug and microspheres CM1 to CM3.

Time (hr)	Formulation Code			
	Pure Drug (%)	CM1 (%)	CM2 (%)	CM3 (%)
1	89.23 ± 2.23	16.12 ± 3.00	11.36 ± 3.00	8.74 ± 2.56
2	100.00 ± 3.45	28.56 ± 3.47	24.78 ± 2.60	19.85 ± 3.00
3	-	41.12 ± 3.01	37.14 ± 2.36	33.98 ± 3.12
4	-	53.78 ± 3.00	48.14 ± 2.45	45.12 ± 1.87
5	-	64.30 ± 3.11	63.30 ± 2.69	59.45 ± 2.00
6	-	75.10 ± 3.01	74.50 ± 2.56	74.7 ± 3.14
7	-	87.56 ± 2.00	83.56 ± 1.99	78.11 ± 2.60
8	-	94.34 ± 2.89	89.19 ± 2.85	81.58 ± 2.36

(n=3), ± RSD

Fig. 9.15 In vitro drug dissolution for pure drug and microspheres CM1 to CM3

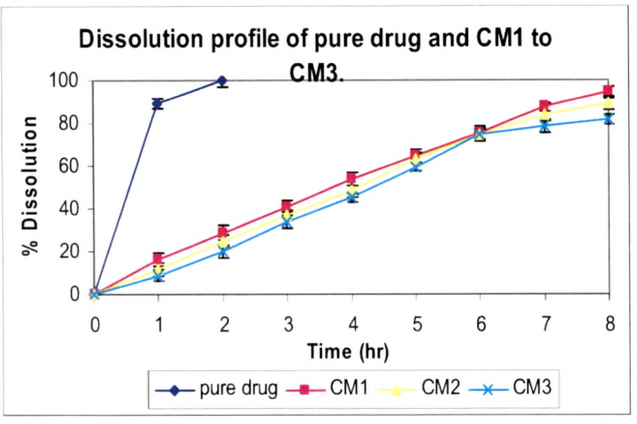


Table 9.10 Model fitting for Carvedilol dissolution from chitosan microspheres

Formulation Code	Zero order		First Order		Higuchi		Hixson Crowell		Peppas		
	K_0	r^2	K_1	R^2	K_H	r^2	K_S	r^2	K	r^2	n
CM1	11.36	0.996	0.15	0.921	44.18	0.99	0.28	0.957	0.91	0.996	0.83
CM2	11.49	0.991	0.13	0.965	44.83	0.99	0.3	0.938	1.03	0.99	0.97
CM3	11.09	0.991	0.1	0.978	43.23	0.99	0.32	0.932	1.11	0.989	0.98

9.3.6.2 Bilayer tablets formulated with chitosan microspheres:

In vitro dissolution profiles of bilayer tablets were studied and are shown in Table 9.11 and Fig 9.16. CTM1, CTM2 and CTM3 showed 72.08 ± 3.05%, 65.58 ± 3.01% and 64.58 ± 2.89 % dissolution respectively. Maximum dissolution was observed in CTM1 (72.08 ± 3.05). Pure drug showed 100.00 ± 3.45% dissolution in less than 2 hr.

The dissolution profile exhibited by bilayer tablets was slower than microspheres. It indicates that Carbopol 934P which was used as matrix forming polymer in tablets plays a role in controlling drug dissolution. In tablets, slow in vitro dissolution results could be due to possible ionic interactions among chitosan, a cationic polymer used for the preparation of the microspheres, and the anionic polymers, Carbopol 934P which was used as matrix forming polymer. In fact, it is already known that the cationic nature of chitosan permits the formation of complexes with oppositely charged polymers (Macleod et al., 1999). Therefore

it can be concluded that the dissolution was slow may be because of use of chitosan and Carbopol 934P simultaneously.

It was found in present study that all matrices show extended dissolution behavior. These results can be correlated with Giunchedi et al. They investigated the development of buccal tablets based on chitosan microspheres containing chlorhexidine diacetate which showed the capacity of these formulations to give an extended dissolution of the drug in the buccal cavity (Giunchendi et al., 2002).

Dissolution data was subjected to model fitting by zero order, first order, Higuchi, Hixson Crowell and Peppas and results are shown in Table 9.12.

All the formulations followed nearly zero order kinetics as it shown from r^2 values 0.996, 0.993 and 0.995 for CTM1, CTM2 and CTM3 respectively. When Peppas model was applied, n values (release exponent) were 1.23, 1.45 and 1.62 for CTM1, CTM2 and CTM3 respectively. They followed non-fickian release mechanism with super case II transport i.e. dissolution is the combination of diffusion and chain relaxation (Singh and Ahuja, 2002; Ritger and Peppas, 1987; Jug and Becirevic-Lacan, 2004). Best fit was shown by CTM1 as it shows r^2 very near to 1.00.

Table 9.11 In vitro dissolution of pure drug and tablets CTM1 to CTM3.

Time (hr)	Formulation Code			
	Pure Drug (%)	CTM1 (%)	CTM2 (%)	CTM3 (%)
1	89.23 ± 2.23	5.01 ± 2.36	2.74 ± 2.89	2.11 ± 2.89
2	100.00 ± 3.45	15.36 ± 3.00	10.85± 2.98	8.23 ± 2.87
3		27.23 ± 2.87	22.98± 3.00	17.23 ± 3.00
4		36.98 ± 3.47	34.12± 3.01	29.35 ± 3.01
5		46.87± 3.11	43.45 ± 2.89	39.12 ± 2.89
6		58.01± 3.00	52.70± 2.87	48.36 ± 3.11
7		65.23 ± 2.98	60.11± 2.85	53.69 ± 2.85
8		73.08 ± 3.05	66.58 ± 3.01	64.58 ± 2.89

(n=3) ± RSD

Fig. 9.16 In vitro drug dissolution for pure drug and tablets CTM1 to CTM3

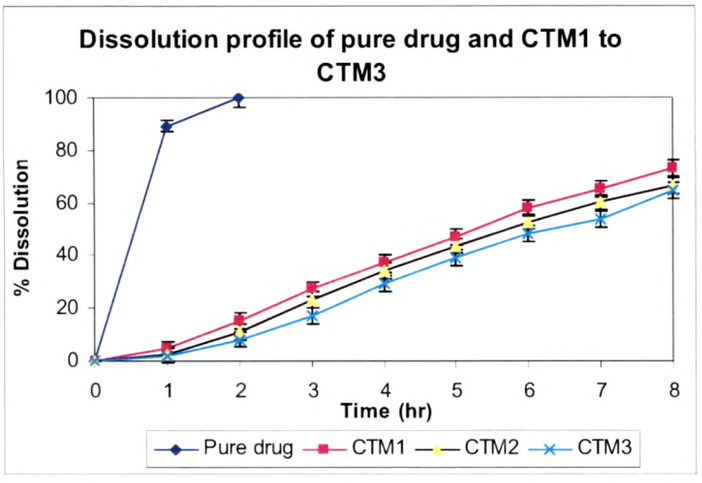


Table 9.12 Model fitting for Carvedilol dissolution from bilayer tablets formulated with microspheres

Formulation Code	Zero Order		First Order		Higuchi		Hixson Crowell		Peppas		
	K_0	r^2	K_1	R^2	K_H	r^2	K_S	r^2	K	r^2	n
CTM1	9.85	0.996	0.07	0.988	38.5	0.992	0.33	0.924	1.29	0.981	1.23
CTM2	9.42	0.993	0.06	0.992	36.96	0.990	0.35	0.908	1.52	0.971	1.45
CTM3	9.13	0.995	0.06	0.970	35.28	0.975	0.37	0.932	1.67	0.986	1.62

9.3.7 Pharmacokinetic Study:

9.3.7.1 Selection of optimized formulation for pharmacokinetic study:

On the basis of *in vitro* parameters such as mucoadhesion, diffusion and in vitro dissolution, it was observed that formulation CTM1 (Carvedilol: Chitosan, 1:1) has satisfactory in vitro mucoadhesive force (50 ± 1.84 x 10^3 dyne cm^{-2}), in vitro diffusion (71.12 ± 2.59 %) and 73.08 ± 3.05 dissolution in 8hr.

On the above basis, CTM1 was finalized to be used for pharmacokinetic studies, histological examination, in-vivo patient acceptability studies on human volunteers and pharmacodynamic studies.

Comparison of plasma profile of oral and bilayer buccal tablets formulated with microspheres.

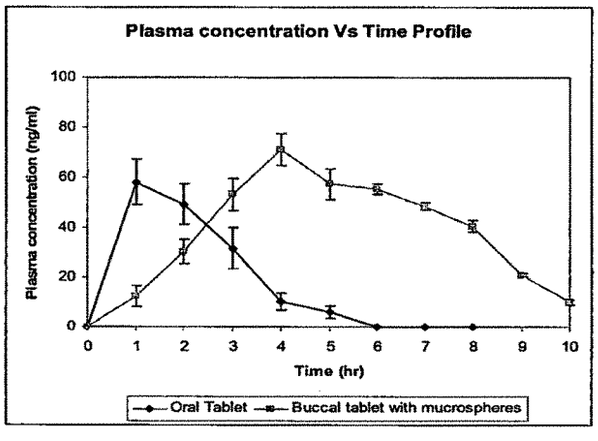
Plasma profile of oral conventional tablets and buccal bilayer tablets formulated with microspheres is shown in Table 9.13 and Fig 9.17. The CTM1 showed sustained release of Carvedilol, as indicated by high t_{max} of 4.0 hr for tablets as compared to 1.00 hr for oral administration and plasma concentration profile. The C_{max} values observed were also higher (71.26 ± 6.45 ng/ml) for Carvedilol bilayer buccal tablets than oral tablets (58.25 ± 9.26 ng/ml) indicating greater absorption. The AUC values after buccal administration of bilayer buccal tablets (390.75 ± 5.23) were significantly higher than that of oral administration (155.22 ± 8.43) which revealed increase in bioavailability coupled with sustained release of Carvedilol by the buccal bilayer tablets.

Administration of Carvedilol to rabbits in the form of bilayer buccal tablets showed about 2.52 fold increase in bioavailability compared with the conventional tablets by oral administration. The one way ANOVA test showed statistically significant differences ($P < 0.01$) between the AUC of oral conventional tablets and bilayer buccal tablets formulated with microspheres. Absorption of Carvedilol from the bilayer buccal tablets formulated with microspheres appeared slow for 1st and 2nd hour. Plasma concentration for 1st and 2nd hr was observed to be 10.36 ± 4.36 and 28.25 ± 4.90 ng/ml for bilayer buccal tablets as compared to 58.25 ± 9.26 and 49.26 ± 8.22 ng/ml for oral conventional tablets. The lag time for the release of Carvedilol can be explained by its retarded in vitro dissolution behavior. Afterwards, plasma concentration of Carvedilol was high i.e. 53.23 ± 6.61 ng/ml at 3rd hr from bilayer buccal tablets as compared to 31.55 ± 8.28 ng/ml from oral conventional tablets. Prolonged plasma levels (10.02 ± 0.95 ng/ml at 10th hr) were exhibited by bilayer buccal tablets formulated with chitosan microspheres.

Table 9.13 Plasma concentration of Carvedilol following administration of oral conventional tablets and bilayer buccal tablets formulated with microspheres.

Time (Hr)	Plasma concentration (ng/ml)	
	Oral Tablet	Buccal tablets formulated with microspheres
1	58.25 ± 9.26	10.36 ± 4.36
2	49.26 ± 8.22	28.25 ± 4.90
3	31.55 ± 8.28	53.23 ± 6.61
4	10.11 ± 3.50	71.26 ± 6.45
5	6.07 ± 2.60	57.55 ± 6.15
6	B.LoQ	55.55 ± 2.05
7	B.LoQ	48.42 ± 1.51
8	B.LoQ	40.23 ± 2.42
9	B.LoQ	20.87 ± 0.66
10	B.LoQ	10.02 ± 0.95
Tmax (Hr)	**1.0 ± 0.2**	**4.0 ± 1.0**
Cmax (ng/ml)	**58.25 ± 9.26**	**71.26 ± 6.45**
AUC (ng/ml/hr)	**155.22 ± 8.43**	**390.75 ± 5.23**

Fig. 9.17 Plasma concentration Vs Time profile following administration of oral conventional tablets and bilayer buccal tablets formulated with microspheres.



9.3.8 Histological study of buccal mucosa:

9.3.8.1 Light microscopy:

Fig 9.18 and 9.19 shows the section of control and sample buccal mucosa. Light microscopy reveals several distinct patterns of maturation in the epithelium of the human oral mucosa based on various regions of the oral cavity. Three distinctive layers of the oral mucosa are the epithelium, basement membrane, and connective tissues are seen in both the figures.

Fig. 9.18 Section of Control Buccal Mucosa.

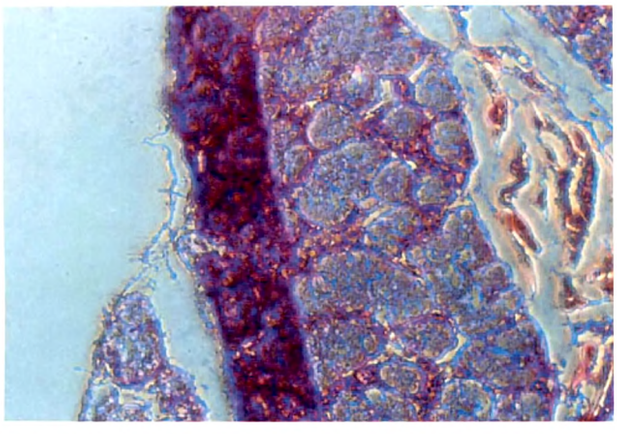
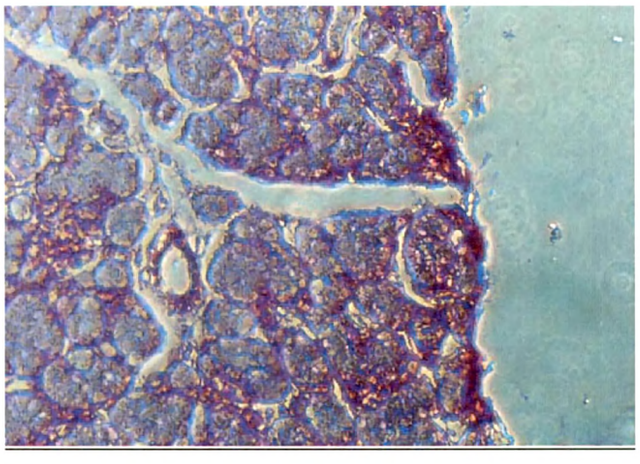


Fig. 9.19 Section of sample Buccal Mucosa.



The tissue specimens examined by light microscopy showed slight modification of the epithelial layer because of use of chitosan, as it improves the paracellular transport by opening the tight junctions in the epithelial layer. It can also be assumed that the slight change in epithelial layer was may be due to retention of drug on mucosa because from the data available in the literature, it appears that the effect of chitosan on mucosa may be due to increasing the retention of the drug at the mucosal surface (Nicolazzo et al., 2005).

It is clear from the results of the permeation experiments that no major alterations in the barrier function of the tissue had been provoked by exposure of the tissue to the formulation.

9.3.8.2 Scanning electron microscopy of buccal mucosa

Fig 9.20 and 9.21 shows the SEM of control and sample buccal mucosa. SEM of the control buccal epithelium revealed the appearance of the superficial cells of the epithelium which represents the major absorption site in the oral cavity.

Treatment of the buccal mucosa with the bilayer buccal tablets formulated with chitosan microspheres showed that the squamous cells are normal and similar to those of the control. But, slight histological changes such as shrinkage of superficial cells appeared in some parts of the tissue which may be because of chitosan. Chitosan is characterized by permeation enhancing effect which opens paracellular junctions and results in shrinkage of superficial layers. From available literature it can be expected that these slight changes may be reversible and will not affect overall structure, surface and function of the buccal mucosa (Attia et al., 2004). **Therefore it can be concluded that Carvedilol bilayer buccal tablets formulated with chitosan microspheres does not cause major damage to the oral buccal mucosa and observed changes may be reversible.**

Fig 9.20 SEM of control Buccal Mucosa.

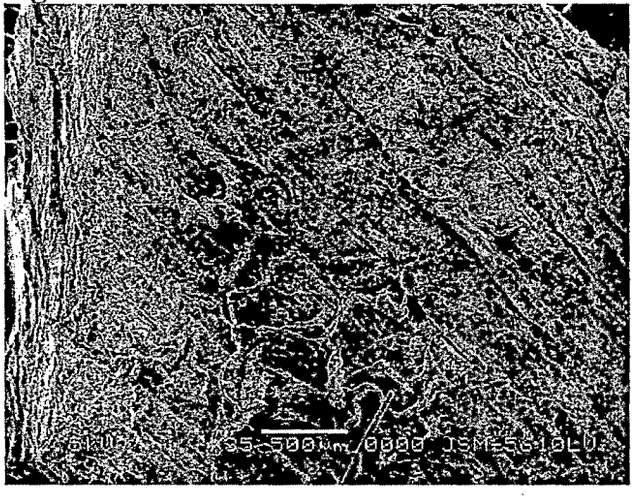


Fig 9.21 SEM of Sample Buccal Mucosa at 35X.

9.3.9 *In vivo* acceptability testing:

The response of volunteers to each subjective parameter was calculated, and obtained results are presented in Table 9.14. Volunteer's response in irritation criteria showed that none of them had complaints. In comfort testing of 80% and 20% volunteers reported comfortable and slightly comfortable levels respectively. None of the volunteers reported moderately comfortable and severely uncomfortable levels. Dryness of mouth was not experienced by any of the volunteers. 20% of volunteers did not experience salivation while 80% reported slight salivary secretion. When volunteers were asked to express their views on heaviness of tablets at the application site, all of them experienced no heaviness. None of the volunteers reported dislodgement of the system during the study for 6 hr.

Based on above results, it can be concluded that the bilayer buccal tablets formulated with chitosan microspheres would be comfortable and acceptable by the patients and retained in the human oral cavity long enough for the drug release to occur.

Table 9.14 Evaluation criteria and results for in vivo acceptability study.

Criteria	Volunteer's response %
Irritation	
None	100
Slight (Tolerated)	-
Moderate	-
Severe (Not tolerated)	-
Comfort	
Very comfortable	-
Comfortable	80
Slightly uncomfortable	20
Moderately uncomfortable	-
Severely uncomfortable	-
Dryness of mouth	
None (Not experienced)	100
Slight (Tolerated)	-
Moderate	-
Severe (Not tolerated)	-
Salivary Secretion	
None (Not experienced)	20
Slight (Tolerated)	80
Moderate (Feeling of discomfort)	-
Severe	-
Heaviness of tablets at the application site	
None (Not experienced)	100
Slight (Tolerated)	-
Moderate (Feeling of discomfort)	-
Severe (Highly discomfort)	-
Dislodgement of the tablets during study (up to 6 hr)	
No	100
Yes	-

9.3.10 Pharmacodynamic studies:

These studies were divided into 2 parts,

3. Development of hypertension in rats for 6 weeks.
4. Treatment with oral conventional tab and buccal bilayer tablets formulated with microspheres (CTM1).

9.3.10.1 Development of fructose induced hypertension:

Results for the development of hypertension were same as described in Carvedilol buccal core in cup tablets.

9.3.10.2 Treatment with oral conventional tablets and buccal bilayer tablets formulated with microspheres.

After development of hypertension in rats, they were treated with oral conventional tablets and bilayer buccal tablets with microspheres. When the hypertensive group of rats was treated with oral conventional tablets, the obtained data is already discussed in pharmacodynamic study part in bilayer buccal patches. When second hypertensive group of rats was treated with bilayer buccal tablets formulated with microspheres, the values obtained (Table 9.15) for MAP, HR and body weight were 111 ± 15 mmHg, 340 ± 27 /min and 200 ± 21 gm at the end of 2 weeks respectively. Triglyceride levels were found to be 86 ± 12 mg/dl. Table 9.16 shows statistical significance ($p<0.001$) between oral conventional tablets and bilayer buccal tablets when one way ANOVA was applied. For 2nd week of treatment with oral conventional tablets, it does not show significant values except for triglycerides. With buccal bilayer tablets all the values were significant.

Hypertensive parameters were also compared in terms of percent reduction in values by administering oral conventional and buccal bilayer tablets and results are depicted in Table 9.17. At the end of 2 weeks, reduction in MAP (mm Hg) was found to be 8.72 and 25.50 % and reduction in HR/min was found 5.12 and 17.07 % by oral conventional and buccal bilayer tablets respectively. 9.61% and 23.07% reduction was found in body Weight (gm) while reduction in triglycerides (mg/dl) was found 33.80 and 59.05 % by oral conventional and buccal bilayer tablets respectively. This clearly indicated that buccal bilayer tablets provided satisfactory treatment as compared to oral conventional tablets.

Treatment with buccal bilayer tablets formulated with microspheres exhibited better results when compared with oral conventional tablets. Possible reasons may be discussed as,

i) Buccal bilayer tablets being sustained release dosage form showed satisfactory results by minimizing fluctuations in the plasma levels as it also seen by pharmacokinetic studies.

ii) Microspheres played an important role in sustaining the release of Carvedilol.

iii) Bilayer formulation design of buccal tablets prevented the loss of drug to GIT through saliva which result in improved bioavailability and in turn increased therapeutic activity.

Table 9.15 Treatment with bilayer buccal tablets formulated with microspheres.

Bilayer buccal tablets			
Parameters	Before treatment (Initial)	After Treatment	
		1W	2W
MAP (mm Hg)	149 ± 08	134 ± 08	111 ± 15
HR/min	410 ± 19	385 ± 21	340 ± 27
Body Weight (gm)	260 ± 24	230 ± 21	200 ± 21
Triglyceride (mg/dl)	210 ± 26	140 ±15	86 ± 12

(± R. S. D.) (n=6).

Table 9.16 Statistical significance at p<0.001 between oral conventional tablets and bilayer buccal tablets.

Statistical significance (p<0.001)				
Parameters	Oral conventional tablets		Bilayer buccal tablets	
	1W	2W	1W	2W
MAP (mm Hg)	NS	NS	NS	S
HR/min	NS	NS	NS	S
Body Weight (gm)	NS	NS	S	S
Triglyceride (mg/dl)	NS	S	S	S

NS: Not significant, S= Significant, n=6.

Table 9.17- % Reduction in Hypertensive Parameters.

% Reduction in Hypertensive Parameters				
Parameters	Oral conventional tablets		Bilayer buccal tablets	
	1W	2W	1W	2W
MAP (mm Hg)	4.69	**8.72**	10.06	**25.50**
HR/min	3.65	**5.12**	6.09	**17.07**
Body Weight (gm)	4.23	**9.61**	11.53	**23.07**
Triglyceride (mg/dl)	13.80	**33.80**	33.33	**59.05**

Fig 9.22 Comparative evaluation of MAP after administration of oral and buccal bilayer tablets.

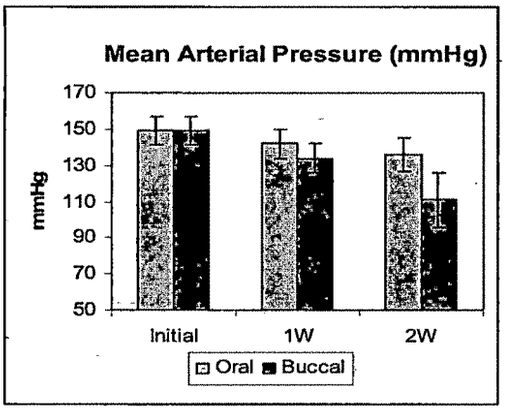


Fig 9.23 Comparative evaluation of heart rate after administration of oral and buccal bilayer tablets.

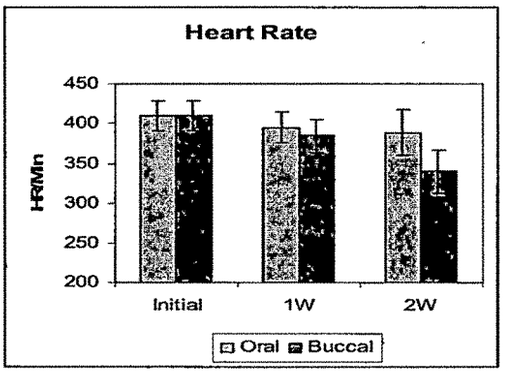


Fig 9.24 Comparative evaluation of triglycerides after administration of oral and buccal bilayer tablets.

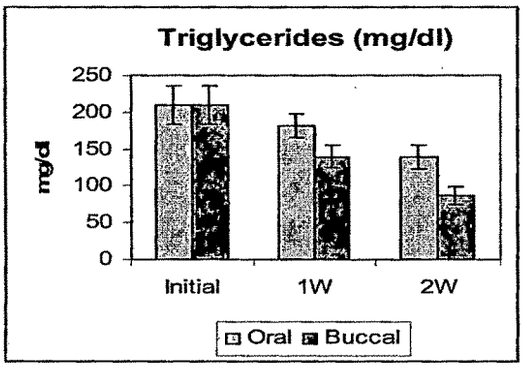
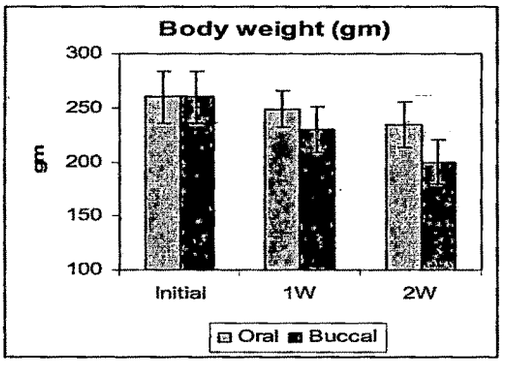


Fig 9.25 Comparative evaluation of body weight after administration of oral and buccal bilayer tablets.



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10.0 RESULTS AND DISCUSSION (PRAVASTATIN SODIUM-PREFORMULATION STUDIES)

10.1 Determination of n-octanol: buffer partition coefficient:

The observed partition coefficient using n-octanol: buffer system was found to be 0.50. This value implies that Pravastatin sodium will show poor permeability across the buccal mucosa. As Pravastatin sodium is hydrophilic in nature, it will have difficulty in permeating through the cell membrane.

10.2 Compatibility studies using FT-IR spectroscopy:

The FT-IR spectra of Pravastatin sodium alone and in combination with Carbopol 934P and HPMC K4M were recorded to evaluate any incompatibility between Pravastatin sodium and polymers. Fig. 10.1 shows the IR spectra of Pravastatin sodium. The principal peaks were at wave numbers 1727, 1579, 1187 cm^{-1} were seen in spectra of plain Pravastatin sodium.

Fig. 10.2 and 10.3 show the IR spectra of physical mixture of Pravastatin sodium and HPMC K4M and Pravastatin sodium and Carbopol934P. All these spectra showed the principal peaks of Pravastatin sodium. This signifies that there is no interaction of Pravastatin sodium with HPMC K4M and Carbopol934P.

Fig 10.1 IR spectra of Pravastatin sodium.

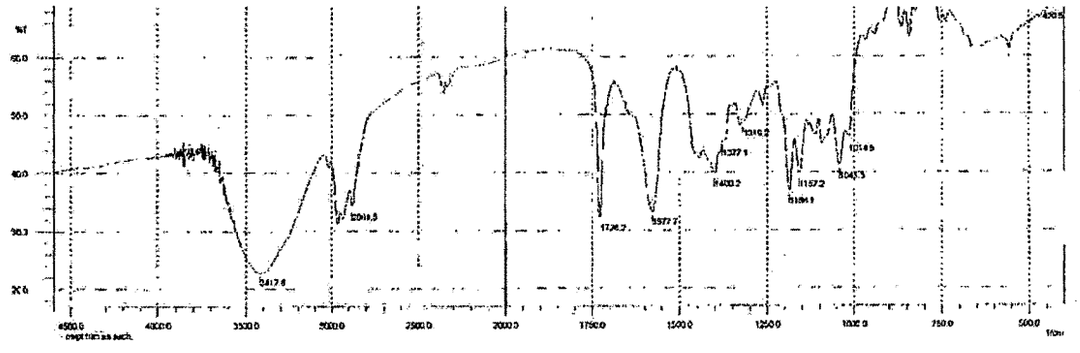


Fig 10.2 IR spectra of physical mixture of Pravastatin sodium and HPMC K4M.

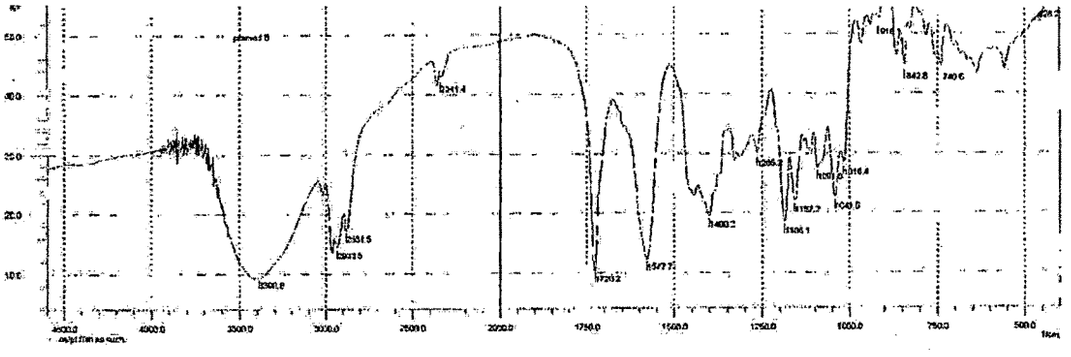
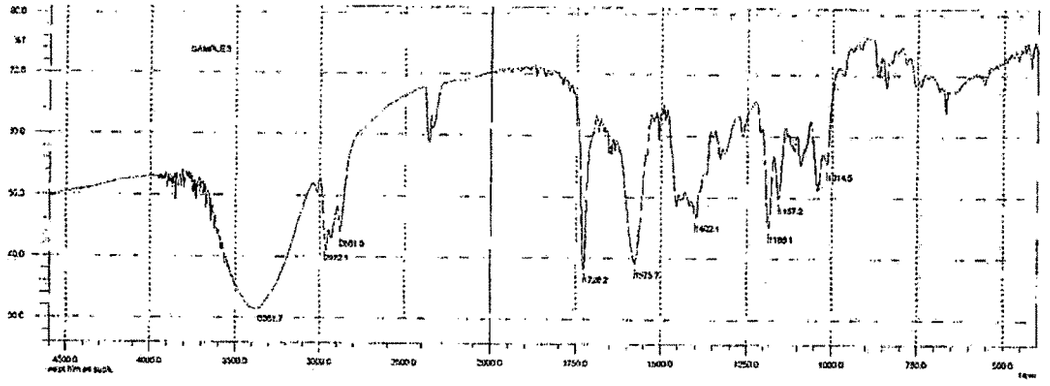


Fig 10.3 IR spectra of physical mixture of Pravastatin sodium and Carbopol 934P.



10.3 SEM study of Pravastatin sodium:

Fig 10.4 shows the SEM of Pravastatin sodium. Pravastatin sodium was observed to be flake like structure. The flakes were small in size and irregular in shape. The particle size ranged from 5 to 500 μ.

Fig 10.4 SEM of Pravastatin sodium

10.4 *In vitro* permeation studies:

The permeation of Pravastatin sodium was studied through sheep buccal mucosa by using Franz diffusion cell. The flux of Pravastatin sodium was found to be 0.18 x 10^{-6} μg cm^{-2} min^{-1} at pH 6.8 indicating poor permeability. Lower flux value through sheep buccal mucosa coupled with lower partition coefficient signifies poor transmucosal permeability of Pravastatin sodium.

Hence it was found necessary to use permeation enhancers to improve the permeability of Pravastatin sodium. Sodium glycocholate and sodium lauryl sulphate were used as permeation enhancers.

10.5 Influence of permeation enhancers on permeability of Pravastatin sodium:

10.5.1 Sodium glycocholate:

Pravastatin sodium was taken at 1 and 5 % concentration in donor compartment and 'a' denotes the 1 and 5 % concentration of Pravastatin sodium with 100mM of sodium glycocholate. Sodium glycocholate was found to enhance the permeability of Pravastatin sodium. Results are shown in Table 10.1.

Table 10.1 Effect of Sodium glycocholate on permeability of Pravastatin sodium.

Concentration of Pravastatin sodium (%)	Steady state flux ($\mu g\ cm^{-2}\ min^{-1}$)	Enhancement Ratio (%)	% Diffusion
1	$0.18 \pm 0.02 \times 10^{-6}$	-	42.36 ± 3.56
5	$0.24 \pm 0.04 \times 10^{-6}$	-	48.36 ± 4.91
1a	$0.26 \pm 0.02 \times 10^{-6}$	144	60.99 ± 4.18
5a	$0.36 \pm 0.04 \times 10^{-6}$	150	72.54 ± 3.87

n=3, ± RSD, a – With 100 mM sodium glycocholate.

With sodium glycocholate, permeability increased from 0.18×10^{-6} to 0.26×10^{-6} $\mu g\ cm^{-2}\ min^{-1}$ for 1% Pravastatin sodium i.e. permeability enhancement ratio is 144%. Percentage diffusion was increased from 42.36 ± 3.56 to 60.99 ± 4.18 %. For 5% Pravastatin sodium, it was found that permeability has been increased from 0.24×10^{-6} to 0.36×10^{-6} $\mu g\ cm^{-2}\ min^{-1}$ i.e. enhancement ratio is 150%. Percentage diffusion was increased from 48.36 ± 4.91 to 72.54 ± 3.87 %. Statistically significant difference ($p < 0.005$) was found for % diffusion of Pravastatin sodium and Pravastatin sodium with sodium glycocholate. Increase in permeability was independent of concentration of Pravastatin sodium because with 1% and 5% concentration of Pravastatin sodium, enhancement ratio was 144% and 150% respectively.

Shojaei et al. found the similar results for buccal acyclovir delivery, with the incorporation of sodium glycocholate as the permeation enhancer (Shojaei et al., 1998). Based on our results, sodium glycocholate will increase the permeation of Pravastatin sodium by paracellular

transport because hydrophilic drug prefer the permeation through paracellular pathway. Sodium glycocholate was shown to enhance the buccal transport of flecainide acetate and not the more lipophilic flecainide base, which was attributed to the different pathways and the ability of the bile salt to affect only the paracellular route (Deneer et al., 2002). The mechanism of permeation activity of sodium glycocholate is by provoking lipid solubilization, both in the intercellular domains and from the cell membranes. The solubilization of lipids in the intercellular space may increase the diffusivity of hydrophilic compounds and thus enhance their overall transport rate (Shojaei et al., 1998).

10.5.2 Sodium lauryl sulphate (SLS):

SLS was found to enhance the permeability of Pravastatin sodium. Results are shown in Table 10.2. With sodium lauryl sulphate, permeability increased from 0.18 x 10^{-6} to 0.27 x 10^{-6} $\mu g/cm^{-2}$ min^{-1} for 1% Pravastatin sodium i.e. enhancement ratio is 150%. Percentage diffusion was increased from 42.36 ± 3.56 to 63.54 ± 5.01 %.

For 5% Pravastatin sodium, it was increased from 0.24 x 10^{-6} to 0.41 x 10^{-6} $\mu g/cm^{-2}$ min^{-1} i.e. enhancement ratio was 155%. Percentage diffusion was increased from 48.36 ± 4.91 to 74.95 ± 4.85%.

Out of sodium glycocholate and sodium lauryl sulphate, sodium lauryl sulphate was found to have slightly higher enhancement ratio but the difference was not statistically significant ($p > 0.1$). Irritation of buccal mucosa with sodium lauryl sulphate is reported in literature (Williams and Barry, 2004). Therefore sodium glycocholate was selected for further studies. It was decided to use sodium glycocholate (100 mM) in all the formulations.

Table 10.2 Effect of Sodium lauryl sulphate on permeability of Pravastatin sodium.

Concentration of Pravastatin sodium (%)	Steady state flux ($\mu g/cm^{-2}$ min^{-1})	Enhancement Ratio (%)	% Diffusion
1	0.18 ± 0.02 x 10^{-6}	-	42.36 ± 3.56
5	0.24 ± 0.03 x 10^{-6}	-	48.36 ± 4.91
1a	0.27 ± 0.02 x 10^{-6}	150	63.54 ± 5.01
5a	0.41 ± 0.03 x 10^{-6}	155	74.95 ± 4.85

n=3, ± RSD, a – With 100 mM sodium lauryl sulphate

10.6 References:

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11.0 RESULTS AND DISCUSSION (PRAVASTATIN SODIUM CORE IN CUP TABLETS -PCT)

11.1 Formulation of Pravastatin sodium core in cup tablets:

11.1.1 Formulation of core (6 mm diameter):

Core tablets were loosely compressed on 8 station D-tooling machine. The obtained physicochemical parameters are shown in Table 11.1. Diameter of tablets ranged from 6.00 ± 0.09 (PCT4) to 6.02 ± 0.10 (PCT1) mm. Thickness values were found in between 3.03 ± 0.03 (PCT1) to 3.09 ± 0.05 (PCT11) mm. Hardness values were in between 2.0 ± 0.50 (PCT11) to 2.5 ± 0.50 (PCT1) Kg/cm^2. Average weight of tablets was within acceptable limits (<7.5 % deviation). Assay values ranged from 97.87 ± 2.09 (PCT5) to 102.02 ± 1.13 % (PCT7). All the physicochemical characteristics were within acceptable limits.

Table 11.1 Physicochemical parameters of core tablets

Parameter	PCT1	PCT2	PCT3	PCT4	PCT5	PCT6	PCT7	PCT8	PCT9	PCT10	PCT11
Diameter (mm)	6.02 ± 0.10	6.02 ± 0.11	6.02 ± 0.10	6.00 ± 0.09	6.02 ± 0.11	6.02 ± 0.11	6.00 ± 0.11	6.01 ± 0.10	6.01 ± 0.09	6.01 ± 0.11	6.01 ± 0.10
Thickness (mm)	3.03 ± 0.03	3.04 ± 0.05	3.04 ± 0.05	3.06 ± 0.04	3.06 ± 0.05	3.08 ± 0.05	3.08 ± 0.05	3.08 ± 0.06	3.08 ± 0.06	3.09 ± 0.05	3.09 ± 0.05
Hardness (Kg/cm^2)	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.5 ± 0.50	2.0 ± 0.50	2.0 ± 0.50
Average weight (mg)	81.49 ± 1.47	81.98 ± 1.40	82.06 ± 2.00	81.12 ± 1.24	80.02 ± 1.06	82.08 ± 1.68	81.55 ± 1.14	78.88 ± 1.12	78.12 ± 1.12	80.13 ± 1.45	79.05 ± 1.41
Assay (%)	99.30 ± 1.16	100.37 ± 2.41	101.96 ± 1.73	100.22 ± 1.84	97.87 ± 2.09	98.88 ± 1.08	102.01 ± 1.88	98.48 ± 1.63	102.02 ± 1.13	99.01 ± 1.17	98.98 ± 1.99

± R.S.D. (n=3), PCT-Pravastatin sodium core in cup tablets

11.1.2 Formation of buccal adhesive cup

Table 11.2 shows physical parameters of cup tablets. Outer and inner diameter of tablets was found to be 10.02 ± 0.33 mm and 6.02 ± 0.31 mm respectively. Obtained thickness values was 4.67 ± 0.04 mm. Hardness values was 2.5 ± 0.50 Kg/cm^2. Average weight was found within acceptable limits (<5% deviation).

Table 11.2 Physical parameters of cup tablets

Parameters	F_{cup}
Outer Diameter (mm,	10.02 ± 0.33
Inner Diameter (mm)	6.02 ± 0.31
Thickness (mm)	4.67 ± 0.04
Hardness (Kg/cm²)	2.5 ± 0.50
Average weight (mg)	320.40 ± 1.58

± R.S.D. (n=3)

11.1.3 Formation of core in cup tablets (Compressing core tablets in cup).

Table 11.3 exhibits physicochemical parameters of core in cup tablets. Diameter of tablets ranged from 10.01 ± 0.10 (PCT3) to 10.10 ± 0.10 (PCT11) mm. Thickness values were found in between 4.41 ± 0.04 to 4.70 ± 0.08 mm. Hardness values were in between 3.0 ± 0.50 (PCT11) to 4.5 ± 0.50 (PCT4) Kg/cm^2. Average weight was within acceptable limits (<5% deviation). All the physicochemical characteristics were within acceptable limits.

Table 11.3 Physicochemical parameters of core in cup tablets.

Parameter	PCT1	PCT2	PCT3	PCT4	PCT5	PCT6	PCT7	PCT8	PCT9	PCT10	PCT11
Diameter (mm)	10.02 ± 0.08	10.03 ± 0.09	10.01 ± 0.10	10.04 ± 0.08	10.04 ± 0.10	10.01 ± 0.09	10.04 ± 0.09	10.06 ± 0.15	10.05 ± 0.08	10.10 ± 0.10	10.10 ± 0.10
Thickness (mm)	4.61 ± 0.05	4.59 ± 0.04	4.52 ± 0.06	4.41 ± 0.04	4.59 ± 0.05	4.61 ± 0.04	4.59 ± 0.05	4.62 ± 0.06	4.62 ± 0.04	4.65 ± 0.09	4.70 ± 0.08
Hardness (Kg/cm²)	3.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.5 ± 0.50	4.0 ± 0.50	4.0 ± 0.50	4.0 ± 0.50	3.0 ± 0.50
Average weight (mg)	401.20 ± 1.81	401.12 ± 1.78	402.06 ± 2.01	402.02 ± 1.76	401.92 ± 1.65	401.78 ± 1.67	400.45 ± 1.87	401.76 ± 1.48	401.98 ± 1.05	402.33 ± 1.35	403.18 ± 1.85
Friability (%)	0.1	0.08	0.08	0.09	0.07	0.09	0.08	0.08	0.08	0.09	0.1

± R.S.D. (n=3)

11.2 Surface pH:

The surface pH of the tablets was determined in order to confirm that the tablets would not cause irritation in and around the buccal mucosa due to extremes in pH (Govender et al., 2005). Table 11.4 showed that the surface pH of the buccal tablets remained fairly constant at a pH of approximately 5.5–6.25 over the 8 h test period. Therefore, this study confirmed that the surface pH of the buccoadhesive tablets was near the neutral conditions of saliva and hence would not alter the pH of the buccal fluids and cause no damage or alteration to the buccal mucosa due to altered pH conditions.

Table 11.4 Surface pH of formulation PCT1 to PCT11.

Formulation Code	Surface pH
PCT1	5.80 ± 0.10
PCT2	5.83 ± 0.13
PCT3	5.92 ± 0.13
PCT4	5.53 ± 0.61
PCT5	6.10 ± 0.11
PCT6	6.22 ± 0.31
PCT7	6.25 ± 0.22
PCT8	6.28 ± 0.23
PCT9	6.13 ± 0.09
PCT10	6.19 ± 0.18
PCT11	6.11 ± 0.31

± R.S.D. (n=3)

11.3 Swelling study:

Swelling is the prerequisite for the mucoadhesive dosage form to adhere to the buccal mucosa. The swelling behaviour of the Pravastatin sodium core in cup buccoadhesive tablets in phosphate buffered saline pH 6.8 ±0.2 was investigated and data is shown in Table 11.5, Fig. 11.1 and 11.2. It can be seen that the Pravastatin sodium tablets displayed 4.82 to 6.13 % swelling. Formulation with HPMC K4M alone showed a comparatively higher swelling as compared to all other formulations i.e. 6.13 ± 0.20 and 5.93 ± 0.20 swelling after 8 hr by PCT1 and PCT11 respectively. Formulations with combination of HPMC and Carbopol 934P showed 4.82 to 5.81 % swelling but it was low as compared to formulations where

HPMC K4M and Carbopol 934P were used alone. Comparatively least swelling was observed in PCT9 (Carbopol 934P: HPMC K4M, 9:1) i.e. 4.82 ± 0.21 % which may be due to less uptake of water. It was observed in PCT1 and PCT11, where HPMC K4M and Carbopol 934P were used alone that there was formation of highly porous structure at the end of study. Ugwoke et al. reported that formation of highly porous structure may loosen adhesive bonds with mucosa and results in weaker adhesion (Ugwoke et al., 2005). In PCT11 where only Carbopol 934P was present, different swelling pattern was seen as compared to other formulations i.e. sharp rise in swelling index after 6th hr of study. This may be due to its ionization constant. At pH 6.8 Carbopol934P will get ionized, which will loosen the polymer integrity/matrix and result in high swelling.

Unlike Carvedilol core in cup tablets, Pravastatin sodium core in cup tablets showed high degree of swelling at 8th hr of exposure to the phosphate buffer saline (PBS) which may be due to high affinity of Pravastatin sodium towards water. Solubilisation of drug from buccal core in cup tablets will result in loose matrix structure and increase in swelling.

It can be concluded that satisfactory swelling was observed with all the formulations containing combination of Carbopol 934P and HPMC K4M. Comparatively higher swelling was seen in PCT1.

Table 11.5 Swelling studies of PCT1 to PCT11.

Formulation Code	% Swelling			
	2 Hrs	4 Hrs	6 Hrs	8 Hrs
PCT1	2.11 ± 0.11	2.89 ± 0.18	4.10 ± 0.19	6.13 ± 0.20
PCT2	2.09 ± 0.11	2.49 ± 0.17	3.79 ± 0.17	5.81 ± 0.19
PCT3	2.08 ± 0.10	2.39 ± 0.14	3.65 ± 0.17	5.65 ± 0.18
PCT4	2.02 ± 0.12	2.34 ± 0.13	3.49 ± 0.18	5.49 ± 0.19
PCT5	2.01 ± 0.10	2.27 ± 0.14	3.44 ± 0.18	5.45 ± 0.18
PCT6	2.23 ± 0.12	2.39 ± 0.16	3.35 ± 0.17	5.41 ± 0.17
PCT7	2.09 ± 0.11	2.33 ± 0.15	3.31 ± 0.18	5.34 ± 0.21
PCT8	2.11 ± 0.12	2.34 ± 0.16	3.19 ± 0.18	5.10 ± 0.20
PCT9	1.89 ± 0.10	2.01 ± 0.15	3.11 ± 0.19	4.82 ± 0.21
PCT10	1.83 ± 0.12	2.05 ± 0.14	2.99 ± 0.19	5.02 ± 0.21
PCT11	1.76 ± 0.09	2.00 ± 0.14	2.78 ± 0.20	5.93 ± 0.20

± R.S.D. (n=3)

Fig 11.1 Swelling profile for PCT1 to PCT6.

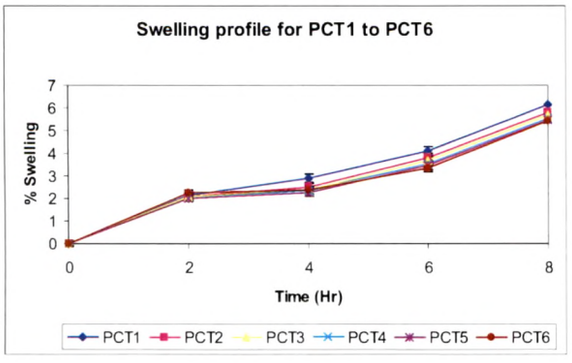
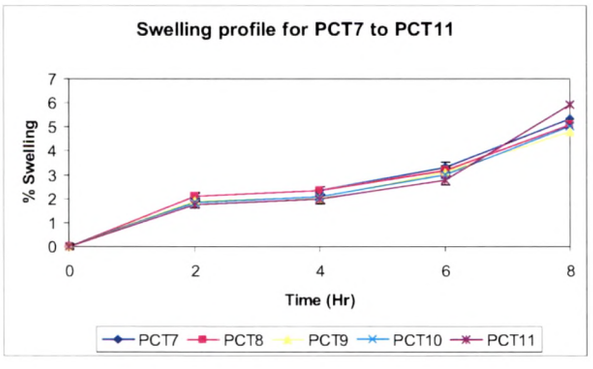


Fig 11.2 Swelling profile for PCT7 to PCT11.



11.4 In vitro Mucoadhesive force:

Mucoadhesion is the first and foremost important and significant prerequisite for the mucoadhesive drug delivery to adhere to mucosa. In this study, sheep buccal mucosa was used as biological membrane to investigate the effect of different polymeric combinations used in formulation on mucoadhesion. Table 11.6 and Fig 11.3 shows in vitro mucoadhesive force for PCT1 to PCT11.

Buccal core in cup tablets containing Carbopol 934P and HPMC K4M at the ratio of 4:6 (PCT5) exhibited comparatively highest mucoadhesion (45 ± 2.58 x 10^3 dyne cm^{-2}) with the buccal mucosa when compared with other formulations. However, all the formulations from PCT3 to PCT8 (in the range of 35 ± 2.14 to 45 ± 2.58 x 10^3 dyne cm^{-2}) exhibited satisfactory mucoadhesion with buccal mucosa. It shows that combination of polymers had demonstrated good mucoadhesive force than individual polymer.

Mucoadhesive force is dependent on many parameters, including the % swelling, pH of medium and degree of ionization of polymer. Moreover, each of the polymers under

consideration is known to exhibit optimum mucoadhesive force at a well-defined state of swelling. Consequently, a change in any of these variables may yield different mucoadhesive strengths (Kockisch et al., 2003). High or inordinate swelling values may lead to weak mucoadhesive force. From swelling studies it was seen that formulation with individual polymers have shown high swelling values i.e. PCT1 (HPMC K4M) - 6.13 ± 0.20 % and PCT11 (Carbopol 934P) - 5.93 ± 0.20 %. The mucoadhesive force shown by this formulations were PCT1 (15 ± 2.36 x 10^3 dyne cm^{-2}) and PCT11 (16 ± 2.14 x 10^3 dyne cm^{-2}). Another factor behind the in vitro mucoadhesive force is pH of medium and ionization of polymer which was seen in PCT11. PCT11, which contains only Carbopol 934P, showed weak mucoadhesive force (16 ± 2.14 x 10^3 dyne cm^{-2}) which may be due to mucoadhesive force of Carbopol 934P is dependent on the pH of surrounding medium. The pH of the buffer solution used in the present study was 6.8, which presumably could have decreased the mucoadhesive force because of the change in the ionization property of carboxylic groups present in Carbopol 934P [pKa of Carbopol 934P is 6.5 (Shojaei and Li, 1997)]. Desai K. G. H. et al., also found weak mucoadhesive force for formulation where only Carbopol 934P was used in the tablets (Desai and Pramodkumar, 2004). The mechanism by which mucoadhesion occurs in Carbopol 934P and HPMC K4M is that of polymer adsorption at an interface, where polymers will reduce the surface energy and can then bind by the formation of bonds, mimicking the natural role of mucins in saliva (Smart, 2005).

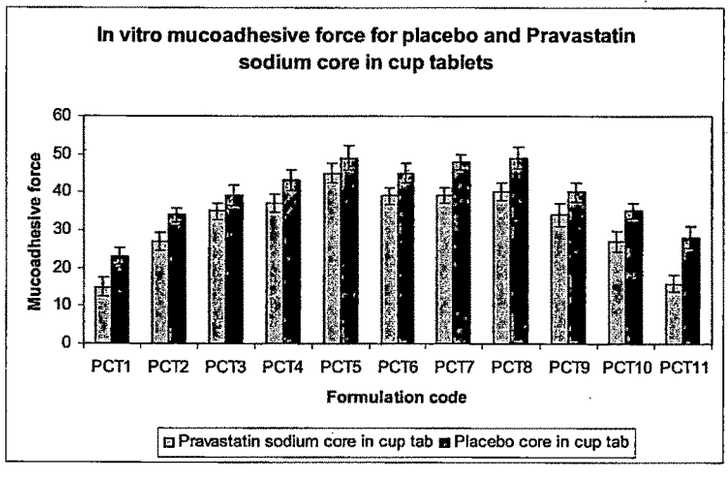
Comparison between the mucoadhesive property of placebo and Pravastatin sodium buccal core in cup tablets indicates that presence of Pravastatin sodium in the formulation had negative effect on the in vitro mucoadhesive force. As water molecule penetrates into the tablets, it will dissolve the water soluble drug particles present at periphery and loosen the matrix, thereby decreasing the mucoadhesive force of tablets. Yong et al concluded that the dispersed drug particles in tablets may have weakened cohesive forces between the polymer chains allowing each chain to hydrate freely and increased the swelling of medicated patches and ultimately decrease in mucoadhesive force (Yong et al., 2001). The values for mucoadhesion of placebo and Pravastatin sodium core in cup tablets were significantly different at $P < 0.1$ indicating that the drug significantly reduced the mucoadhesive property of polymers.

Table 11.6 In vitro mucoadhesive force of Pravastatin sodium and placebo buccal tablets.

Formulation Code	In vitro mucoadhesive force ($\times 10^3$ dyne cm^{-2})		Formulation Code (Placebo)
	Pravastatin sodium Tablets	Placebo Tablets	
PCT1	15 ± 2.36	23 ± 2.23	P-PCT1
PCT2	27 ± 2.45	34 ± 1.88	P-PCT2
PCT3	35 ± 2.14	39 ± 2.69	P-PCT3
PCT4	37 ± 2.35	43 ± 2.68	P-PCT4
PCT5	45 ± 2.58	49 ± 3.15	P-PCT5
PCT6	39 ± 2.11	45 ± 2.69	P-PCT6
PCT7	39 ± 2.01	48 ± 2.01	P-PCT7
PCT8	40 ± 2.35	49 ± 2.78	P-PCT8
PCT9	34 ± 3.01	40 ± 2.45	P-PCT9
PCT10	27 ± 2.58	35 ± 2.01	P-PCT10
PCT11	16 ± 2.14	28 ± 2.88	P-PCT11

± **R.S.D.** (n=3)

Fig 11.3 In vitro mucoadhesive force for placebo and Pravastatin sodium and core in cup tablets



11.5 *In vitro* diffusion:

To study the *in vitro* diffusion of the prepared formulations, Franz diffusion cell was used. Table 11.7 and Fig 11.4 shows the *in vitro* diffusion values for PCT1 to PCT11 and pure drug and pure drug with enhancer diffusion studied through sheep buccal mucosa.

Pure drug has shown less diffusion i.e. 49.36 ± 4.87 %. Pravastatin sodium being a BCS – class III drug having a hydrophilic property has showed a less diffusion because of its lower flux 0.18 x 10^{-6} at pH 6.8 as shown by earlier preformulation studies. Pure drug with permeation enhancer i.e. sodium glycocholate has shown satisfactory diffusion (80.23 ± 3.15%). The marked difference in diffusion property of pure drug and pure drug with permeation enhancer is because of effect of sodium glycocholate which is acting as permeation enhancer. Sodium glycocholate improves diffusion is by opening mucosal non-selective porous pathway. Shojaei et al. reported that increased permeation of hydrophilic compounds due to sodium glycocholate is by wide opening of porous pathway (Shojaei et al., 1998). Aungst and Rogers reported that sodium glycocholate is effective in enhancing transbuccal diffusion of insulin (Aungst and Rogers, 1989).

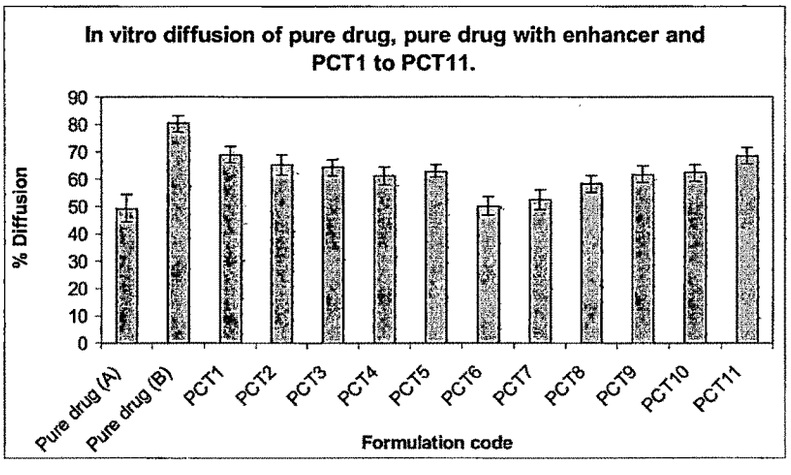
Formulations containing HPMC K4 (PCT1) and Carbopol 934P (PCT11) alone showed highest diffusion (68.98 ± 2.89 and 68.54 ± 3.11 %) respectively as compared to other prepared formulations. The combination of Carbopol 934P and HPMC K4M (PCT2 to PCT10) showed less diffusion of drug as compared to formulations where Carbopol 934P and HPMC K4M were used alone. It implies that combination of polymers have better control over drug diffusion than individual polymers because combination of polymers imparts better matrix structure to tablets. Sustained release of Pravastatin sodium can be expected from combination of Carbopol 934P and HPMC K4M than with individual polymers.

Table 11.7 In vitro diffusion for PCT1 to PCT11 and pure drug at 8th hr.

Formulation Code	% Diffusion ± RSD
Pure drug (Without enhancer)	49.36 ± 4.87
Pure drug (With enhancer)	80.23 ± 3.15
PCT1	68.98 ± 2.89
PCT2	65.23 ± 3.56
PCT3	64.32± 3.01
PCT4	61.29 ± 3.12
PCT5	62.98 ± 2.14
PCT6	50.08 ± 3.41
PCT7	52.35 ± 3.56
PCT8	58.24 ± 3.12
PCT9	61.65 ± 2.99
PCT10	62.23 ± 3.16
PCT11	68.54 ± 3.11

± R.S.D. (n=3), Note: All formulations studied with enhancer (sodium glycocholate).

Fig 11.4 In vitro diffusion for pure drug and PCT1 to PCT11.



Pure drug (A): Pure drug without enhancer.
Pure drug (B): Pure drug with enhancer.

11.6 In vitro dissolution profile:

In vitro dissolution profile of pure drug and formulations (PCT1 to PCT11) is shown in Table 11.8a, 11.8b, Fig 11.5, 11.6 and 11.6. Maximum Pravastatin sodium dissolution was observed in formulation containing HPMC K4M alone, it released 86.49±2.00 % in 5 hr and 100 ± 4.00 % of the drug in 6 h. This could be attributed to its high swelling (6.13 ± 0.20 %), as greater swelling of the matrices leads to faster release of the drug (Agarwal and Mishra, 1999).

Formulation (PCT11) containing Carbopol 934P alone also showed fast dissolution of drug, It released 84.25±2.68 % in 5 hr and 100±3.24 % in 6 hrs due to its high swelling (5.93 ± 0.20 %). During this study it was observed that formulation PCT11 did not maintain its integrity and tended to collapse.

Formulations (PCT2 to PCT10) where combinations of polymers were used extended the drug dissolution up to 8th hr. This may be because the combination of two polymers imparts better matrix characteristics to the tablets than individual polymer. Strong matrix integrity will inhibit the entry of dissolution media and delay the release of drug. Singh et al. reported that combination of Carbopol and HPMC fairly regulated the Metoprolol tartarate release up to 10 hr (Singh and Ahuja, 2002). Formulations (PCT9 and PCT10) where Carbopol 934P was used in high concentration initially showed high release as compared to formulations where HPMC K4M was used in high concentration (PCT2 and PCT3).

To investigate the kinetics of Pravastatin sodium dissolution from core in cup buccal tablets, the dissolution data was applied to zero order, first order, Higuchi (suited for the modeling of drug release from a homogeneous planar matrix, assuming that the matrix does not dissolve), Hixson-Crowell (models drug release from systems with dissolution-rate limitations) and Korsmeyer Peppas (diffusion and polymer relaxation phenomena or anomalous transport) models and best fit was determined (Kockisch et al., 2005). The values of r^2, K and n are listed in table 11.9.

The results indicate that the dissolution mechanism changed with the type and amount of polymer incorporated in the formulation and this can be reflected by the observed values of release exponent (n). For PCT1, which contains only HPMC K4M, n value was 0.61; indicating non-fickian release i.e. drug dissolution is the combination of erosion and

diffusion. PCT11 where only HPMC is present showed n value as 0.50, followed non-fickian release.

When concentration of Carbopol 934P was gradually increased in the formulation, n values were found to increase from 0.72 to 0.99 and when Carbopol 934P was further increased n values were found to decrease from 0.99 to 0.59. Formulations PCT2 to PCT10 showed n values in between 0.72 to 0.99 indicating they follow non-fickian diffusion pattern. Dissolution pattern followed nearly zero order kinetics with non-fickian release implies diffusion being a dominant release mechanism. Singh and Ahuja also reported non-fickian release approaching zero order for the dissolution of Diltiazem hydrochloride, a water soluble drug (Singh and Ahuja, 2002).

Formulation PCT5 showed best fit as it showed $r^2 = 0.998$ for zero order with non-fickian release implying diffusion and erosion mechanism. Peppas model for PCT5 showed n = 0.99 implies non-fickian diffusion pattern.

Table 11.8a In vitro dissolution of pure drug and formulations PCT1 to PCT5.

Formulation Code	Pure Drug	PCT1	PCT2	PCT3	PCT4	PCT5
Time (hr)	Pravastatin sodium dissolution					
1	99.63±2.23	23.10 ±2.56	19.87±1.88	17.02±2.00	17.08±3.00	10.01±2.89
2	-	42.23 ±3.00	31.23±3.00	29.11±2.56	28.11±3.47	21.23±3.02
3	-	52.45±4.00	47.01±3.02	43.14±3.00	39.23±4.00	34.14±3.00
4	-	68.36±1.87	61.14±3.47	54.89±4.01	52.14±3.00	46.89±3.65
5	-	86.49±2.00	79.25±4.01	71.45±1.87	68.32±3.11	60.15±4.00
6	-	100.00±4.00	94.11±3.00	91.32±2.00	85.16±2.56	72.32±3.00
7	-	-	99.85±3.5	99.01±4.00	98.19±2.01	83.11±2.85
8	-	-	-	99.87±3.84	99.01±3.00	92.11±3.84

n=3, ± R.S.D.

Table 11.8b In vitro dissolution of formulations PCT6 to PCT11.

Formulation Code	PCT6	PCT7	PCT8	PCT9	PCT10	PCT11
Time (hr)	Pravastatin sodium dissolution					
1	14.11±2.00	16.01±1.87	18.01±4.01	20.11±2.89	26.29±4.00	29.11±2.69
2	23.01±2.00	28.23±2.00	30.23±3.99	32.25±2.36	38.11±2.60	41.23±2.87
3	37.21±2.89	41.42±4.00	44.14±3.00	46.31±3.01	52.28±2.36	56.69±3.01
4	49.54±4.00	54.32±3.84	57.56±4.00	62.21±4.11	67.12±2.45	72.14±3.25
5	66.36±1.99	68.14±4.00	71.12±1.87	77.11±4.01	80.27±2.69	84.25±2.68
6	81.14±2.00	80.11±2.56	83.24±2.00	90.47±2.56	95.14±2.56	100±3.24
7	98.25±3.98	94.01±2.85	94.96±3.98	99.85±2.85	99.98±1.99	-
8	99.79±4.00	99.82±4.10	99.98±3.84	99.89±4.01	99.98±2.85	-

n=3, ± R.S.D.

Fig 11.5 In vitro drug dissolution of pure drug, PCT1, PCT2, PCT3 and PCT4.

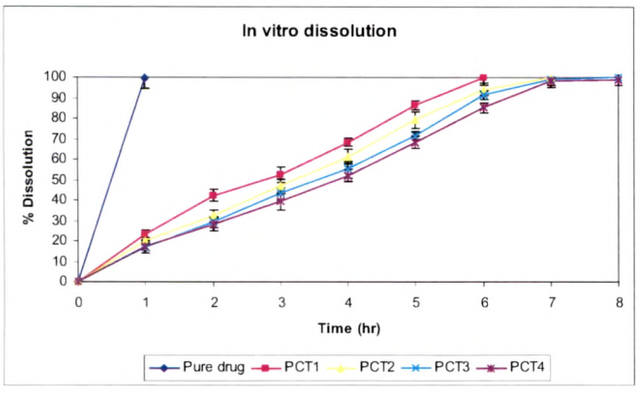


Fig 11.6 In vitro drug dissolution of pure drug, PCT5, PCT6, PCT7 and PCT8.

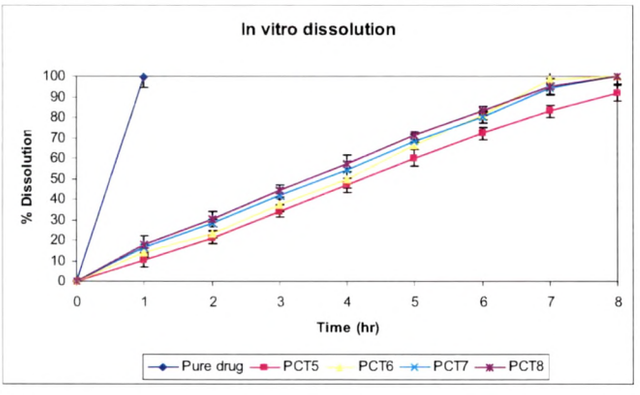


Fig 11.7 In vitro drug dissolution of pure drug, PCT9, PCT10 and PCT11.

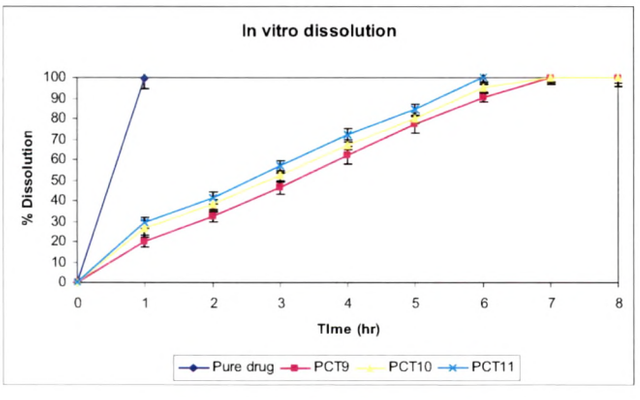


Table 11.9 Model fitting of Pravastatin sodium dissolution from core in cup buccal tablets

Formulation Code	Zero Order		First Order		Higuchi		Hixson Crowell		Korsmeyer Peppas		
	K_0	r^2	K_1	R^2	K_H	r^2	K_S	r^2	K_k	r^2	n
PCT1	11.76	0.932	0.32	0.770	46.66	0.965	0.25	0.892	0.74	0.981	0.61
PCT2	14.14	0.991	0.20	0.862	51.92	0.979	0.32	0.969	0.86	0.992	0.72
PCT3	12.79	0.981	0.16	0.873	49.76	0.977	0.30	0.951	0.89	0.994	0.78
PCT4	12.59	0.990	0.19	0.835	48.78	0.977	0.29	0.963	0.89	0.994	0.79
PCT5	12.04	0.998	0.11	0.953	46.67	0.985	0.32	0.953	1.08	0.998	0.99
PCT6	13.26	0.991	0.19	0.809	51.19	0.970	0.32	0.968	1.00	0.990	0.89
PCT7	12.44	0.996	0.17	0.868	48.26	0.985	0.29	0.961	0.90	0.998	0.80
PCT8	12.24	0.993	0.18	0.880	47.64	0.989	0.28	0.955	0.85	0.997	0.75
PCT9	11.84	0.982	0.23	0.836	46.24	0.986	0.26	0.950	0.76	0.994	0.66
PCT10	11.51	0.962	0.21	0.857	45.25	0.978	0.24	0.932	0.69	0.987	0.59
PCT11	10.50	0.941	0.31	0.747	41.58	0.971	0.21	0.912	0.59	0.985	0.50

11.7 Pharmacokinetic Study

The plasma concentration profile for Pravastatin sodium oral conventional tablets and buccal core in cup tablets in rabbits are shown in Table 11.10 and Fig 11.8. After the administration of Pravastatin sodium oral conventional tablets, t_{max} was observed 1.00 hr indicated a rapid absorption. t_{max} of buccal core in cup tablets was 3.00 hr indicating slow absorption but it was sustained which was seen by plasma concentration profile. The C_{max} were higher (72.36 ± 9.68 ng/ml) for core in cup tablets than oral tablets (67.40 ± 9.23 ng/ml) implying better absorption than oral conventional tablets.

The AUC values (270.28 ± 10.98 ng/ml/hr) after buccal administration of core in cup tablets was significantly higher than that of oral administration (130.33 ± 10.25 ng/ml/hr) which indicates increase in bioavailability of the buccal formulations. Pravastatin sodium showed 2.07 fold increased in bioavailability by core in cup tablets through buccal route in rabbits. The one way ANOVA test showed statistically significant differences ($P < 0.005$) between the AUC of oral conventional tablets and buccal core in cup tablets.

The absorption from oral tablets was high as seen by plasma concentration of 67.40 ± 9.23 ng/ml at 1st hr but it falls off rapidly as seen by plasma concentration of 21.13 ± 5.36 and 13.56 ± 5.11 ng/ml at 3rd and 4th hr respectively. The absorption from the buccal core in cup tablets in the initial phase appeared to be slightly slow i.e. 13.01 ± 4.23 ng/ml plasma concentration at 1st hr. This is may be due to less diffusion of Pravastatin sodium through

buccal mucosa. The fast absorption in the latter phase might be explained by permeation enhancing effect of sodium glycocholate.

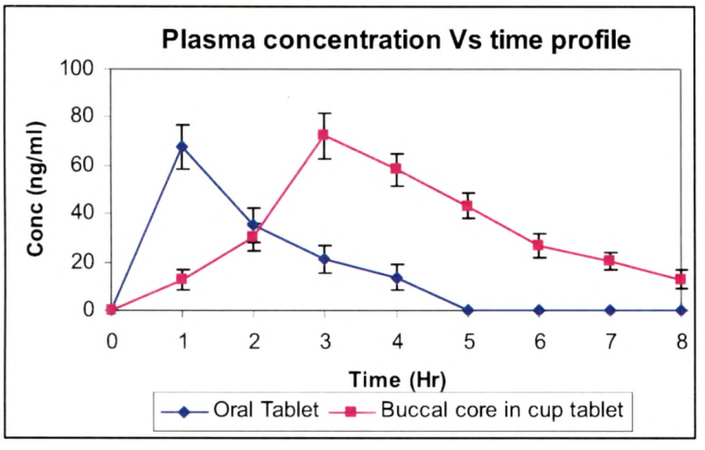
The results demonstrated in pharmacokinetic studies prove the justification of administering Pravastatin sodium through the buccal route as a useful alternative to the oral route for avoiding pre-systemic metabolism, improving bioavailability and sustaining activity.

Table 11.10 Plasma concentration of Pravastatin sodium (ng/ml) following administration of oral tablets and buccal core in cup tablets.

Time (Hr)	Plasma concentration (ng/ml)	
	Oral Tablets	Buccal core in cup tablets
1	67.40 ± 9.23	13.01 ± 4.23
2	35.02 ± 6.89	30.12 ± 5.69
3	21.13 ± 5.36	72.36 ± 9.68
4	13.56 ± 5.11	58.36 ± 6.69
5	B.LoQ	43.12 ± 5.12
6	B.LoQ	26.51 ± 4.87
7	B.LoQ	20.36 ± 3.69
8	B.LoQ	12.9 ± 3.98
t_{max} (Hr)	**1.00 ±0.20**	**3.00 ± 1.00**
C_{max} (ng/ml)	**67.40 ± 9.23**	**72.36 ± 9.68**
AUC (ng/ml/hr)	**130.33 ± 10.25**	**270.28 ± 10.98**

± R.S.D., n=6, B.LoQ-Below limit of quantitation.

Fig 11.8 Plasma concentration Vs Time profile for Pravastatin sodium oral conventional and buccal core in cup tablets.



11.8 Histological study of buccal mucosa:

11.8.1 Light microscopy:

Fig 11.9 and 11.10 shows section of control buccal mucosa and section of sample mucosa. Description of control buccal mucosa is already given in Carvedilol core in cup tablets.

Sample mucosa appeared to be slightly different when compared with control mucosa. Sections showed little modification in the epithelial layer may be because of use of sodium glycocholate which was used a permeation enhancer. It was reported that sodium glycocholate to the buccal epithelium was found to provoke lipid solubilization, both in the intercellular domains and from the cell membranes (Hoogstraate et al., 1996; Gibaldi and Feldman, 1970). The solubilization of these lipids in the intercellular space may increase the diffusivity of hydrophilic compounds and results in slight disruption of superficial cells.

It is clear from the observations of the sections examined by light microscopy that the buccal formulation provoked no major alteration in the barrier function of the mucosa.

Fig 11.9 Section of control buccal mucosa.

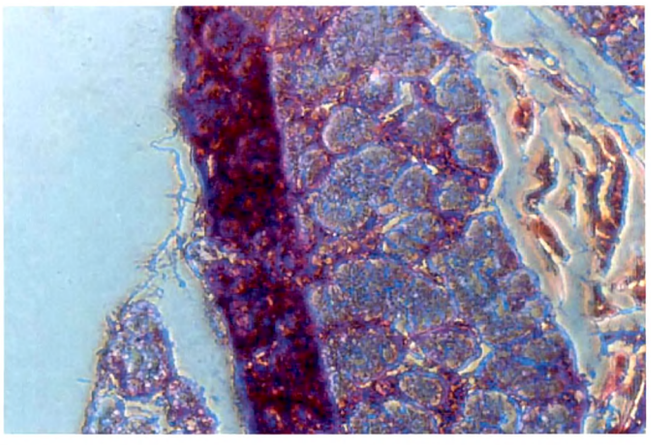
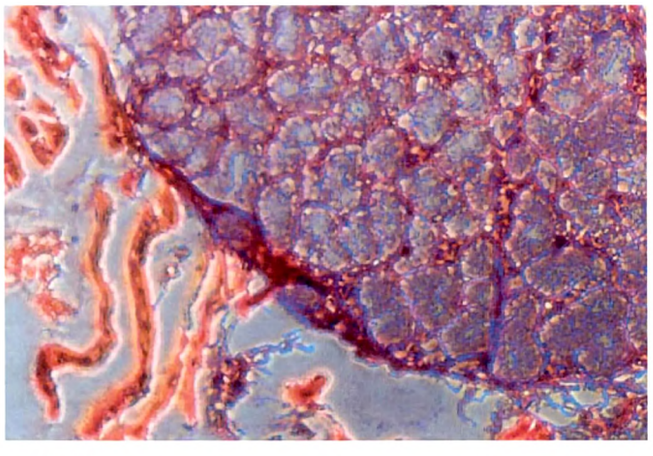


Fig 11.10 Section of sample buccal mucosa.



11.8.2 Scanning electron microscopy of buccal mucosa:

Fig 11.11 and 11.12 shows SEM of control buccal mucosa and sample buccal mucosa respectively. Description of control buccal mucosa was given in Carvedilol core in cup tablets.

Sample buccal mucosa showed that the squamous cells are normal and to some extent similar to those of the control. But, slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue. These changes may be due to use of permeation enhancing effect of sodium glycocholate by effectively decreasing resistance to paracellular pathway. Mechanism by which sodium glycocholate act is by solubilization of intercellular lipids (Jasti et al., 2000) which may have altered the structure of buccal mucosa. Enhancing effect of the sodium glycocholate also depends on the degree of their membrane irritation potential and the rate of penetration of enhancer through the mucosa and the

increase in the fluidity of the intercellular lipids, thereby facilitating diffusion of the drug through the epithelium (Attia et al., 2004). The altered structure of buccal mucosa resulted in shrinkage of squamous cells and desquamation of superficial layer in sample mucosa.

From available literature it can be expected that these slight changes may be reversible and not affected overall structure, surface and function of the buccal mucosa (Attia et al., 2004). Zhang et al proved the effective enhancement of drug diffusion but with significant tissue recovery by application of permeation enhancer (Zhang, 1994).

Fig. 11.11 SEM of Control Buccal Mucosa:

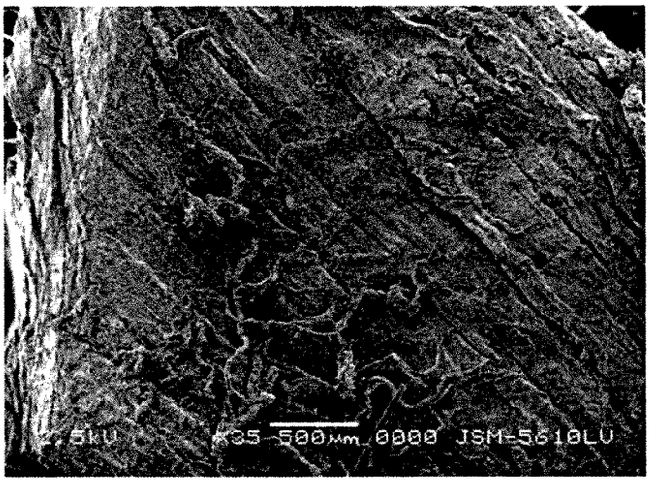
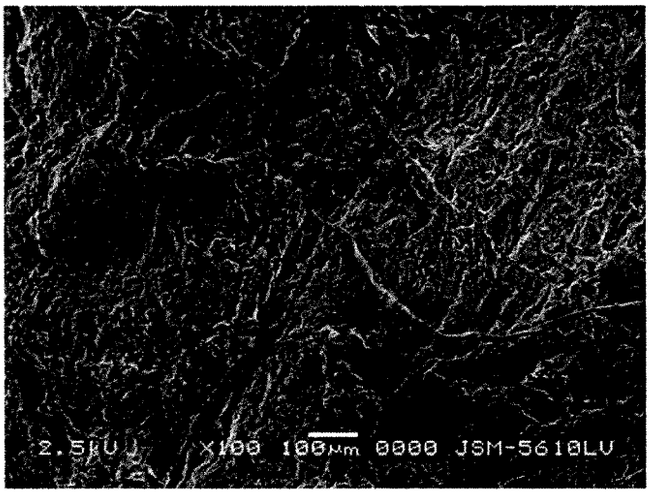


Fig. 11.12 SEM of Control Buccal Mucosa:



11.9 *In vivo* acceptability testing:

The same response from volunteers can be expected as showed in Carvedilol buccal core in cup tablets as the dimension and qualitative composition of tablets was nearly same.

11.10 Pharmacodynamic studies:

These studies were divided into 2 stages,

Stage 1: Induction of hyperlipidemia.

Stage 2: Treatment with conventional oral tablets (10.0 mg/ once a day) and buccal core in cup tablets (10.0 mg/ once a day) and comparison of conventional and buccal formulation in terms of lowering of hyperlipidemic parameters.

11.10.1 Stage 1: Induction of hyperlipidemia.

Hyperlipidemic rabbits show elevation in triglycerides (TG), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels. High density lipoproteins (HDL) levels were constant. Parameters like TG, VLDL, LDL and HDL were measured at start and at every 2nd week during the study. Table 11.11 and Fig 11.13 shows hyperlipidemic parameters for Control (without cholesterol intake) group and Table 11.12 and Fig 11.14 shows hyperlipidemic parameters for Test (with cholesterol intake) group.

At the end of 8 weeks of cholesterol administration, the TG of test group was 191.14 ± 9.00 mg/dL, as compared to 112.11 ± 7.9 mg/dL in the rabbits before the cholesterol intake and TG of control group was observed to be 110.55 ± 9.45 mg/dL. VLDL of test group was observed to be 38.03 ± 3.23 mg/dL as compared to 21.44 ± 3.23 mg/dL in the control. After 8 weeks of cholesterol administration LDL levels were observed to be 20.10 ± 2.21 mg/dL as compared to 9.01 ± 2.44 mg/dL in control group. HDL levels were not increased, as it was 13.14 ± 1.44 mg/dL and 13.87 ± 1.11 mg/dL for test and control group respectively. Generally HDL levels are not increased after cholesterol intake. Yi-Ping et al. found no change in HDL levels after 10 weeks in cholesterol fed rabbits (Yi-Ping et al., 2000).

Control group maintained their TG, LDL and VLDL levels without major changes. Thus cholesterol induced hypertension was developed in rats after 8 weeks. These hyperlipidemic rabbits were then used for studying effect of Pravastatin sodium when administered in the form of oral conventional as well as buccal core in cup tablets.

Table 11.11 Hyperlipidemic parameters for Control (without cholesterol intake) group.

Parameters	At start	During study			
	0W	2W	4W	6W	8W
HDL (mg/dL)	13.25 ± 1.01	13.36 ± 1.02	13.11 ± 1.45	13.44 ± 2.41	13.87 ± 1.11
TG (mg/dL)	112.28 ± 7.90	110.14 ± 8.23	112.14 ± 9.45	110.1 ± 8.21	110.55 ± 9.45
VLDL (mg/dL)	22.36 ± 2.01	21.14 ± 2.14	22.55 ± 3.44	22.15 ± 2.11	21.44 ± 3.23
LDL (mg/dL)	8.14 ± 1.1	8.11 ± 1.00	8.01 ± 1.0	8.23 ± 1.01	9.01 ± 2.44

(n=6), ± R.S.D.

Fig 11.13 Graphical representation of hyperlipidemic parameters for Control (without cholesterol intake) group.

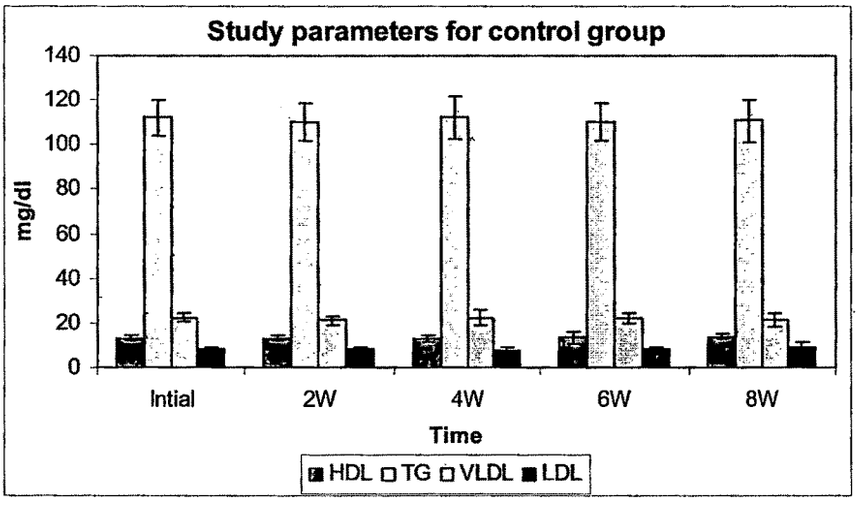
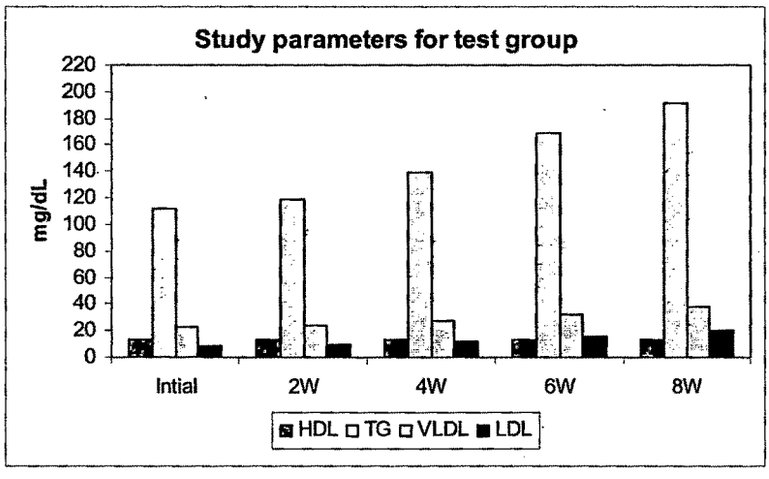


Table 11.12 Hyperlipidemic parameters for test (cholesterol intake) group.

Parameters	At start	During study			
	0W	2W	4W	6W	8W
HDL (mg/dL)	13.21 ± 1.01	13.11 ± 1.03	13.24 ± 1.45	13.23 ± 2.57	13.14 ± 1.44
TG (mg/dL)	112.11 ± 7.9	119.45 ± 8.14	139.21 ± 9.69	168.47 ± 9.00	191.14 ± 9.00
VLDL (mg/dL)	22.01 ± 2.01	24.32 ± 2.5	27.41 ± 3.00	32.15 ± 2.01	38.03 ± 3.23
LDL (mg/dL)	8.2 ± 1.1	9.14 ± 1.23	12.23 ± 1.54	15.12 ± 1.14	20.10 ± 2.21

(n=6), ± R.S.D.

Fig 11.14 Graphical representation of hyperlipidemic parameters for Test (cholesterol intake) group.



11.10.2 Treatment of hyperlipidemia with oral conventional tab and buccal core in cup tablets.

After developing hyperlipidemia in rabbits, they were treated with Pravastatin sodium oral conventional and buccal core-in-cup tablets and observations are recorded in Table 11.13 and 11.14. Fig 11.15, 11.16, 11.17 and 11.18 shows comparative evaluation of HDL, TG, VLDL and LDL respectively after administration of oral conventional and buccal core in cup tablets.

When the hyperlipidemic group was treated with oral conventional tablets for 4 weeks, slight reduction of hyperlipidemic parameters was found (Table 11.13). At the end of 4 weeks, observed TG, VLDL and LDL were 161.24 ± 11.02 mg/dL, 32.14 ± 2.85 mg/dL and 17.01 ± 2.54 mg/dL respectively. HDL levels were found to be 13.23 ± 1.00 mg/dl which was nearly constant and not changed. These HDL results can be correlated with Kuroda M. et al. They found no change in HDL levels after 4 weeks of Pravastatin sodium conventional treatment (Kuroda et al., 1992).

When second hyperlipidemic group of rabbits was treated with core in cup buccal tablets, considerable reduction of hyperlipidemia was found (Table 11.14). The observed values for TG, VLDL and LDL were 143.58 ± 11.89 mg/dL, 29.24± 2.88 mg/dL and 10.02± 2.59 mg/dL respectively. The one way ANOVA test showed statistically significant differences ($P < 0.005$) between the results of oral conventional tablets and buccal core in cup tablets as shown in table 11.15. It can be seen from statistical significant ($p<0.005$) data that the significant effect shown by oral conventional tablets in 4 weeks is equivalent to effect shown by buccal core in cup tablets in 2 weeks. Further the statistical significance was observed in all the parameters except HDL in 4 weeks when treated with buccal core in cup tablets while it was not observed in treatment by oral conventional tablets.

Hyperlipidemic parameters were also compared in terms of percent reduction by administering Pravastatin sodium oral conventional and buccal core in cup tablets (Table 11.16).

At the end of 4 weeks, reduction in TG (mg/dL) was found 15.70 and 25.13 % by oral conventional and buccal core in cup tablets respectively. Reduction in VLDL was found to be 15.78 and 23.68 % while 15.00 % and 50.24 % reduction was found in LDL by oral conventional and buccal core in cup tablets respectively. HDL values were remained as it was earlier i.e. before treatment. This clearly indicated that buccal core in cup tablets provided better antihyperlipidemic treatment as compared to oral conventional tablets.

Buccal core in cup tablets decreased the elevated lipid profile of hyperlipidemic rabbits significantly as compared to oral conventional tablets and the effect continued for 8 hours as seen by plasma concentration profile by pharmacokinetic studies. This clearly indicates that the buccal core in cup tablets release the drug gradually over a period of time, which results in prolonged control of hyperlipidemia.

Oral Pravastatin sodium is rapidly and extensively absorbed following oral administration, but has absolute bioavailability of approximately 18% due to a significant degree of first-pass metabolism (Schachter, 2004). As reported in the literature presence of food reduces absorption of Pravastatin sodium and in turn bioavailability (Pan et al, 1990). The satisfactory increase in therapeutic effect of Pravastatin sodium buccal core in cup tablets may be due to bypassing first pass metabolism and avoidance of food factor which affects absorption. The current study revealed increase in therapeutic activity of Pravastatin sodium when it was administered through buccal route which bypasses the first pass metabolism, and hence resulted in increased bioavailability (as evidenced by pharmacokinetic studies). Buccal core in cup tablets being sustained release dosage form showed optimized treatment by a sustained control over a lipid profile of hyperlipidemic rabbits. Thus, the results showed that Pravastatin sodium buccal core-in-cup tablets are more effective in the treatment for hyperlipidemia when compared with oral conventional tablets.

Table 11.13 Treatment with Pravastatin sodium oral conventional tablets

Treatment with oral conventional tablets			
Parameters	**Before Treatment (Initial)**	**After Treatment**	
		2W	**4W**
HDL (mg/dL)	13.14 ± 1.44	13.01 ± 1.01	13.23 ± 1.00
TG (mg/dL)	191.14 ± 9.00	185.56 ± 10.12	161.24 ± 11.02
VLDL (mg/dL)	38.03 ± 3.23	36.12 ± 3.01	32.14 ± 2.85
LDL (mg/dL)	20.10 ± 2.21	19.14 ± 2.11	17.01 ± 2.54

(n=6), ± R.S.D.

Table 11.14 Treatment with Pravastatin sodium buccal core in cup tablets

Treatment with buccal core in cup tablets			
Parameters	**Before Treatment (Initial)**	**After Treatment**	
		2W	**4W**
HDL (mg/dL)	13.14 ± 1.44	13.10 ± 1.1	14.01± 1.01
TG (mg/dL)	191.14 ± 9.00	170.94 ± 9.63	143.58 ± 11.89
VLDL (mg/dL)	38.03 ± 3.23	33.15 ± 2.63	29.24± 2.88
LDL (mg/dL)	20.10 ± 2.21	13.01± 2.56	10.02± 2.59

(n=6), ± R.S.D.

Table 11.15 Statistical significance at $p<0.005$ between oral conventional tablets and core in cup buccal tablets.

Statistical significance ($p<0.005$)				
Parameters	Oral conventional tablets		Core in cup buccal tablets	
	2W	4W	2W	4W
HDL (mg/dL)	NS	NS	NS	NS
TG (mg/dL)	NS	S	S	S
VLDL (mg/dL)	NS	NS	NS	S
LDL (mg/dL)	NS	NS	NS	S

NS: Not significant, S= Significant, (n=6).

Table 11.16 - % Reduction in Hyperlipidemic Parameters

% Reduction in Hypertensive Parameters				
Parameters	Oral conventional tablets		Buccal core in cup tablets	
	2W	4W	2W	4W
HDL (mg/dL)	0	0	0	0
TG (mg/dL)	3.14	15.70	10.99	25.13
VLDL (mg/dL)	5.26	15.78	13.15	23.68
LDL (mg/dL)	5.00	15.00	35.00	50.24

(n=6)

Fig 11.15 Comparative evaluation of high density lipoprotein (HDL) after administration of oral and buccal core in cup tablets.

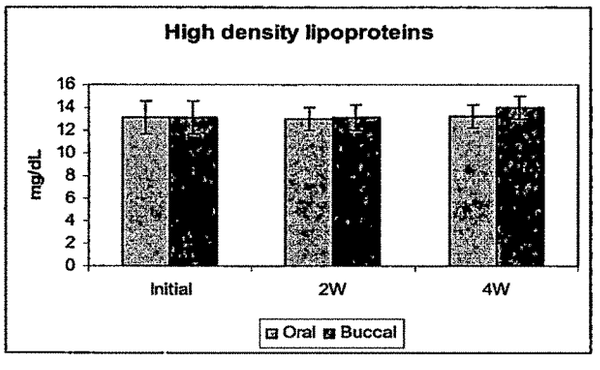


Fig 11.16 Comparative evaluation of triglycerides (TG) after administration of oral and buccal core in cup tablets.

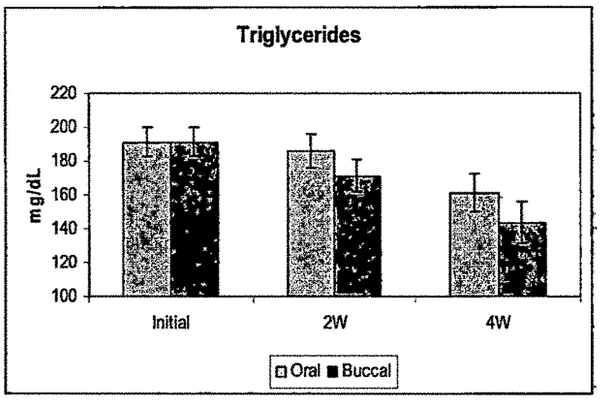


Fig 11.17 Comparative evaluation of very low density lipoproteins (VLDL) after administration of oral and buccal core in cup tablets.

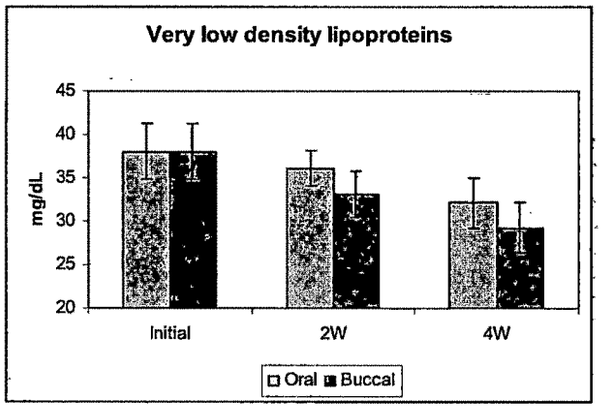
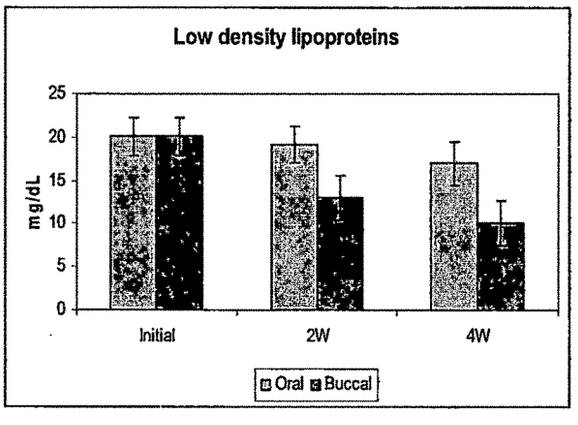


Fig 11.18 Comparative evaluation of low density lipoproteins (LDL) after administration of oral and buccal core in cup tablets.



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12.0 RESULTS AND DISCUSSION (PRAVASTATIN SODIUM BILAYER BUCCAL PATCHES -PBP)

12.2 Medicated layer:

Physicochemical parameters such as diameter, thickness, average weight and assay values are given in table 12.1. It was found that all the parameters were within acceptable limits.

Table 12.1 Physiochemical parameters of medicated layer.

Parameters	PBP1	PBP2	PBP3	PBP4	PBP5	PBP6	PBP7	PBP8	PBP9	PBP10	PBP11
Diameter (mm)	14.10 ± 0.12	14.11 ± 0.10	14.01 ± 0.10	14.10 ± 0.10	14.01 ± 0.14	14.14 ± 0.09	14.05 ± 0.17	14.09 ± 0.15	14.01 ± 0.09	14.11 ± 0.13	14.01 ± 0.09
Thickness (mm)	1.01 ± 0.06	1.01 ± 0.04	1.03 ± 0.07	1.01 ± 0.04	1.03 ± 0.07	1.06 ± 0.01	1.07 ± 0.05	1.01 ± 0.06	1.06 ± 0.07	1.08 ± 0.08	1.09 ± 0.06
Average weight (mg)	90.21 ± 1.96	98.38 ± 1.80	91.65 ± 2.01	92.11 ± 0.12	92.22 ± 1.85	92.56 ± 1.44	95.55 ± 1.78	85.97 ± 1.72	85.99 ± 1.02	92.31 ± 1.47	97.00 ± 1.77
Assay (%)	98.14 ± 1.12	98.18 ± 2.01	102.21 ± 1.13	97.89 ± 1.12	97.11 ± 2.47	98.38 ± 1.33	103.55 ± 1.58	98.99 ± 1.14	102.12 ± 1.15	99.32 ± 2.04	99.12 ± 1.09

± R.S.D., (n=3)

12.1.2 Bilayer patches:

Physical parameters such as thickness, diameter and average weight of the bilayer patches were studied and listed in Table 12.2. As expected, final thickness was slightly increased e.g. for medicated layer of PBP1 it was 1.01 ± 0.06 mm and for bilayer patch it was observed to be 1.69 ± 0.09 mm. Similarly average weight increased from 90.21 ± 1.96 mg to 134.11 ± 2.01 mg for the bilayer patches.

Table 12.2 Physical parameters of bilayer patches.

Parameters	PBP1	PBP2	PBP3	PBP4	PBP5	PBP6	PBP7	PBP8	PBP9	PBP10	PBP11
Diameter (mm)	14.01 ± 0.10	14.00 ± 0.21	14.02 ± 0.12	14.14 ± 0.15	14.12 ± 0.10	14.00 ± 0.19	13.49 ± 0.16	14.44 ± 0.14	14.32 ± 0.16	14.41 ± 0.09	13.98 ± 0.18
Thickness (mm)	1.69 ± 0.09	1.69 ± 0.10	1.81 ± 0.10	1.79 ± 0.10	1.80 ± 0.08	1.71 ± 0.09	1.70 ± 0.15	1.70 ± 0.08	1.78 ± 0.10	1.73 ± 0.09	1.75 ± 0.14
*Average weight (mg)	134.11 ± 2.01	136.38 ± 1.88	138.47 ± 2.06	132.10 ± 2.11	139.01 ± 1.84	140.50 ± 2.01	140.50 ± 1.68	142.89 ± 2.11	139.10 ± 2.00	139.36 ± 2.11	138.90 ± 1.51

± R.S.D., (n=3).

12.2 Evaluation of buccal bilayer patches:

12.2.1 Mechanical properties of bilayer buccal patches:

The mechanical properties such as tensile strength (TS), elastic modulus (EM), elongation at break (E/B), folding endurance (FE) and strain (SN) were evaluated and obtained data is shown in Table 12.3. Fig 12.1, 12.2, 12.3, 12.4 and 12.5 shows TS, EM, E/B, FE and SN of formulation PBP1 to PBP11. Increase in Carbopol 934P content was found to initially increase and then reduce the TS and EM. It also increased FE, E/B and SN significantly, indicative of a weaker, more elastic, flexible and softer film. A reverse pattern was seen in the HPMC films i.e. increase in HPMC K4M content initially reduce TS and EM indicating different mechanical properties of Carbopol 934P and HPMC K4M. There was no significant decrease in TS when the Carbopol 934P content was increased from 50% to 70% rather it decreased TS. When Carbopol 934P was increased from 30% to 50%, there was not much increase in the E/B but when it was further increased to 60%, a significant increase in the E/B value was observed. Increase in E/B value indicates soft and flexible patch (Khan T.A., 2000). Further increase in Carbopol 934P content resulted in decreased E/B.

Increase in the mean value of SN was seen when the Carbopol 934P content was increased to 50%. Although there was no significant difference between films of 30% and 40% Carbopol 934P, further increase in Carbopol 934P content was found to decrease SN. FE values increased with increase in Carbopol 934P content in the formulation and exhibited best FE values at Carbopol 934P: HPMC ratio of 4:6. FE values were found to decrease when there was excess amount of Carbopol 934P in the formulation. These results indicated that Carbopol 934P generally reduced the strength while increased the softness, elasticity and flexibility of HPMC patches when both the polymers were used simultaneously. The greater elasticity exhibited by films containing higher Carbopol 934P content could be related to its conformation and configuration, which is highly crosslinked (Peh and Wong, 1999). It can be concluded that HPMC K4M and Carbopol 934P must be optimized properly in order to impart satisfactory mechanical properties to patches.

Mechanical properties exhibited by Carvedilol bilayer patches were slightly higher than that of Pravastatin sodium bilayer buccal patches indicating that properties of drug affects mechanical properties. The incorporation of water soluble drug (Pravastatin sodium) may have made the film slightly weak and soft than poorly water soluble drug (Carvedilol).

Mechanical properties of PBP5 were found to be suitable as it demonstrated relatively high TS (6.62 ± 0.59 $kgmm^{-2}$), high E/B (130.23 ± 4.98 % mm^{-2}), high FE (296 ± 25) and high SN (1.99 ± 0.35 kg) but a low EM (3.66 ± 0.10 $kgmm^{-2}$) indicating that the patch had both strength as well as elasticity. Hence, PBP5 was considered as optimized batch.

Table 12.3 Mechanical properties of Pravastatin sodium bilayer buccal patches

Formulation code	Tensile Strength ($kgmm^{-2}$)	Elastic modulus ($kgmm^{-2}$)	Elongation at break (% mm^{-2})	Strain (kg)	Folding endurance (no of folds)
PBP1	3.00 ± 0.37	2.06 ± 0.10	40.02 ± 2.56	1.20 ± 0.36	200 ± 19
PBP2	4.00 ± 0.51	5.01 ± 0.09	60.40 ± 2.36	1.03 ± 0.28	214 ± 20
PBP3	6.12 ± 0.77	5.02 ± 0.09	102.26 ± 7.12	1.0 ± 0.29	218 ± 19
PBP4	6.00 ± 0.35	4.28 ± 0.12	114.56 ± 5.63	1.60 ±0.31	229 ± 14
PBP5	6.62 ± 0.59	3.66 ± 0.10	130.23 ± 4.98	1.99 ± 0.35	296 ± 25
PBP6	5.52 ± 0.36	4.69 ± 0.09	107.36 ± 5.32	1.82 ± 0.39	285 ± 21
PBP7	5.92 ± 0.31	4.04 ± 0.10	101.36 ± 7.49	1.45 ± 0.34	278 ± 21
PBP8	5.45 ± 0.52	4.32 ± 0.10	100.23 ± 4.63	1.84 ± 0.29	266 ± 22
PBP9	5.01 ± 0.48	3.65 ± 0.11	95.23 ± 2.89	1.65 ± 0.31	220 ± 11
PBP10	4.20 ± 0.49	2.13 ± 0.11	72.23 ± 2.11	1.38 ± 0.39	221 ± 21
PBP11	3.08 ± 0.31	2.05 ± 0.09	64.12 ± 1.92	1.89 ± 0.31	218 ± 11

± R.S.D., (n=3)

Fig 12.1 Tensile strength for PBP1 to PBP11.

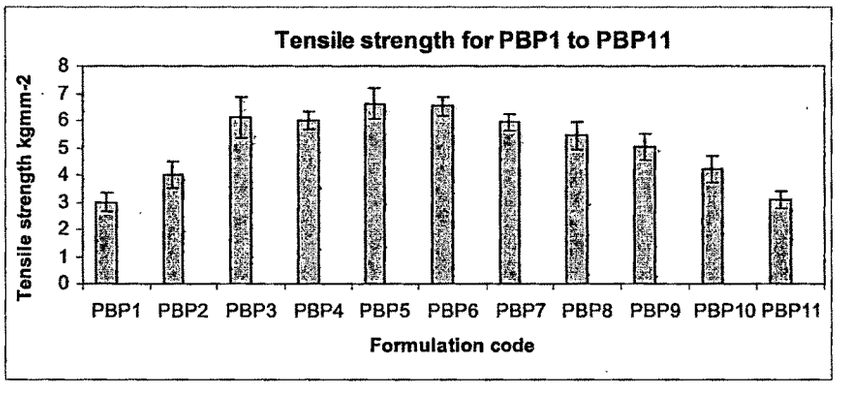


Fig 12.2 Elastic modulus for PBP1 to PBP11.

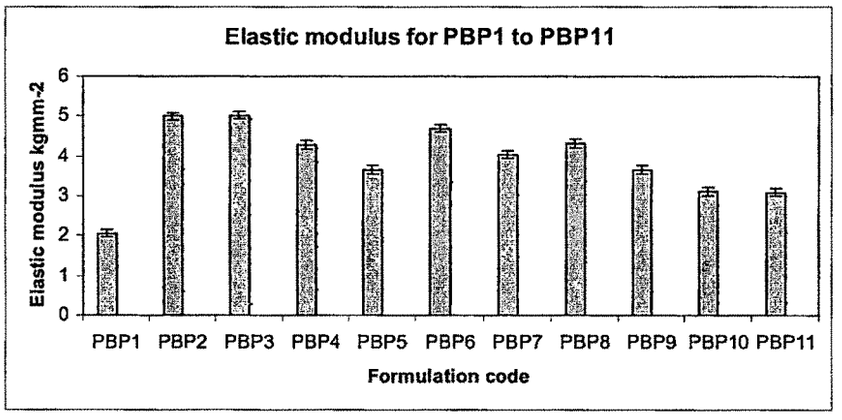


Fig 12.3 Elongation at break for PBP1 to PBP11.

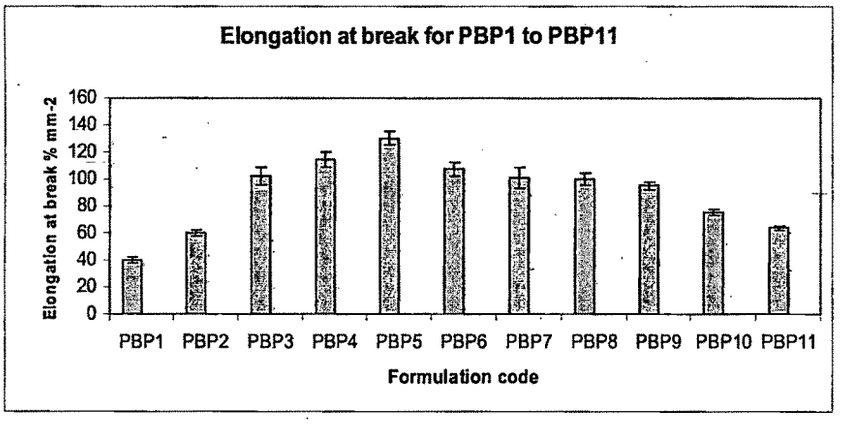


Fig 12.4 Folding endurance for PBP1 to PBP11.

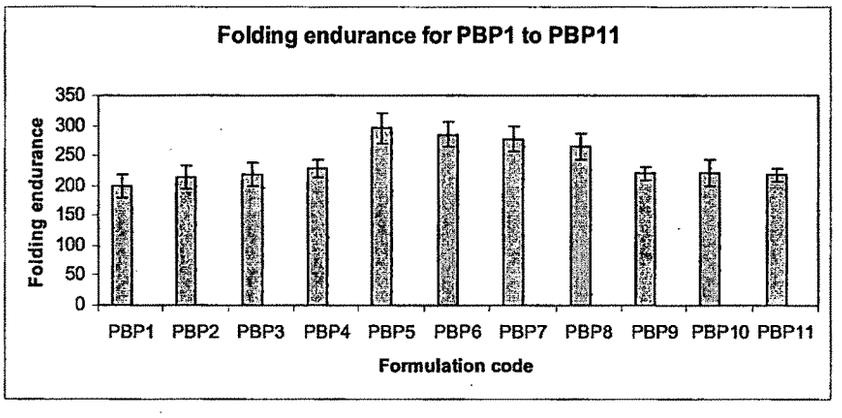
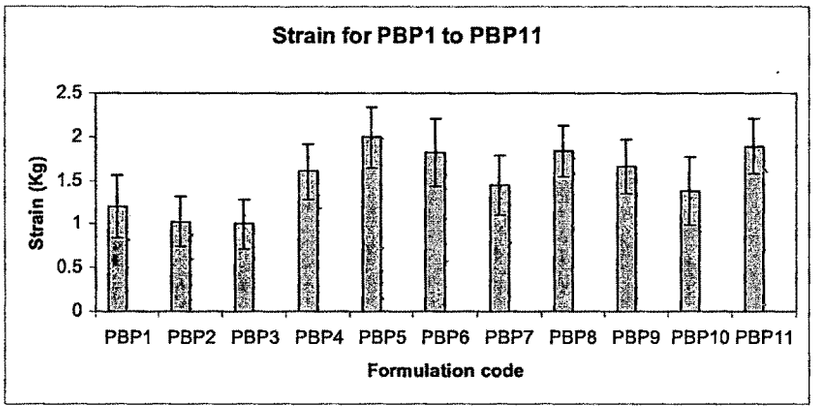


Fig 12.5 Strain for PBP1 to PBP11.



12.3 Surface pH:

Table 12.4 shows that the surface pH of the bilayer buccal patches remained fairly constant at a pH of approximately 5.55 – 6.15. Therefore, this study confirmed that the surface pH of the buccoadhesive patches was near the neutral conditions of saliva and hence would not alter the pH of the buccal fluids and cause no damage or alteration to the buccal mucosa due to altered pH conditions.

Table 12.4 Surface pH of PBP1 to PBP11

Formulation Code	Surface pH
PBP1	5.87 ± 0.11
PBP2	6.08 ± 0.12
PBP3	6.01 ± 0.19
PBP4	6.02 ± 0.27
PBP5	6.00 ± 0.19
PBP6	6.15 ± 0.21
PBP7	5.96 ± 0.25
PBP8	5.80 ± 0.11
PBP9	5.71 ± 0.12
PBP10	5.70 ± 0.20
PBP11	5.55 ± 0.11

± R.S.D. (n=3)

12.4 Swelling:

The swelling behavior of the Pravastatin sodium bilayer buccal and placebo patches in phosphate buffered saline (pH 6.8 ± 0.2) were investigated. Table 12.5 and 12.6 shows % swelling of Pravastatin sodium and placebo patches respectively.

Fig 12.6 and 12.7 shows % swelling with time for PBP1 to PBP6 and PBP7 to PBP11 of Pravastatin sodium bilayer patches respectively. Fig 12.8 and 12.9 shows % swelling with time for P-PBP1 to P-PBP6 and P-PBP7 to P-PBP11 of placebo Pravastatin sodium bilayer patches respectively.

It was seen from data that Pravastatin sodium patches displayed 5.50 to 6.23 % swelling. Formulation with HPMC K4M alone (PBP1) showed highest swelling i.e. 6.23 ± 0.19 % swelling after 8 hrs. Formulation with Carbopol 934P alone (PBP11) showed 6.13 ± 0.20 % swelling. Formulations with combination of Carbopol 934P and HPMC K4M show 5.50 to 6.09 % swelling, slightly less swelling than formulations with individual polymers.

A maximum degree of swelling was achieved after 2 to 4 hr of exposure to the phosphate buffer saline. At the end of study, when matrix structure was physically evaluated, it was found to be very porous in nature. In placebo patches, 4.06 to 5.78 % swelling was observed. Here also, formulation with HPMC K4M alone (P-PBP1) and Carbopol 934P alone (P-PBP2) showed highest swelling i.e. 5.78 ± 0.26 and 5.78 ± 0.20 % swelling after 8 hr.

When swelling behavior of Pravastatin sodium and placebo patches were compared, it was found that addition of drug to the patches increased their swelling. At the end of 8 hr, it was found that Pravastatin sodium patches (PBP1) showed 6.23 ± 0.19 % swelling while that of placebo patches (P-PBP1) was 5.78 ± 0.26 %. This can be attributed to the fact that dispersed drug particles may have weakened cohesive forces between the polymer chains allowing each chain to hydrate freely and increased the swelling of medicated patches. Yong C.S. et al also found high % swelling for medicated patches than placebo patches (Yong et al., 2001).

Slight difference in swelling pattern of Pravastatin sodium and Carvedilol patches were found. It shows that formulations with Pravastatin sodium, a water soluble drug shows higher swelling values than with water insoluble drug, Carvedilol. This may be due to ability of water soluble drug to weaken cohesive forces between polymer chains after uptake of water due to increased porosity.

Table 12.5 Swelling studies of Pravastatin sodium bilayer patches.

Formulation Code	% Swelling			
	2 Hrs	4 Hrs	6 Hrs	8 Hrs
PBP1	2.21 ± 0.11	2.99 ± 0.15	5.20 ± 0.15	6.23 ± 0.19
PBP2	2.19 ± 0.12	2.57 ± 0.17	3.98 ± 0.14	5.91 ± 0.19
PBP3	2.17 ± 0.10	2.58 ± 0.14	3.85 ± 0.19	5.85 ± 0.17
PBP4	2.02 ± 0.16	2.45 ± 0.16	3.65 ± 0.19	5.78 ± 0.19
PBP5	2.10 ± 0.18	2.24 ± 0.14	3.64 ± 0.20	5.75 ± 0.18
PBP6	2.33 ± 0.19	2.39 ± 0.15	3.55 ± 0.19	5.61 ± 0.17
PBP7	2.29 ± 0.21	2.38 ± 0.14	3.51 ± 0.20	5.60 ± 0.21
PBP8	2.21 ± 0.11	2.32 ± 0.17	3.39 ± 0.38	5.50 ± 0.20
PBP9	1.99 ± 0.15	2.09 ± 0.18	3.31 ± 0.29	6.02 ± 0.21
PBP10	1.93 ± 0.15	2.10 ± 0.16	3.99 ± 0.19	6.09 ± 0.21
PBP11	1.86 ± 0.19	2.09 ± 0.04	5.78 ± 0.20	6.13 ± 0.20

± R.S.D., n=3

Table 12.6 Swelling studies of placebo patches.

Formulation Code	% Swelling			
	2 Hrs	4 Hrs	6 Hrs	8 Hrs
P-PBP1	1.56 ± 0.09	2.71 ± 0.16	4.89 ± 0.27	5.78 ± 0.26
P-PBP2	1.19 ± 0.11	2.14 ± 0.09	3.57 ± 0.18	5.31 ± 0.59
P-PBP3	1.29 ± 0.09	2.16 ± 0.23	3.49 ± 0.16	4.67 ± 0.30
P-PBP4	1.35 ± 0.10	2.31 ± 0.21	3.30 ± 0.20	4.31 ± 0.30
P-PBP5	1.36 ± 0.11	2.01 ± 0.15	3.10 ± 0.10	4.11 ± 0.18
P-PBP6	1.12 ± 0.10	2.30 ± 0.10	3.14 ± 0.25	4.77 ± 0.20
P-PBP7	1.11 ± 0.16	2.02 ± 0.25	3.39 ± 0.15	4.81 ± 0.31
P-PBP8	1.10 ± 0.05	2.00 ± 0.14	3.21 ± 0.24	4.80 ± 0.29
P-PBP9	0.89 ± 0.20	1.99 ± 0.12	3.01 ± 0.17	4.51 ± 0.31
P-PBP10	0.90 ± 0.30	1.61 ± 0.27	2.61 ± 0.12	4.06 ± 0.30
P-PBP11	0.88 ± 0.11	1.55 ± 0.11	2.51 ± 0.18	5.78 ± 0.20

± R.S.D., n=3, P-PBP: Placebo-Pravastatin sodium Buccal Patches

Fig 12.6 Swelling Profile for PBP1 to PBP6 of Pravastatin sodium bilayer patches.

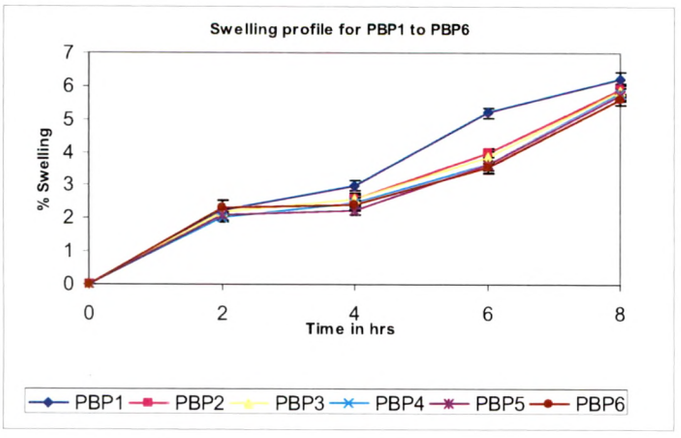


Fig 12.7 Swelling Profile for PBP7 to PBP11 of Pravastatin sodium bilayer patches.

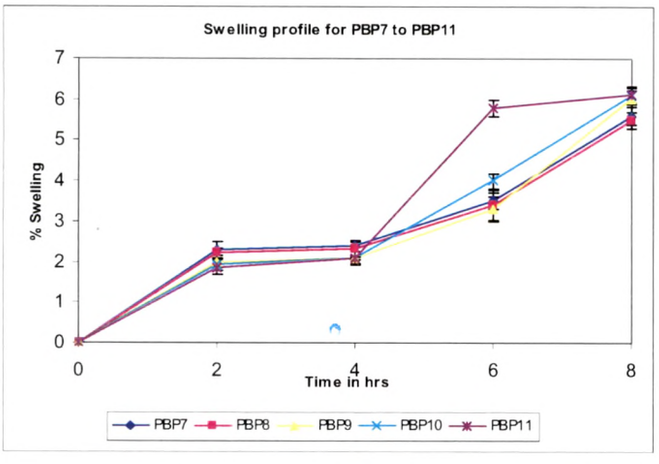


Fig 12.8 Swelling Profile for P-PBP1 to P-PBP6 of placebo patches.

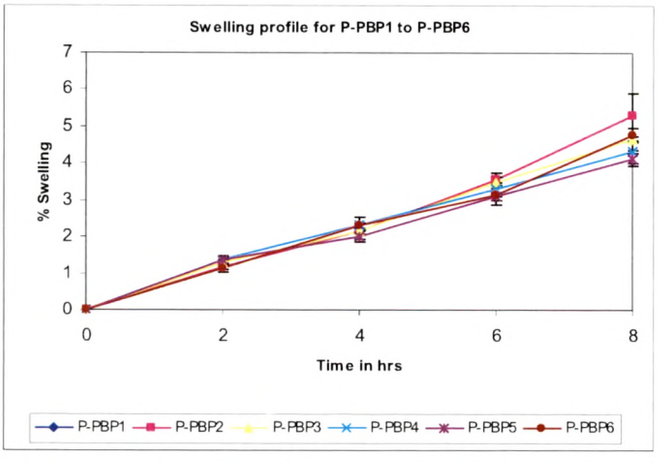
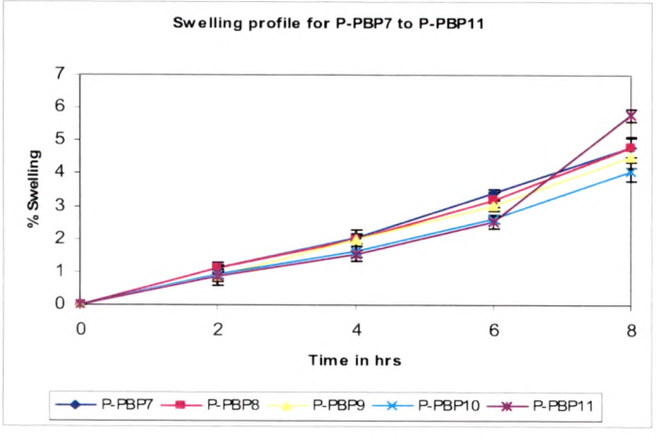


Fig 12.9 Swelling Profile for P-PBP7 to P-PBP11 of placebo patches.



12.5 In vitro mucoadhesive force:

Table 12.7 and Fig 12.10 show in vitro mucoadhesive force for Pravastatin sodium and placebo buccal patches. All the formulations from PBP4 to PBP8 (38 ± 1.98 to 44 ± 1.56 x 10^3 dyne cm^{-2}) exhibited good mucoadhesion (>35 x 10^3 dyne cm^{-2}) required by dosage form to adhere to buccal mucosa (Adel et al., 2004). However, the patch containing Carbopol 934P and HPMC K4M at the ratio of 4:6 (PBP5) exhibited highest mucoadhesive force (44 ± 1.56 x 10^3 dyne cm^{-2}) with buccal mucosa when compared with other ratios.

Formulation PBP1 shows weak in vitro mucoadhesive force of 22 ± 2.13 x 10^3 dyne cm^{-2}. Formulation PBP11 which contains only Carbopol 934P also showed weak mucoadhesive force (25 ± 1.95 x 10^3 dyne cm^{-2}) which may be because mucoadhesive force of Carbopol 934P is dependent on the pH of experimental medium. If pH of medium is more than ionization constant of Carbopol 934P (6.00) then Carbopol 934P will ionized and loose its integrity (Desai and Pramodkumar, 2004). This will result in loss of hydrogen bonding with the mucus and consequently lower mucoadhesive force.

Swelling affects the mucoadhesive force (Valenta, 2005) of formulation as it was seen in PBP1. The formulation (PBP1) containing only HPMC showed less mucoadhesive force (22 ± 2.13 x 10^3 dyne cm^{-2}) may be because higher swelling (6.23 ± 0.19 %) of polymer which may have weakened its adhesive property.

It was noted that the presence of Pravastatin sodium in patches reduced mucoadhesive force when compared with placebo patches. In PBP1 mucoadhesive force of 22 ± 2.13 x 10^3 dyne cm^{-2} was obtained while that of placebo was 29 ± 2.03 x 10^3 dyne cm^{-2}. PBP11 shows 25 ±

1.95 x 10^3 dyne cm^{-2} of mucoadhesive force while placebo patch shows 32 ± 1.67 x 10^3 dyne cm^{-2}. Average difference of 7 x 10^3 dyne cm^{-2} mucoadhesive force was obtained between Pravastatin sodium and placebo patches. Buccal tablets containing a testosterone showed a significantly lower mucoadhesive force in comparison with the placebo formulation (Voorspoels et al., 1996).

The Pravastatin sodium particles may have weakened cohesive forces between the polymer chains allowing each chain to hydrate freely (Yong et al., 2001) and increased the swelling of Pravastatin sodium patches. This implies that the addition of Pravastatin sodium was found to decrease mucoadhesive force because of increase swelling of patches as compared to placebo patches.

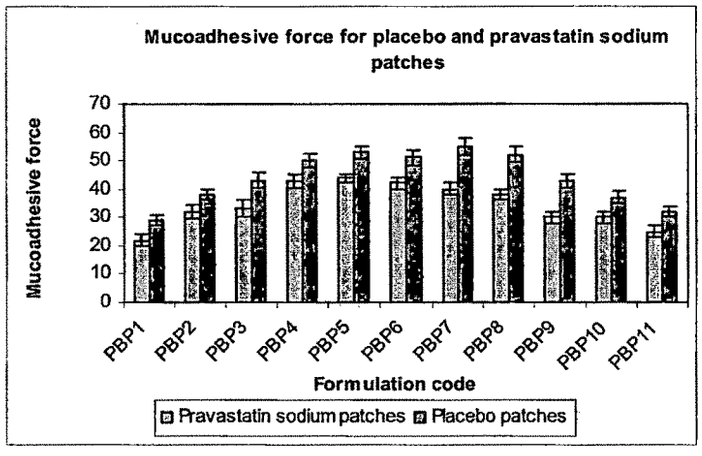
In vitro mucoadhesive force shown by Carvedilol buccal patches was slightly high as compared to Pravastatin sodium bilayer buccal patches e.g. CBP3 shows 38 ± 3.01 x 10^3 dyne cm^{-2} while that of PBP3 was 33 ± 3.02 x 10^3 dyne cm^{-2}, shows that solubility of drug affects mucoadhesive force. Formulations with highly water soluble drug shows less mucoadhesive force than formulations with water insoluble drug.

Table 12.7 In vitro mucoadhesive force for PBP1 to PBP11.

Formulation Code	Mucoadhesive force (x 10^3 dyne cm^{-2})		Formulation Code
	Pravastatin sodium Patches	Placebo Patches	
PBP1	22 ± 2.13	29 ± 2.03	**P-PBP1**
PBP2	32 ± 2.36	38 ± 1.98	**P-PBP2**
PBP3	33 ± 3.02	43 ± 2.69	**P-PBP3**
PBP4	43 ± 2.47	50 ± 2.58	**P-PBP4**
PBP5	44 ± 1.56	53 ± 2.15	**P-PBP5**
PBP6	42 ± 2.05	51 ± 2.59	**P-PBP6**
PBP7	40 ± 2.08	55 ± 3.10	**P-PBP7**
PBP8	38 ± 1.98	52 ± 2.69	**P-PBP8**
PBP9	30 ± 2.01	43 ± 2.38	**P-PBP9**
PBP10	30 ± 2.01	37 ± 2.14	**P-PBP10**
PBP11	25 ± 1.95	32 ± 1.67	**P-PBP11**

± R.S.D., n=3

Fig 12.10 In vitro mucoadhesive force for placebo and Pravastatin sodium buccal patches.



12.6 In vitro diffusion:

Table 12.8 and Fig 12.11 show the *in vitro* diffusion for pure drug, PBP1 to PBP11 studied through sheep buccal mucosa up to 8 hr. Formulations containing HPMC K4 (PBP1) alone showed highest diffusion (71.78 ± 2.47 %). Formulation (PBP6) showed least diffusion (52.28 ± 3.21 %).

Pure drug has showed less diffusion i.e. 49.36 ± 4.87 %. Pravastatin sodium being a BCS – class III drug having a hydrophilic property has showed a less diffusion. Pure drug with permeation enhancer i.e. sodium glycocholate has shown satisfactory diffusion (80.23 ± 3.15%). The marked difference in diffusion property of pure drug and pure drug with permeation enhancer is because of effect of sodium glycocholate. Aungst and Rogers reported that sodium glycocholate is effective in enhancing transbuccal diffusion of insulin (Aungst and Rogers, 1989). Acyclovir permeability increased 9 times with sodium glycocholate in the concentration of 100 mM (Jasti et al., 2000). Mechanism by which sodium glycocholate improve diffusion is by opening mucosal non-selective porous pathway. Shojaei et al. reported that increased permeation of hydrophilic compounds due to sodium glycocholate is by wide opening of porous pathway (Shojaei and Berner, 1998).

Formulations with combination of Carbopol 934P and HPMC K4M (PBP2 to PBP10) has shown 52.28 ± 3.21 % to 71.78 ± 2.47 % diffusion of Pravastatin sodium while formulations with individual polymers i.e. PBP1 (HPMC K4M) and PBP11 (Carbopol 934P) showed 71.78 ± 2.47 % and 70.59 ± 3.54 % diffusion respectively. Obtained diffusion values for

formulation PBP2 to PBP10 shows that combination of polymers plays a part in sustaining diffusion of Pravastatin sodium up to 8 hr. It also highlight that combination of polymers have control over sustaining drug diffusion than individual polymers because combination of polymers imparts better matrix structure to patches.

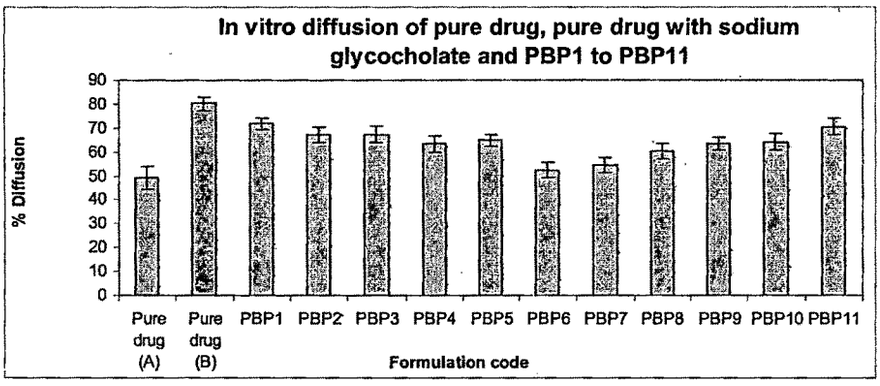
The in vitro diffusion values obtained for bilayer patches are slightly higher than core in cup tablet e.g. core in cup tablet (PCT1) showed 68.98 ± 2.89% diffusion while bilayer patches (PBP1) showed 71.78 ± 2.47% diffusion in 8 hr. This may be because of available surface area of formulation available for diffusion. The diameter of Core in cup tablet is 6 mm while that of bilayer patch is 14mm. Thickness of the core tablet, in core in cup tablet was 3.00 mm while that of medicated layer in bilayer patches was 1.00 mm, therefore diffusional path lengh for Pravastatin sodium in core in cup tablets was higher than that of bilayer patches.

Table 12.8 In vitro diffusion for pure drug, pure drug with sodium glycocholate and PBP1 to PBP11.

Formulation Code	% Diffusion ± RSD
Pure drug (Without sodium glycocholate)	49.36 ± 4.87
Pure drug (With sodium glycocholate)	80.23 ± 3.15
PBP1	71.78 ± 2.47
PBP2	67.33 ± 3.16
PBP3	67.39± 3.45
PBP4	63.39 ± 3.35
PBP5	64.88 ± 2.34
PBP6	52.28 ± 3.21
PBP7	54.39 ± 3.14
PBP8	60.34 ± 3.23
PBP9	63.55 ± 2.44
PBP10	64.29 ± 3.25
PBP11	70.59 ± 3.54

± R.S.D. (n=3), Note: All formulations studied with sodium glycocholate.

Fig 12.11 In vitro diffusion of pure drug, pure drug with sodium glycocholate and PBP1 to PBP11.



Pure drug (A): Pure drug without sodium glycocholate.

Pure drug (B): Pure drug with sodium glycocholate.

12.7 In vitro dissolution profile:

In vitro dissolution profile of formulations is shown in Table 12.9a and 12.9b, Fig 12.12, 12.13 and 12.14. PBP11 released the drug at fastest rate with 100.00 ± 2.56 % in 6 hr. This could be attributed to the high swelling (6.13 ± 0.20 %) of HPMC K4M as greater swelling of the matrices leads to faster dissolution of the drug (Agarwal and Mishra, 1999). Also PBP1 showed 99.98 ± 3.14 % dissolution in 6 hr may be due to its high swelling (6.23 ± 0.19 %). There was formation of gel and collapsing of formulation at 6th hr of study. The possible reason behind it is that there might be ionization of Carbopol 934P at experimental pH (6.8) which is higher than its ionization constant (pKa-6.0).

Combinations of Carbopol 934P and HPMC K4M show % dissolution from 94.12 ± 2.11 to 99.90 ± 2.01 in 7 to 8 hr. It was seen that formulations with combinations of polymers sustained Pravastatin sodium dissolution. On this basis it can be concluded that combination of polymers imparts better matrix characteristics to the patches. Strong matrix integrity will inhibit the entry of dissolution media and delay the dissolution of drug.

To investigate the kinetics of Pravastatin sodium release from bilayered buccal patches, the release data was applied to zero order, first order, Higuchi, Hixson-Crowell and Korsmeyer Peppas models and best fit was determined. The values of r^2, K and n are listed in table 12.10.

All the formulations showed n values in between 0.5 to 1.0 i.e. 0.51 to 0.86 indicating that they followed nonfickian diffusion pattern. The results indicate that the release mechanism changed with the type and amount of polymer incorporated in the formulation and this can be reflected by the observed values of release exponent (n). When concentration of Carbopol 934P was gradually increased in the formulation, n values were found to increase.

For PBP1, which contained only HPMC K4M, n value was 0.51 while that of F5 was 0.86 where HPMC K4M: Carbopol 934P; 6:4 was used. In PBP11 where only Carbopol 934P is present, showed n value as 0.57 implying non-fickian release. PBP2 to PBP10 where combination of polymers were used showed n value between 0.62 and 0.86. This shows that release pattern followed non-fickian release; implying diffusion is dominant release mechanism.

None of the formulations followed first order release and Hixson-Crowell kinetics as seen from its r^2 values. For zero order most of the formulations showed r^2 very near 1.0, this implies that they followed nearly zero order kinetics. Formulation F5 showed best fit as it showed $r^2 = 0.996$ for zero order model implied drug release is by diffusion mechanism. Peppas model for F5 showed n = 0.86 implies non-fickian diffusion pattern. Singh and Ahuja formulated controlled release matrices of Diltiazem hydrochloride, a water soluble drug with Carbopol 934P and HPMC K4M and reported non-fickian release approaching nearly zero order (Singh and Ahuja, 2002).

Table 12.9a In vitro dissolution of pure drug and formulations PBP1 to PBP5.

Formulation Code	Pure Drug	PBP1	PBP2	PBP3	PBP4	PBP5
Time (hr)	Pravastatin sodium dissolution					
1	99.63 ± 2.23	31.05 ± 2.69	25.87 ± 2.36	22.02 ± 3.12	21.00 ± 3.01	14.01 ± 2.36
2		46.63 ± 3.11	37.11 ± 2.56	35.25 ± 3.01	32.23 ± 2.69	25.28 ± 3.11
3	-	58.49 ± 3.41	51.11 ± 2.24	47.77 ± 2.56	43.15 ± 3.12	38.14 ± 2.58
4	-	71.36 ± 3.14	64.87 ± 2.69	59.63 ± 2.98	56.01 ± 2.45	51.12 ± 3.14
5	-	84.49 ± 3.02	81.31 ± 2.98	75.23 ± 3.01	69.98 ± 2.13	64.01 ± 2.29
6	-	95.98 ± 3.14	99.90 ± 2.01	95.12 ± 2.69	84.01 ± 2.69	76.09 ± 3.58
7	-	-	-	99.01 ± 2.56	93.16 ± 2.44	87.04 ± 4.01
8	-	-	-	-	99.01 ± 3.14	94.12 ± 2.11

± R.S.D. (n=3)

Table 12.9b In vitro dissolution of formulations PBP6 to PBP11.

Formulation Code	PBP6	PBP7	PBP8	PBP9	PBP10	PBP11
Time (hr)	Pravastatin sodium dissolution					
1	16.01 ± 2.66	18.01 ± 3.01	22.01 ± 2.69	26.18 ± 3.12	29.78 ± 3.45	32.00 ± 3.01
2	27.98 ± 2.45	29.40 ± 2.59	34.14 ± 2.98	38.89 ± 2.56	41.71 ± 3.22	44.28 ± 2.56
3	39.42 ± 2.59	42.14 ± 2.98	46.56 ± 3.12	51.78 ± 3.45	56.49 ± 2.59	60.24 ± 2.69
4	54.32 ± 3.01	57.56 ± 2.87	59.01 ± 3.45	67.45 ± 2.78	71.89 ± 2.01	74.36 ± 2.59
5	69.14 ± 3.24	71.12 ± 3.01	72.11 ± 2.68	79.78 ± 2.69	80.99 ± 2.09	87.24 ± 2.58
6	83.11 ± 3.06	87.14 ± 3.26	84.89 ± 2.45	95.14 ± 2.58	99.14 ± 2.89	100.00 ±2.56
7	94.01 ± 2.58	98.87 ± 4.06	97.78 ± 2.36	98.63 ±3.01		
8	99.82 ± 2.98	99.98 ± 2.36	99.89 ± 2.11	99.98 ± 3.11		

± R.S.D. (n=3)

Fig 12.12 In vitro dissolution of pure drug, PBP1, PBP2, PBP3 and PBP4.

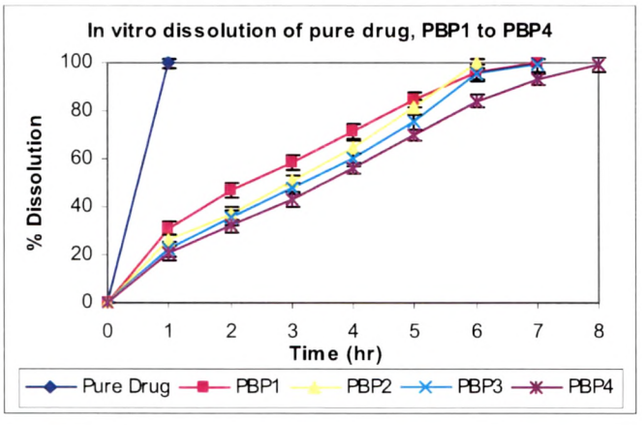


Fig 12.13 In vitro dissolution of pure drug, PBP5, PBP6, PBP7 and PBP8.

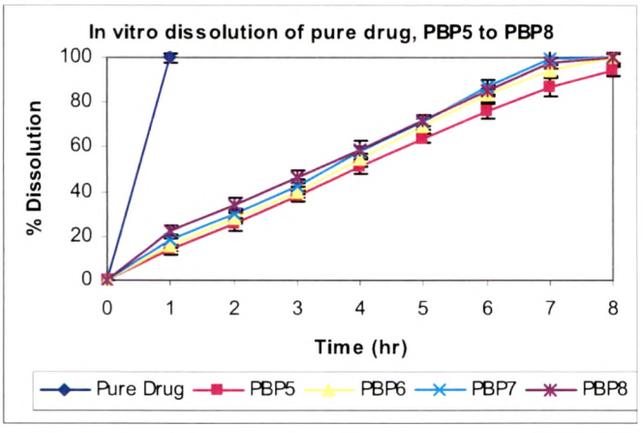


Fig 12.14 In vitro dissolution of pure drug, PBP9, PBP10 and PBP11.

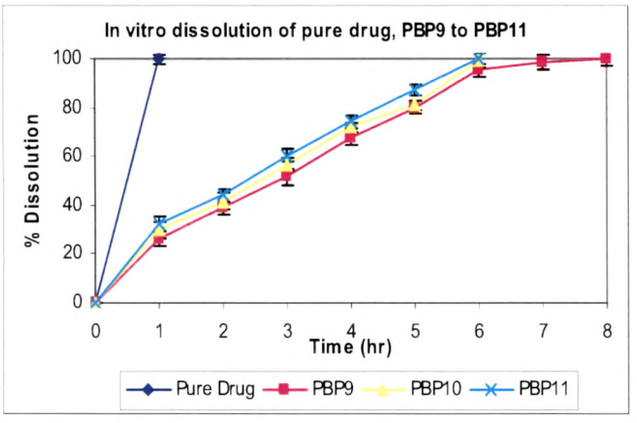


Table 12.10 Model fitting of Pravastatin sodium dissolution from bilayer buccal patches

Formulation Code	Zero order		First Order		Higuchi		Hixson Crowell		Korsmeyer Peppas		
	K_0 (h^{-1})	r^2	K_1 (h^{-1})	R^2	K_H ($mg/h^{-1/2}$)	r^2	K_S (h^{-3})	r^2	K_k (h^{-n})	r^2	n
PBP1	11.84	0.986	0.22	0.877	43.90	0.992	0.24	0.957	0.62	0.996	0.51
PBP2	14.75	0.993	0.14	0.934	50.45	0.958	0.33	0.990	0.75	0.979	0.62
PBP3	13.36	0.993	0.28	0.808	48.92	0.974	0.30	0.978	0.79	0.992	0.67
PBP4	11.57	0.993	0.23	0.826	45.56	0.981	0.27	0.969	0.78	0.991	0.70
PBP5	11.86	0.996	0.15	0.920	46.03	0.987	0.30	0.958	0.94	0.998	0.86
PBP6	12.65	0.993	0.30	0.722	49.05	0.982	0.30	0.961	0.91	0.995	0.81
PBP7	12.73	0.984	0.43	0.721	49.49	0.978	0.29	0.956	0.87	0.992	0.76
PBP8	11.80	0.990	0.34	0.751	45.88	0.984	0.26	0.962	0.76	0.994	0.67
PBP9	11.40	0.963	0.43	0.769	44.83	0.979	0.24	0.931	0.69	0.988	0.59
PBP10	13.71	0.995	0.32	0.713	47.26	0.976	0.30	0.987	0.10	0.973	0.59
PBP11	14.14	0.993	0.18	0.944	48.80	0.976	0.30	0.979	0.10	0.966	0.57

12.8 Pharmacokinetic Study

12.8.1 Selection of optimized formulation for pharmacokinetic study:

On the basis of *in vitro* parameters such as mechanical properties, mucoadhesive force, diffusion and in vitro dissolution, it was concluded that PBP5 (Carbopol 934P: HPMC, 4:6) has excellent mechanical properties, in vitro mucoadhesive force (44 ± 1.56 x 10^3 dyne cm^{-2}), diffusion (64.88 ± 2.34 %) and 94.12 ± 2.11 % dissolution in 8 hr. On the above basis, PBP5 was finalized to be used for pharmacokinetic studies, histological examination, in-vivo patient acceptability studies on human volunteers and pharmacodynamic studies.

12.8.2 Comparison of plasma profile of oral tablets and buccal bilayer patches.

The plasma concentration profile for Pravastatin sodium oral conventional tablets and buccal bilayer patches in rabbits are shown in Table 12.11 and Fig 12.15. After the administration of Pravastatin sodium oral conventional tablets, t_{max} was observed to be 1.00 hr, indicating rapid absorption while t_{max} of buccal patches was 3.00 hr, indicating slow absorption but it was sustained which was seen by plasma concentration profile.
The C_{max} were higher (75.63 ± 6.98 ng/ml) for buccal bilayer patches than oral tablets (67.40 ± 9.23 ng/ml) implying better absorption than oral conventional tablets.

The AUC values (311.10 ± 5.89 ng/ml/hr) of buccal bilayer patches was significantly higher than that of oral tablet administration (130.33 ± 10.25 ng/ml/hr) which indicates increase in bioavailability of Pravastatin sodium from the buccal formulation. Pravastatin sodium bilayer patches showed 2.38 fold increased in bioavailability in rabbits. The one way ANOVA test showed statistically significant differences ($P<0.05$) between the AUC of oral conventional tablets and buccal bilayer patches.

The absorption from oral tablets was high as seen by plasma concentration of 67.40 ± 9.23 ng/ml at 1st hr but it falls off rapidly as seen by plasma concentration of 21.13 ± 5.36 and 13.56 ± 5.11 ng/ml at 3rd and 4th hr respectively. The absorption from the buccal patches was slightly slow in the initial phase i.e. 17.12 ± 4.69 ng/ml plasma concentration at 1st hr.
When Pravastatin sodium buccal patches and buccal core in cup tablet was compared for its pharmacokinetic efficacy, it was found that Pravastatin sodium buccal patches (311.10 ± 5.89 ng/ml/hr) showed slightly higher bioavailability than buccal core in cup tablet (270.28 ± 10.98 ng/ml/hr). This may be because greater surface area was available for absorption of

Pravastatin sodium from the patch (14.00 mm) when compared with core in cup tablets (6.00 mm).

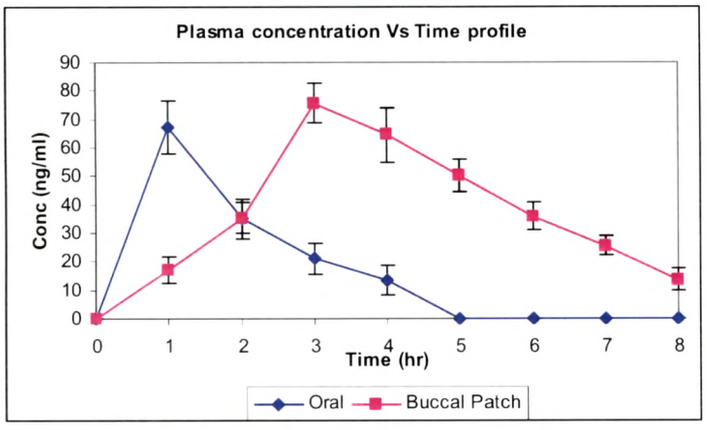
The results obtained from pharmacokinetic studies prove the justification of administering Pravastatin sodium through the buccal route as a useful alternative to the oral route for avoiding pre-systemic metabolism, improving bioavailability and sustaining activity.

Table 12.11 Plasma concentration of Pravastatin sodium (ng/ml) following administration of oral tablets and buccal patches.

Time (Hr)	Plasma concentration (ng/ml)	
	Oral Tablet	Buccal Patch
1	67.40 ± 9.23	17.12 ± 4.69
2	35.02 ± 6.89	35.25 ± 5.36
3	21.13 ± 5.36	75.63 ± 6.98
4	13.56 ± 5.11	64.63 ± 9.58
5	B.LoQ	50.25 ± 5.69
6	B.LoQ	35.85 ± 5.01
7	B.LoQ	25.56 ± 3.54
8	B.LoQ	13.63 ± 4.01
T_{max} (Hr)	**1.00 ±0.20**	**3.0 ± 1.0**
C_{max} (ng/ml)	**67.40 ± 9.23**	**75.63 ± 6.98**
AUC (ng/ml/hr)	**130.33 ± 10.25**	**311.10 ± 5.89**

± R.S.D., n=6, B. LoQ-Below limit of quantitation.

Fig 12.15 Plasma concentration Vs Time profile for oral conventional tablets and Pravastatin sodium bilayer buccal patches (PBP5).



12.9 Histological study of buccal mucosa:

12.9.1 Light microscopy:

Fig 12.16 and 12.17 shows section of control and sample mucosa (treated with formulation) respectively. Sample mucosa appeared to be different when compared with control mucosa. Sections showed little modification in the epithelial layer i.e. slight disruption of epithelial layer, may be because of use of sodium glycocholate which was used a permeation enhancer. Propylene glycol might have caused certain disruption of cells of epithelium. The observed changes may be reversible as seen from available literature (Attia et al., 2004).

It is clear from the observations of the sections examined by light microscopy that the buccal formulation provoked no major alteration in the barrier function of the mucosa.

Fig 12.16 Section of Control Buccal Mucosa.

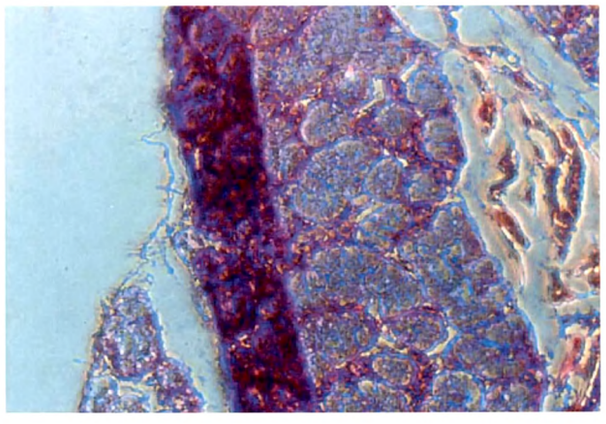
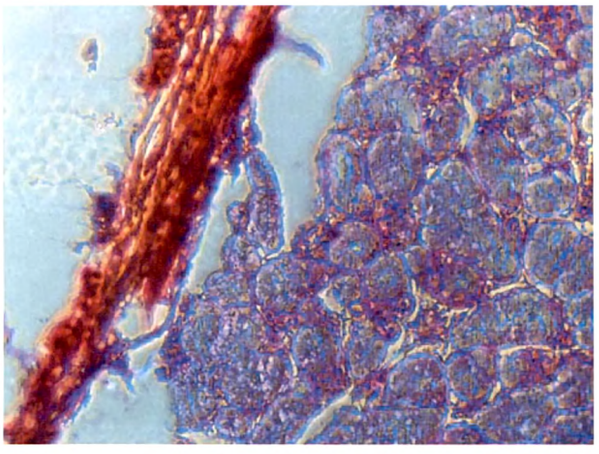


Fig 12.17 Section of Sample Buccal Mucosa.



12.9.2 Scanning electron microscopy of buccal mucosa:

Fig 12.18 and 12.19 shows SEM of control buccal mucosa and sample (treated with formulation) buccal mucosa respectively. Slight histological changes such as shrinkage of superficial cells appeared in epithelial parts of the tissue. These changes may be due to use of permeation enhancing effect of sodium glycocholate. Mechanism by which sodium glycocholate act is by solubilization of intercellular lipids (Jasti et al., 2000) which may have altered the structure of buccal mucosa. From available literature it can be expected that these slight changes may be reversible (Attia et al., 2004) and not permanently affect overall structure, surface and function of the buccal mucosa.

Fig 12.18 SEM of Control Buccal Mucosa

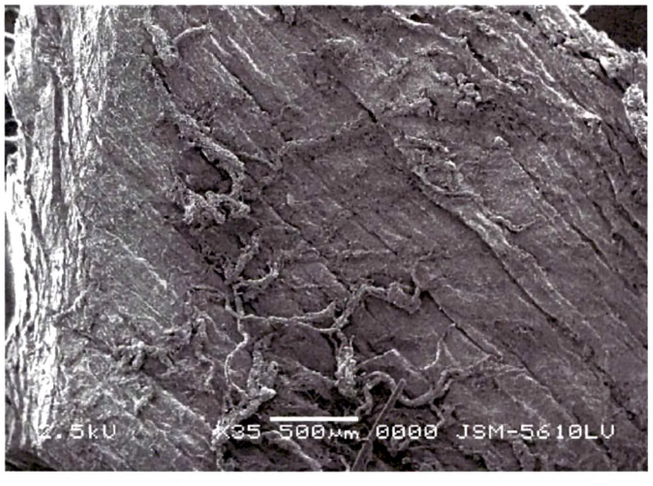
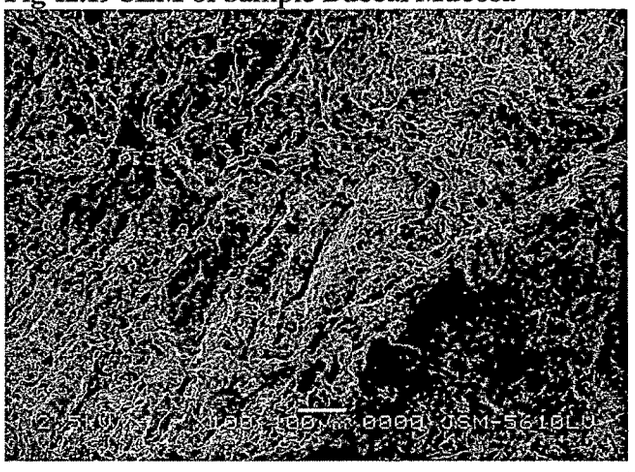


Fig 12.19 SEM of Sample Buccal Mucosa



12.10 Pharmacodynamic studies:

These studies were divided into 2 stages,

Stage 1: Induction of hyperlipidemia.

Stage 2: Treatment with conventional oral tablets (10.0 mg/ once a day) and bilayer buccal patches (10.0 mg/ once a day) and comparison of conventional and buccal formulation in terms of reducing of hyperlipidemic parameters.

12.10.1 Induction of hyperlipidemia.

This is same as Pravastatin sodium buccal core in cup tablets.

12.10.2 Treatment of hyperlipidemia with oral conventional tablets and bilayer buccal patches.

Hyperlipidemic rabbits were treated with Pravastatin sodium oral conventional tablets and bilayer buccal patches and observations are recorded in Table 12.12. and 12.13. Fig 12.20, 12.21, 12.22 and 12.23 shows comparative evaluation of HDL, TG, VLDL and LDL respectively after administration of oral conventional tablets and bilayer buccal patches.

When the hyperlipidemic group was treated with oral conventional tablets for 4 weeks, slight reduction of hyperlipidemic parameters was found (Table 12.12). At the end of 4 weeks, observed TG, VLDL and LDL were 161.24 ± 11.02 mg/dL, 32.14 ± 2.85 mg/dL and 17.01 ± 2.54 mg/dL respectively. HDL levels were found to be 13.23 ± 1.00 mg/dl which was nearly constant and not changed. These HDL results can be correlated with Kuroda M. et al. They found no change in HDL levels after 4 weeks of Pravastatin sodium conventional treatment (Kuroda et al., 1992).

When second hyperlipidemic group of rabbits was treated with bilayer buccal patches, considerable reduction of hyperlipidemic parameters was found (Table 12.13). At the end of 4 weeks, the observed values for TG, VLDL and LDL were 131.10 ± 10.23 mg/dL, 26.00 ± 2.56 mg/dL and 8.99 ± 3.01mg/dL respectively. The one way ANOVA test showed statistically significant differences ($P < 0.005$) between the results of oral conventional tablets and buccal bilayer patches as shown in table 12.14.

When treated with oral conventional tablets for 4 weeks, significance was shown only for TG parameter while buccal bilayer patches shown significance for all parameters. Buccal bilayer patches decreased the elevated lipid profile of hyperlipidemic rabbits significantly as compared to oral conventional tablets and the effect continued for 8 hours as seen by plasma concentration profile by pharmacokinetic studies.

Hyperlipidemic parameters were also compared in terms of percent reduction by administering Pravastatin sodium oral conventional tablets and buccal bilayer patches (Table 12.15). At the end of 4 weeks, reduction in TG (mg/dL) was found 15.70 and 31.41 % by oral conventional tablets and buccal bilayer patches respectively. Reduction in VLDL was found to be 15.78 and 31.57 % while 15.00 % and 55.22 % reduction was found in LDL by oral conventional tablets and buccal bilayer patches respectively. HDL values were remained as it was earlier i.e. before treatment. This clearly indicated that buccal bilayer patches provided better antihyperlipidemic treatment as compared to oral conventional tablet.

The current study revealed increase in therapeutic activity of Pravastatin sodium when it was administered through buccal route which bypasses the first pass metabolism, and hence resulted in increased bioavailability (as evidenced by pharmacokinetic studies). Buccal bilayer patches being sustained release dosage form showed optimized treatment by a sustained control over a lipid profile of hyperlipidemic rabbits.

Thus, the results showed that Pravastatin sodium buccal bilayer patches showed satisfactory treatment for hyperlipidemia when compared with oral conventional tablets.

Table 12.12 Treatment with oral conventional tablets

Treatment with oral conventional tablet			
Parameters	Before Treatment (Initial)	After Treatment	
		2W	4W
HDL (mg/dL)	13.14 ± 1.44	13.01 ± 1.01	13.23 ± 1.00
TG (mg/dL)	191.14 ± 9.00	185.56 ± 10.12	161.24 ± 11.02
VLDL (mg/dL)	38.03 ± 3.23	36.12 ± 3.01	32.14 ± 2.85
LDL (mg/dL)	20.10 ± 2.21	19.14 ± 2.11	17.01 ± 2.54

(n=6), ± R.S.D.

Table 12.13 Treatment with bilayer buccal patches

Treatment with bilayer buccal patches			
Parameters	Before Treatment (Initial)	After Treatment	
		2W	4W
HDL (mg/dL)	13.14 ± 1.44	13.01± 1.2	13.99 ± 1.1
TG (mg/dL)	191.14 ± 9.00	157.90± 8.99	131.10 ± 10.23
VLDL (mg/dL)	38.03 ± 3.23	28..99± 2.98	26.00 ± 2.56
LDL (mg/dL)	20.10 ± 2.21	10.01± 2.01	8.99 ± 3.01

(n=6), ± R.S.D.

Table 12.14 Statistical significance at $p<0.005$ between oral conventional tablets and bilayer buccal patches.

Statistical significance ($p<0.005$)				
	Oral conventional tablets		Buccal bilayer patches	
Parameters	2W	4W	2W	4W
HDL (mg/dL)	NS	NS	NS	NS
TG (mg/dL)	NS	S	S	S
VLDL (mg/dL)	NS	NS	S	S
LDL (mg/dL)	NS	NS	S	S

NS: Not significant, S= Significant, (n=6).

Table 12.15 % Reduction in Hyperlipidemic Parameters

% Reduction in Hypertensive Parameters				
	Oral conventional tablets		Buccal bilayer patches	
Parameters	2W	4W	2W	4W
HDL (mg/dL)	0	0	0	0
TG (mg/dL)	3.14	15.70	17.27	31.41
VLDL (mg/dL)	5.26	15.78	23.68	31.57
LDL (mg/dL)	5.00	15.00	50.24	55.22

(n=6)

Fig 12.20 Comparative evaluation of high density lipoprotein (HDL) after administration of oral and buccal bilayer patches.

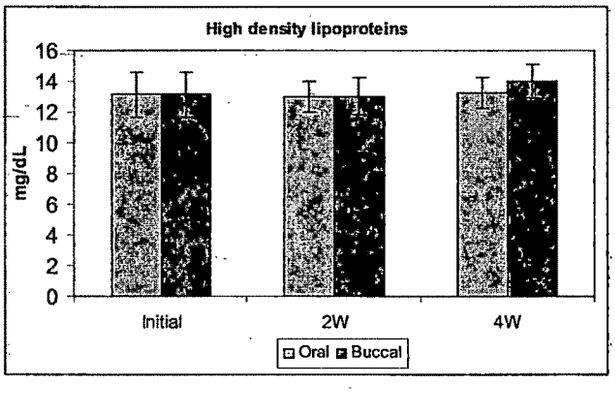


Fig 12.21 Comparative evaluation of triglycerides (TG) after administration of oral and buccal bilayer patches.

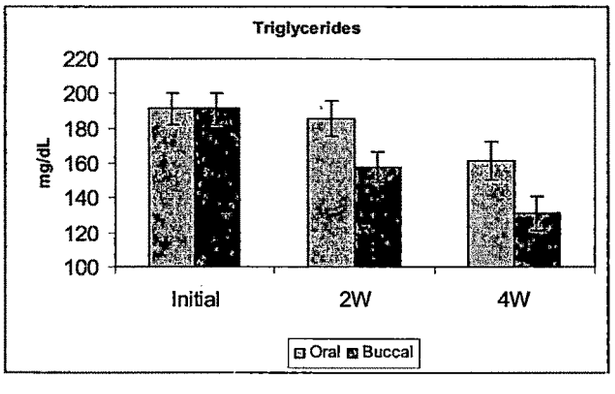


Fig 12.22 Comparative evaluation of very low density lipoproteins (VLDL) after administration of oral and buccal bilayer patches.

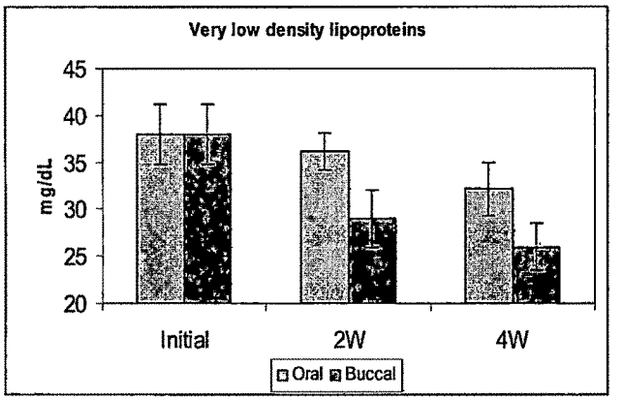
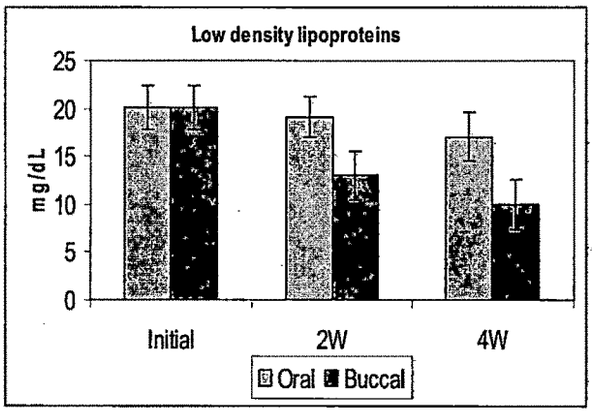


Fig 12.23 Comparative evaluation of low density lipoproteins (LDL) after administration of oral and buccal bilayer patches.



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