Chapter 5
Results & Discussion
(5-Fluorouracil)

5.1 PERMEATION STUDIES OF FLUOROURACIL ACROSS BUCCAL MUCOSA

The permeation profile of FU is shown in Fig 5.1a-d (Marked as control). 52.3 ±8.1 % FU was found to permeate through the buccal mucosa in 8 hours with permeability coefficient of 0.799 (±0.31) x 10^{-4} cm/s (Table 5.1). The results showed that the permeation of FU across buccal mucosa was poor. Hence there was need of penetration enhancement.

5.1.1 *Permeation Enhancement of FU*

In order to improve buccal permeation and hence absorption of FU, various permeation enhancers such as SDS (Sodium Dodecyl Sulphate), Oleic acid, SDC(Sodium Deoxycholate), STGC (Sodium tauroglychocholate) were used and their enhancement factors (EF) were calculated. Fig.5.1.a-d show penetration enhancement in presence of various penetration enhancers. EF as a function of the Kp is plotted in a graph (Fig. 5.2).

In case of SDS (0.5%, 1.0%, and 1.5%), significant improvement ($p<0.05$, ANOVA followed by Dunnett's Multiple Comparison Test) in permeation was discerned. As the concentration of SDS was increased, increase in permeation was observed. SDS at 1.5 % showed 75 ± 8.2 % of FU permeation with Kp value 1.13 (±0.74) x 10^{-4} cm/s and EF as 1.41 (Fig. 5.1a and Fig.5.2). **Surfactants** can interact with the mucosal sites in different ways. They can cause protein denaturation or extraction, enzyme inactivation, swelling of tissue and extraction of lipid components (Ganem Quintanar et al, 1998).

On using oleic acid, permeability coefficient and EF values were 0.879 (±0.58) x 10^{-4} cm/s and 1.08 respectively (Table 5.1, Fig.5.1b and Fig.5.2) indicating no significant improvement ($p >0.05$). **Oleic acid** acts by reducing the lipid packing order in buccal epithelial cell membranes (Turunen et al, 1994).

SDC showed 68 ± 5.6% permeation across the buccal mucosa, while the Kp and EF values were 3.17(±0.69) x 10^{-4} cm/s and 1.21 respectively, which was significantly different from control (*p<0.05*).

When STGC was used, highly significant increase (*p<0.001*) in permeation was observed, with 90.13 ± 7.2 % FU permeated through the mucosal membrane. Kp [5.80 (±0.62) x 10^{-4} cm/s] and EF (1.679) values were also increased significantly. This increase was observed at 3% concentration of STGC, and at higher concentration (5%), no further enhancement in permeation was observed.

Thus, both bile salts i.e. SDC and STGC, displayed improvement in permeation as evident from Kp and EF values. SDC and STGC at the concentrations of 4% and 3% respectively, were able to enhance the buccal permeation of FU significantly. **Bile salts** act by interaction with the intercellular or membrane lipids thus increasing the permeability of the permeant through the epithelium (Senel, S. et al, 1997). The effect of bile salts on the permeability barrier has been reported to be reversible and dependent on the concentration of the bile salts (Zhang et al., 1994; Hoogstraate et al., 1990; Shin et al., 2000b). However, recovery from potential alterations induced by bile salts exposure is possible only in-vivo (Senel et al., 1998).

In case of SDS and oleic acid, it was observed that on increasing the concentration, the enhancement effect also increased i.e. the permeation enhancement effects were concentration dependent for SDS and Oleic acid.

The order of permeation enhancement was STGC > SDS > SDC > Oleic acid. Permeability of mucosa to FU was rather low {kp 0.799(±0.31) x 10^{-4} cm/s}, whereas permeability increased most significantly (*p<0.001*) in the presence of STGC. The value of Kp augmented 7.25 fold in presence of STGC as compared to FU alone. Hence STGC was selected for further studies.

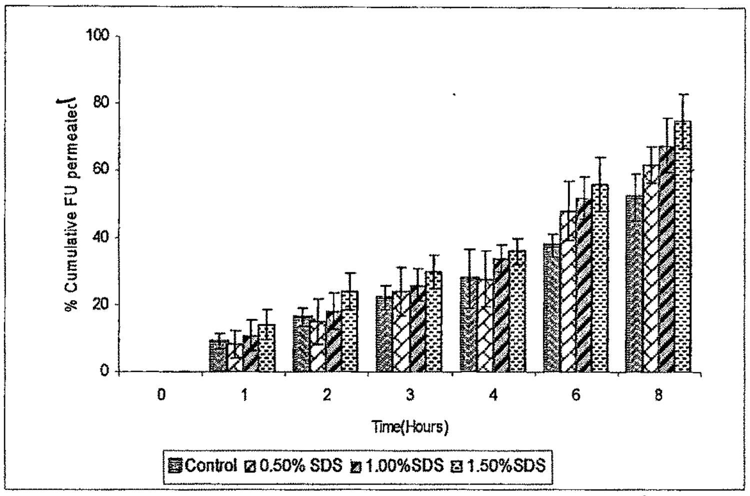

Fig 5.1a. **Permeation of FU in presence of SDS (Sodium Dodecyl Sulphate) across buccal mucosa**

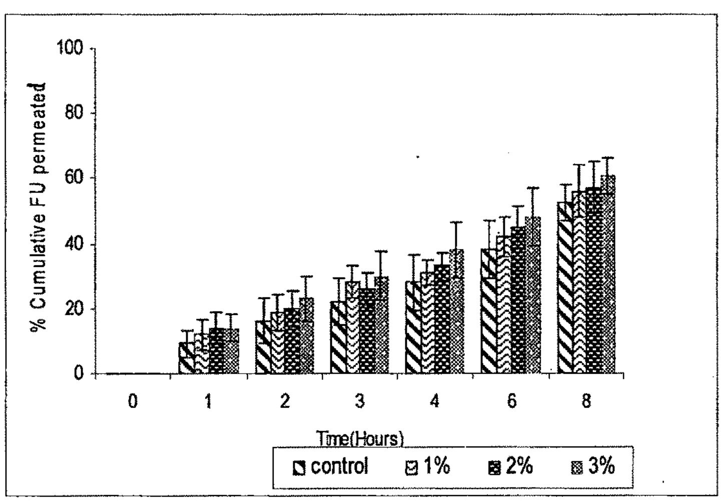

Fig 5.1b. **Permeation of FU in presence of Oleic acid across buccal mucosa**

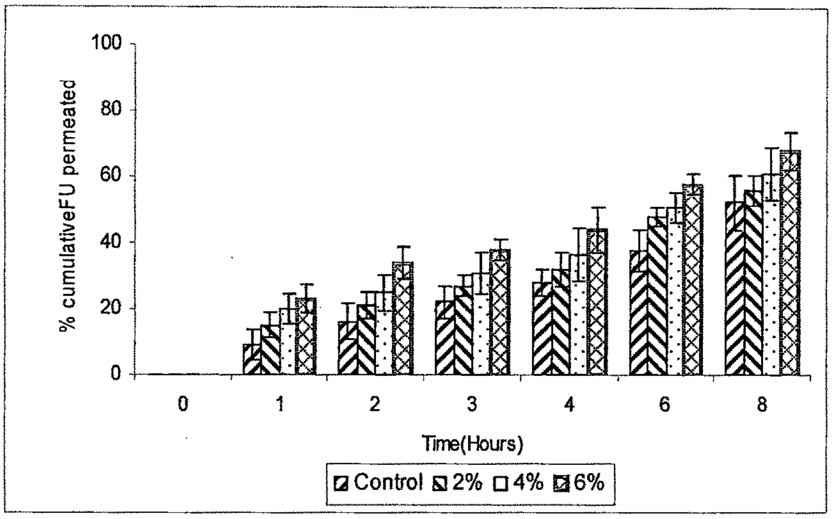

Fig 5.1c. Permeation of FU in presence of SDC (Sodium Deoxycholate) across buccal mucosa

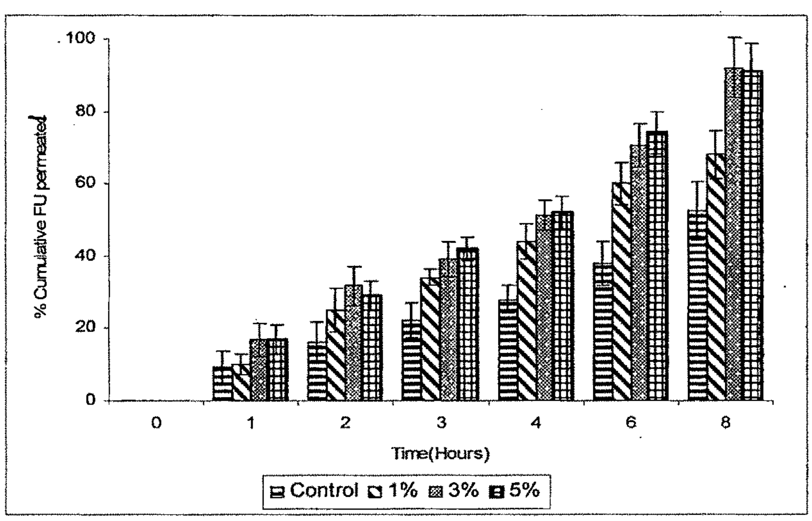

Fig 5.1d. Permeation of FU in presence of STGC (Sodium tauroglychocholate) across buccal mucosa

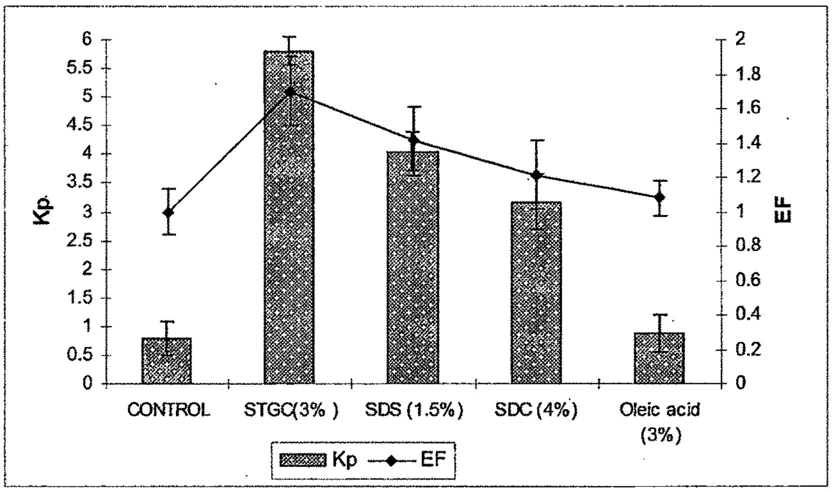

Fig 5. 2. Permeability coefficients (Kp) and Enhancement Factor (EF) for FU across buccal mucosa in presence of optimum concentration of permeation enhancers

Table 5.1: Permeability of FU across buccal mucosa in presence of penetration enhancers

Batch	Permeability Coefficient, Kp ($x10^{-4}$cm/s)	Enhancement factor (EF)
Control	0.799(±0.31)	1
STGC (1%)	0.99 (±0.49)	1.25
STGC (3%)	5.80 (±0.62)	1.68
STGC (5%)	2.93 (±0.53)	1.62
SDS (0.5%)	3.21 (±0.48)	1.25
SDS (1%)	3.44 (±0.82)	1.30
SDS (1.5%)	4.13 (±0.74)	1.41
SDC (2%)	3.01 (±0.22)	1.05
SDC (4%)	3.17(±0.69)	1.21
SDC (6%)	3.07(±0.81)	1.12
Oleic acid (1%)	0.815 (±0.95)	1.03
Oleic acid (2%)	0.869 (±0.54)	1.06
Oleic acid (3%)	0.879 (±0.58)	1.08

5.2 FORMULATION OF FU BUCCAL BIOADHESIVE PATCHES

The patches were prepared by solvent casting technique and consisted of two layers, medicated layer and backing layer. Optimization of formulation composition was carried out by varying the ratios of HPMC K4M and Gantrez MS 955 (Table 4.2) to get the desired characteristics (viz. Release, Bioadhesion and other physicochemical properties). The plasticizer (glycerine) composition was finalized by film casting method by studying the mechanical strength using tensile strength tester. The FU patches containing excess glycerine tended to be semi-solids with poor mechanical properties, while the patches with too low glycerin content yielded brittle films. Thus, an intermediate level of plasticizer, giving the film both flexibility and strength, was required. To ensure that the chosen glycerine content was compatible with the requirements of strong adherence to buccal mucosal tissue, the bioadhesive force and tensile strengths of patches plasticised with various amounts of glycerin were determined (Table 5.2). As expected, the patches that were too heavily plasticised had a low bioadhesive force as well as poor tensile strength. However, those with low to intermediate levels of glycerin adhered strongly to buccal tissue. Thus 0.5%w/w glycerin was added as plasticizer in the formulations.

Table 5.2. Influence of glycerine plasticization on the bioadhesive strength and tensile strength.

%w/w	Bioadhesion(x10^3 dynes/cm^2)	Tensile strength(g)
0.2	7.95±0.98	Patch too brittle
0.3	8.13±1.14	421±24
0.4	9.1±1.52	489±25
0.5	10.40±1.20	612±12
0.6	10.12±1.85	598±31
0.8	7.4±0.65	465±18
1.0	6.24±0.84	431±34

Physicochemical characterization parameters such as thickness, average weight, assay, pH, bioadhesion, tensile strength values are given in Table 5.3.

During patch formulation, the incorporation of FU into the bioadhesive layer presented many problems, mainly due to its limited aqueous solubility. Crystallization occurred from aqueous gels during drying yielding large, unevenly distributed crystals in the bioadhesive matrix layer. This may be due to slow solvent loss, which was overcome by employing volatile solvents, such as ethanol, which gave excellent crystal distributions. Unfortunately, this approach caused a 'custard skin' effect in these gels, which split and gave a resultant film of uneven thickness. The problem was eliminated by balancing the effects of crystallization from aqueous gels with fissuring from the volatile solvent gels. An ethanol/water blend (1:3) was found to produce films with small crystal size, and a visually even crystal distribution. Casting from this solvent blend produced a film with no evidence of fissuring. Similar problems during patch formulation have been reported earlier by Woolfson et al (1995) when they formulated FU containing bioadhesive patches for cervical delivery.

The assay values were within pharmacopoeial limits (95 % - 105%). Surface pH ranged from 5.5-6.2 depicting the suitability of formulations when applied to buccal mucosa (Table 5.3). Patch diameter for all batches was in the range of 16 ± 0.2mm.

5.2.1 *Morphology of Patch*

The scanning electron photomicrograph of the film at 1000 X magnification showed smooth surface with some white particles (probably due to presence of drug in crystallized form) but was without any scratches or transverse striations (Fig 5.3).

Table 5.3. Physicochemical parameters of FU containing mucoadhesive patches.

	Parameters						
Batch	**Assay**	**pH**	**Tensile strength(g)**	**Folding Endurance**	**Mucoadhesion ($x10^3$ dynes/cm^2)**	**Patch Thickness (mm)**	**Average weight (mg)**
FP1	99.01 ±1.62	5.59±0.20	705±27	198±22	3.65±2.60	1.29±0.12	160±5.2
FP2	98.01 ±2.31	5.55±0.11	625±14	212±18	4.05±0.40	1.28±0.10	161±4.8
FP3	97.32 ±3.12	5.83±0.17	614±21	254±25	4.89±1.25	1.24±0.09	145±7.6
FP4	99.87 ±3.60	5.63±0.06	546±12	206±12	5.49±1.56	1.19±0.09	137±7.7
FP5	101.32 ±3.92	5.92±0.12	565±15	232±15	6.36±1.90	1.17±0.14	129±4.5
FP6	99.38 ±1.23	6.05±0.24	580±12	245±35	8.34±1.20	1.16±0.12	112±8.2
FP7	96.77 ±1.1	5.84±0.06	591±12	289±14	11.47±1.50	1.18±0.14	126±3.9
FP8	98.16 ±2.84	5.79±0.08	599±19	258±10	12.07±1.43	1.25±0.11	142±4.5
FP9	97.61 ±1.98	6.07±0.15	624±22	234±21	13.74±2.01	1.24±0.07	149±2.1
FP10	99.35 ±2.20	6.18±0.21	634±28	291±8	14.86±1.40	1.31±0.08	162±5.7
FP11	97.91 ±1.11	5.91±0.20	655±18	204±15	14.99±2.50	1.35±0.15	178±3.9

(n=3; Mean ± S.E.M.).

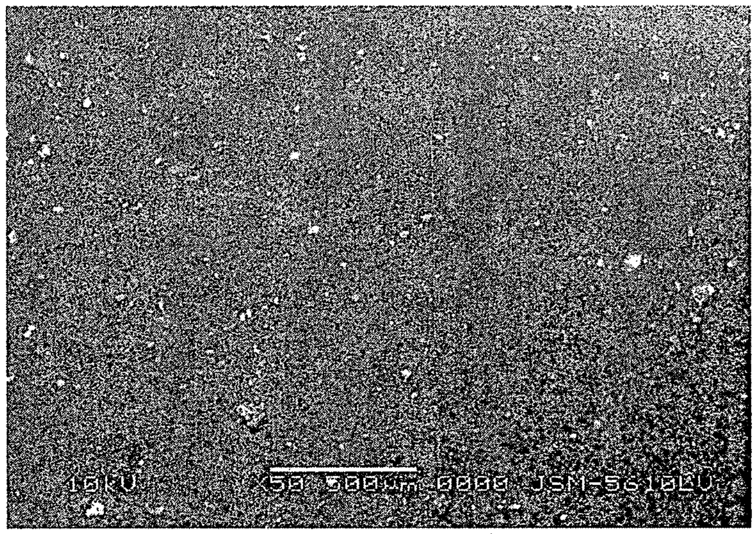

Fig.5.3a. Scanning Electron Photomicrograph of FU patch at 50 X magnification.

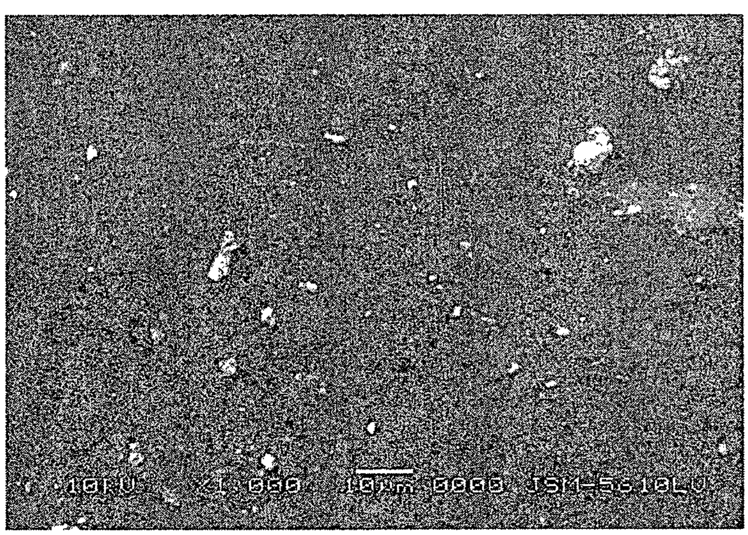

Fig.5.3b. Scanning Electron Photomicrograph of FU patch at 1000 X magnification.

5.2.2 *Swelling studies:*

The swelling capacity of the patch components contributed highly to the swelling degree of the patch. Swelling profiles is helpful to interpret the mechanism of bioadhesion and understanding release from formulations (Tur and Chang, 1998). The hydrophilic components have a stronger bioadhesive force compared to the hydrophobic components due to their high swelling capacity because polymer swelling depends upon the water imbibition which in turn increases the diffusion and interpenetration of macromolecules.

In this study, an agar plate was chosen as the simple model of the mucosa as it can retain an amount of water that resembles the secreting fluid in and around the buccal mucosa required for bioadhesion and subsequent swelling of the formulation to provide adequate release of drug (Agrawal and Mishra, 1999). The swelling profiles are shown in Fig 5.4a and 5.4b. The swelling index of the prepared buccoadhesive patches was in the order FP11 >FP1> FP10> FP9> FP8> FP2> FP7> FP3> FP4> FP5> FP6. Visual and tactile observation of the patches from all batches confirmed that swelling was dominant in these formulations and that the polymer developed a viscous gel when exposed to the dissolution media. All patches were smooth and slippery to touch. Swelling results in an increased diffusional path length, within the dosage form, through which the drug must pass, prior to being released (Khamanga and Walker, 2006).

At the initial stage, the swelling occured rapidly due to the hydration of patches containing HPMC and Gantrez. It was also observed that the swelling rate increased with an increase in HPMC content of the patches, and similar trend was observed with Gantrez (Fig 5.4a and 5.4b). The maximum swelling was attained in FP11containing highest proportion of Gantrez (Swelling index=2.4±0.09), while the batch containing highest proportion of HPMC (FP1) displayed lesser swelling index (2.24 ±0.08). In all cases the swelling index (SI) was proportional to the time the dosage form was exposed to the test medium.Differences in swelling of the tested polymers could be explained by the difference in resistance of the matrix network structure to the movement of water molecules.

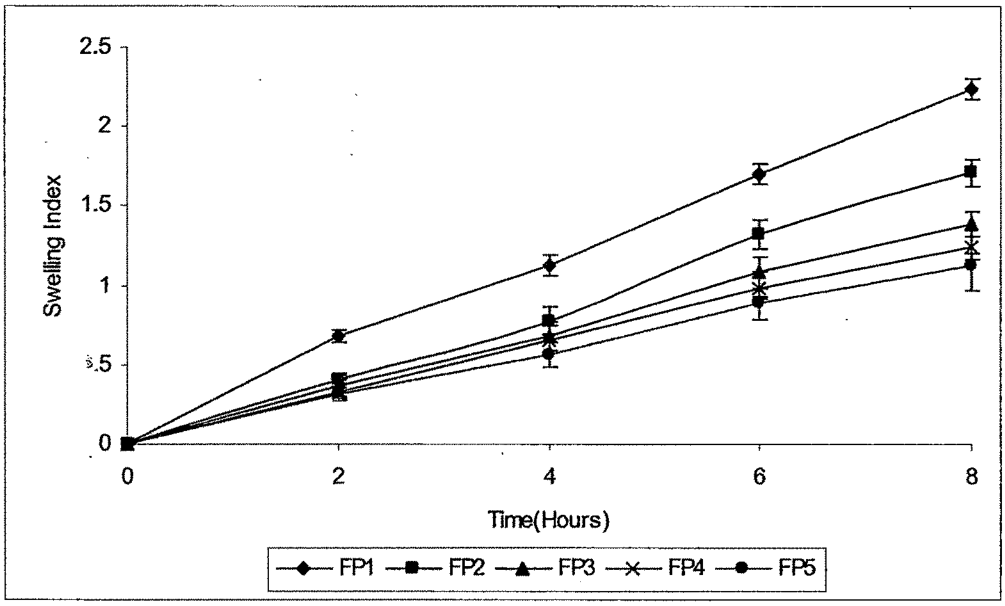

Fig 5.4a. Swelling index of FU patches (FP1-FP5)

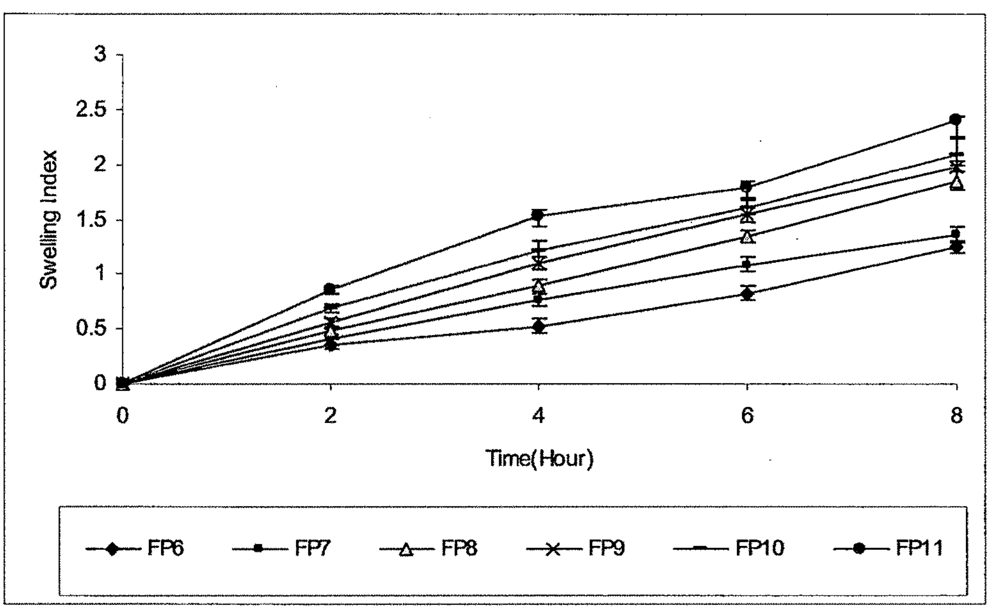

Fig 5.4b. Swelling index of FU patches (FP6-FP11)

5.2.3 *Mucoadhesion:*

Bioadhesive force means the force with which formulation bind to buccal mucous membranes. The stronger the bioadhesive force is, the more it can attach to the buccal mucous membranes. Since buccal mucous membranes consist of oligosaccharide chains with sialic acid, the polymers with hydrophilic groups such as carboxyl and hydroxyl group can bind strongly to oligosaccharide chains, resulting in a strong bioadhesive force.

The data shown in Table 5.3 depicts an increasing trend in the bioadhesive strength with increase in the amount of polymer(s). Maximum bioadhesive strength was seen with the highest levels of Gantrez. Fig. 5.5 construes an increasing trend in the bioadhesive strength with the decrease in the amount of HPMC content (FP1-FP5), while keeping the Gantrez content constant. This may be due to availability of more number of free carboxylic acid groups (present in Gantrez) for bioadhesion. But when HPMC proportion was kept constant (FP6-FP11), very sharp and significant ($p<0.05$) rise in mucoadhesion was noticed with an increase in Gantrez proportion. Maximum mucoadhesive force ($14.99 \pm 2.50 \times 10^3$ dynes/cm^2) was exhibited by FP11 containing HPMC and Gantrez in 1:2.5 proportion. Thus, the bioadhesiveness appeared to increase with a corresponding increase in the Gantrez content. The increase in bioadhesion is more pronounced in FP6-FP9, after which no significant increase is noticed. This could be due to the fact that higher bioadhesive concentrations probably require a longer wetting period with a greater volume of water.

Increasing the polymer amount may provide more adhesive sites and polymer chains for interpenetration with mucin resulting consequently in the aggrandization of bioadhesive strength. The results showed that there was significant difference ($p < 0.05$) in bioadhesion due to Gantrez. Gantrez polymers are soluble in water, providing a large adhesive surface for maximum contact with the mucin (the glycoprotein predominant in the mucous layer) and may provide excellent bioadhesiveness due to presence of carboxyl groups.

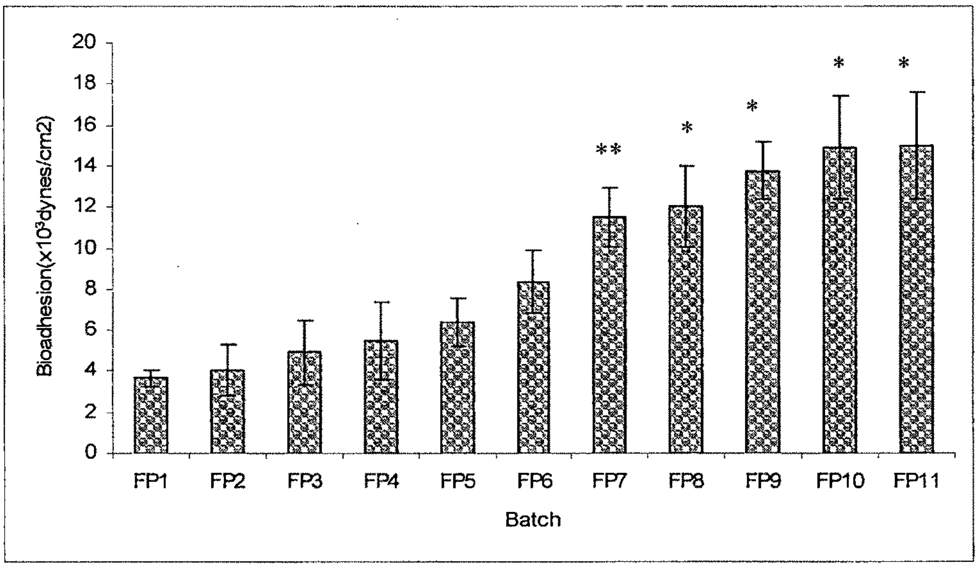

Fig. 5.5. The bioadhesive strength between the mucosa and FU patches.
Values are expressed as mean ± S.E.M. (n = 3).
*$p<0.001$versus FP1; ** $p<0.05$ versus FP1 (Dunnet's test)

5.2.4 *In vitro release study:*

In vitro release profile of the designed formulations is shown in Fig.5.6a and 5.6b. In formulation FP1, containing polymers (HPMC: Gantrez) in 2.5:1 proportion, 78±2.5 % FU was released in 8 hours. As the HPMC proportion was reduced, there was increase in release as evident in release profile of formulations FP1- FP5 (Fig.5.6a). 97.82 ±4.5 % FU was released in 8 hours from FP 6 containing HPMC and Gantrez in 1:1 proportion. With bioadhesive patches containing 1:1 ratio of HPMC: Gantrez, a sustained release effect can be seen compared to the other formulations. Batches FP7-FP11 contained increasing amount of Gantrez and fixed amount of HPMC. Release profile depicts decrease in release with an increase in Gantrez content (Fig.5.6b). In all these cases, the rate of drug release was increased when the polymers were incorporated at low concentrations, but was decreased when incorporated at higher concentrations. Increasing the concentration of Gantrez will raise the micro-viscosity of the patch and provide a more concentrated matrix from which the drug will be released more slowly. Examination of the patches during the dissolution studies revealed that the patches showed considerable swelling and gel formation, especially when the Gantrez polymers were incorporated at higher concentrations. This may help to explain the decrease in drug-release rate observed with higher concentrations of the polymers. Considerable erosion was noticed from the patches in the later phase of dissolution.

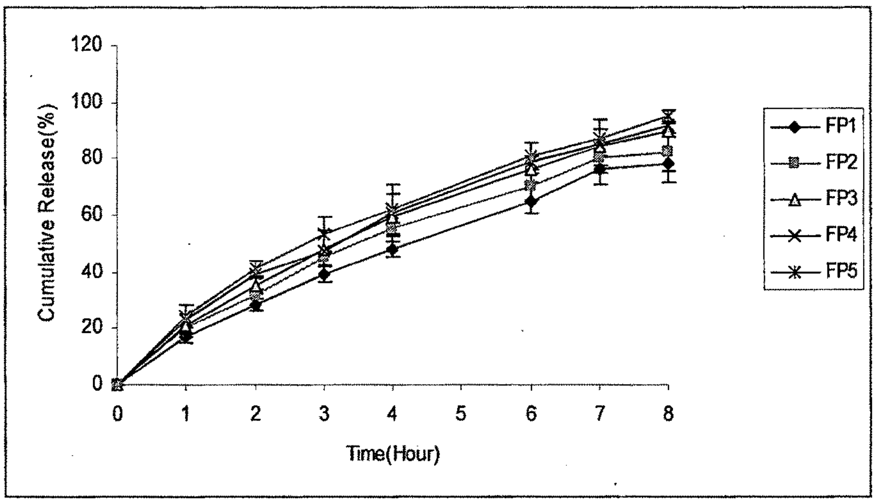

Fig.5. 6a. ***In vitro*** **release profiles of FU from buccoadhesive patches (FP1-FP5) in phosphate buffer of pH 6.8 (values are expressed as mean ± SEM; n = 3)**

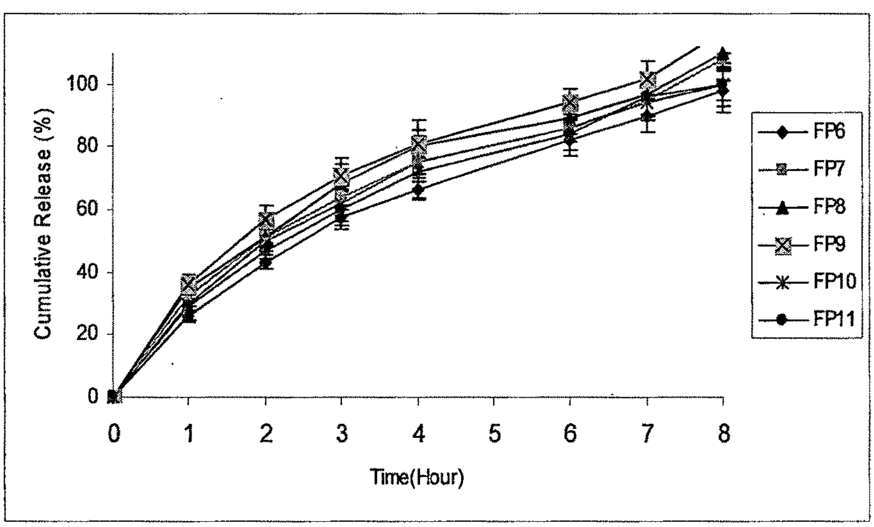

Fig 5.6b. ***In vitro*** **release profiles of FU from buccoadhesive patches (FP6-FP11)in phosphate buffer of pH 6.8 (values are expressed as mean ±SEM; n= 3).**

To investigate the kinetics of FU release from patches the release data was applied to, zero order, first order, Higuchi, and Korsmeyer Peppas models and best fit was determined. The values of R^2, k and n are listed in Table 5.4. For the determination of the exponent n, the portion of the release curve where $Mt/M_\infty<0.6$ was used. To use this equation, it is also necessary that release occur in one dimensional manner and that the system width/thickness or length/thickness relation (aspect ratio) be at least 10. (Peppas, 1985). Here the release occurred in one dimensional way and the aspect ratio was >10. When n approximates to 0.5, a Fickian/diffusion-controlled release is implied, where $0.5 < n < 1.0$, non-Fickian transport and $n = 1$ for zero order (case II transport). When the value of n approaches 1.0, phenomenologically one may conclude that the release is approaching zero order.

The goodness of fit for various models investigated for FU patch formulations ranked in the order of Higuchi ≈ Peppas > zero order > first-order. All the kinetic models, other than the first order, fitted well. The values of diffusional exponent 'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.52 and 0.76 indicating non-fickian behaviour.i.e drug release is the combination of erosion and diffusion.

5.2.5 *In vitro diffusion of FU from patches across buccal mucosa:*

The penetration of FU from patch formulation (FP6) through buccal mucosa (Fig. 5.7) was found very low (Kp=1.5426 (±0.34) $\times 10^{-5}$ cm/sec) when compared with that of FU solution (0.799 (±0.31) $\times 10^{-4}$ cm/sec) (Fig.5.1a; Table 5.1) and the difference was statistically significant ($p < 0.05$). This could be attributed to the fact that the complexity of the composition of the mucosa offered more resistance to the penetrating drug molecules during the diffusion process.

Table 5.4. Release Kinetics of 5-Fluorouracil containing bioadhesive Patches

Formulation code	Zero order model		First order model		Higuchi model		Peppas model		
	k_0	r^2	k_1	r^2	k_h	r^2	n	r^2	k
FP1	9.1610	0.9958	0.0875	0.9352	35.763	0.9295	0.7323	0.9983	0.2173
FP2	9.831	0.986	0.0893	0.8962	38.74	0.9982	0.7627	0.9971	0.2110
FP3	9.7329	0.984	0.0824	0.8987	38.415	0.9993	0.7037	0.9978	0.2390
FP4	9.7055	0.9859	0.0785	0.9105	38.215	0.9965	0.665	0.9966	0.2553
FP5	9.7363	0.9814	0.076	0.8892	38.479	0.9993	0.6502	0.9960	0.2639
FP6	9.7387	0.9728	0.0729	0.8787	38.624	0.9975	0.6281	0.9929	0.2770
FP7	9.1541	0.9589	0.0666	08598	38.415	0.9920	0.5779	0.9867	0.3060
FP8	9.7808	0.9667	0.0646	0.8842	38.735	0.9885	0.5540	0.9900	0.3101
FP9	9.4623	0.9514	0.0597	0.8647	37.653	0.9822	0.5156	0.9834	0.3379
FP10	10.305	0.9639	0.0621	0.8819	40.836	0.9869	0.5323	0.9886	0.3230
FP11	11.247	0.9869	0.0647	0.8787	44.554	0.9914	0.5789	0.9894	0.2994

r^2 represents correlation coefficient;.k_0, release rate constant of zero order kinetic (% h^{-1}); k_1, release rate constant of first order kinetic (%h^{-1});k_h, release rate constant of Higuchi's square root of time (%$h^{-1/2}$). Release rate was calculated from the slope of the respective plots.

(n=3; Mean ± S.E.M.).

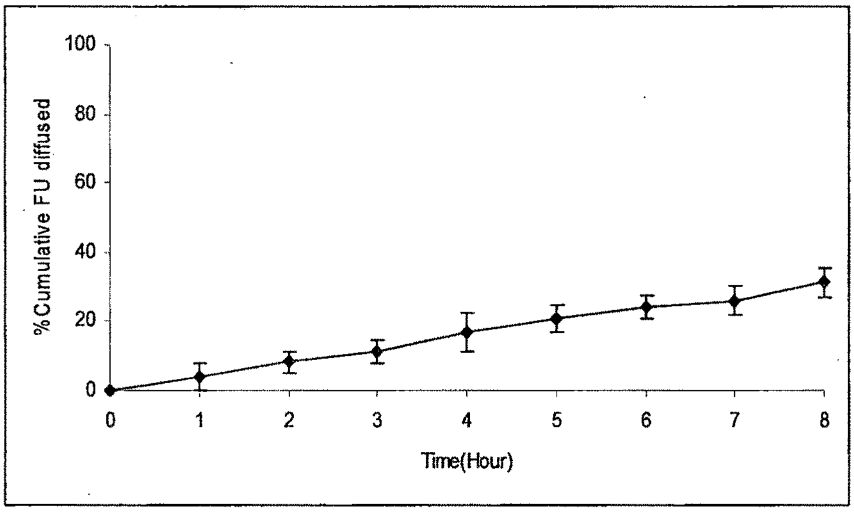

Fig. 5.7. ***In vitro*** **diffusion of FU from patches across buccal mucosal membrane.**

Data are expressed as mean ± S.E.M. for n=3.

The formulation (FP6) was prepared further using STGC as a permeation enhancer (0.05M) for *in vivo* studies (Section 5.1). The bioadhesive strength of these patches was 7.62±0.95 dynes/cm^2, tensile strength 557± 19g and moisture content 13.2± 2.8% w/w. No significant difference in adhesive bond strength, tensile strength and moisture content was observed between STGC containing and STGC free patches.

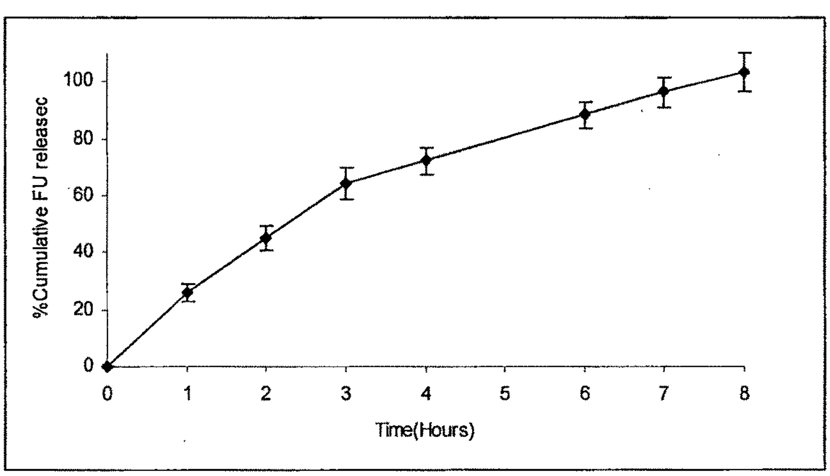

Fig 5.8. ***In vitro*** **release profiles of FU from buccoadhesive patches containing STGC in phosphate buffer of pH 6.8 (values are expressed as mean ±SEM).**

Addition of STGC to patch formulations resulted in an increase in release rate (Fig 5.8). 45.16 ±3.46%, 72.18 ±5.19% and 103.1± 4.8% FU was released from FU patch formulations containing STGC as compared to 42.76 ± 4.2%, 66.45 ± 3.1% and 97.82 ± 4.2% from STGC free patches after 2, 4 and 8 hours, respectively. These results are in agreement with those given in literature (Ikinci et al, 2000).

5.2.6 ***Compatibility studies:***

5.2.6.1 ***Differential Scanning Calorimetry (DSC):*** DSC thermogram of FU and its physical mixture with polymers and STGC (patch components) are presented in Fig 5.9. The DSC thermogram of FU (Fig. 5.9a) shows an endothermic peak at 283.5°C corresponding to its melting temperature, which was also detected in physical mixture of FU with components of patch (FU: Gantrez: HPMC:STGC = 1:2:2:1) at the same temperature. This indicated absence of any kind of interaction between drug and polymers.

5.2.6.2 ***Fourier Transform Infrared (FTIR) Spectroscopy:*** IR spectra of FU, its physical mixture with various polymers and STGC(patch components) and patch are presented in Fig 5.10 Pure FU spectra showed sharp characteristic peaks at 812 (CH out of plane), 1243 (CH in plane), 1425, 1655 and 1718 (C=O, C=N- stretch), and 3122 cm^{-1} (NH stretch). All the above characteristic peaks appeared in the spectra of physical mixtures of FU with components of patch (FU: Gantrez: HPMC: STGC = 1:2:2:1) and FU patch at same wavenumber indicating no modification or interaction between the drug and polymers, even after processing into a patch.

5.2.6.3 ***X-ray diffraction (XRD) spectroscopy:*** X-ray diffraction (XRD) spectra of pure compound and binary systems with carriers are presented in Fig 5.11. The X-ray diffractogram of FU has sharp peaks at diffraction angles (2θ) 16.16°, 18.92°, 20.51°, 21.78°, and 28.49° showing a typical crystalline pattern. All major characteristic crystalline peaks also appeared in the diffractogram of both physical mixture of patch components (FU: Gantrez: HPMC: STGC = 1:2:2:1) and patch formulations. Moreover, the relative intensity and 2θ angle of these peaks remained unchanged (Table 5.5), clearly suggesting that there is no change in FU's crystalline structure.

Table 5.5. Characteristic XRD peaks of FU and its physical mixture with patch components and patch

S.No.	Formulation	Characteristic peaks at 2θ (degrees) *Intensity in Parentheses
1.	FU	16.16°(31.1),18.92°(30.5),20.51°(23.5),21.78°(21.4), and 28.49°(100.00)
2.	FU patch physical mix	16.18°(15.6),18.96°(13.1),20.53°(12.7),21.8°(13.8), and 28.51°(100.00)
3.	FU patch	16.18°(7.94), 18.98°(10.1), 20.48°(8.89), 21.95°(3.88), and 28.47°(53.21)

5.2.7 *Stability study:*

Patches exhibited drug content within acceptable limits of 95-105% over the storage period at different conditions. Table 5.6 enlists different parameters examined and observations during the study. No changes in physical appearance were observed during study. Tensile strength reduced on storage at 40 ± 2°C/ 75% ± 5% RH from 557±19.0 to 489 ±18.98 g. This may be due to significant increase ($p<0.05$) in moisture content from 13.2±2.8 %w/w (initial) to 24±2.8%w/w on storage at this condition. Insignificant difference ($p>0.05$) in *in vitro* release was observed at all conditions (Table 5.6). Hence it is concluded that three month storage under these conditions does not change the release behavior and drug content despite an obvious decrease in tensile strength.

Table 5.6 Stability results for FU bioadhesive patches (FP 6).

Parameter	Initial	3 Months	3 Months
		30 ± 2°C/ 65±5%RH	40 ±2°C/ 75 ±5%RH
Tensile strength (g)	557(±19.0)	540(± 27.32)	469(±18.98)
Moisture content(%w/w)	13.2(± 2.8)	15(±5.4)	24(±2.8)
Assay(%w/w)	99.23 (± 0.207)	97.34 (± 0.155)	97.25(±0.242)
Release (8 h)	103.1(±4.8)	105.48(±7.58)	103.84(±4.65)

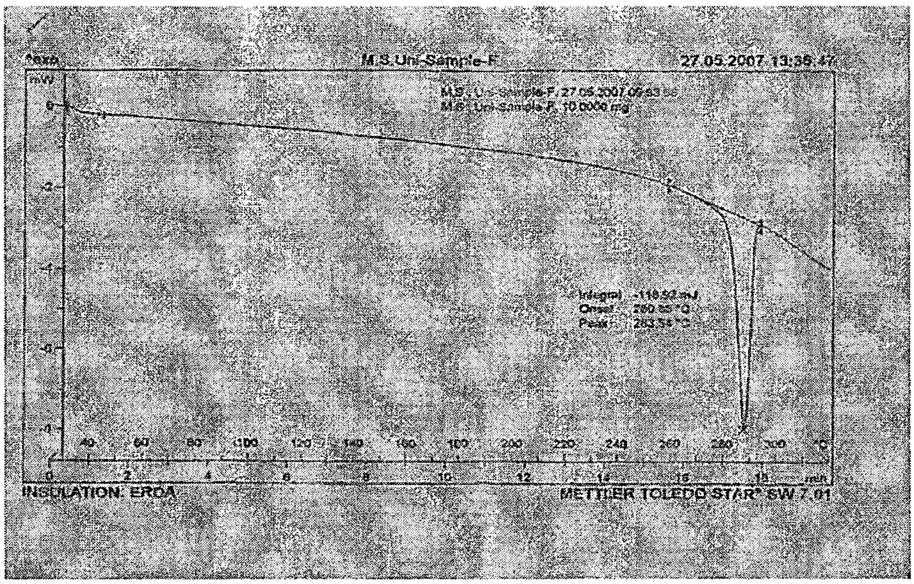

Fig. 5.9a. DSC Thermogram of FU

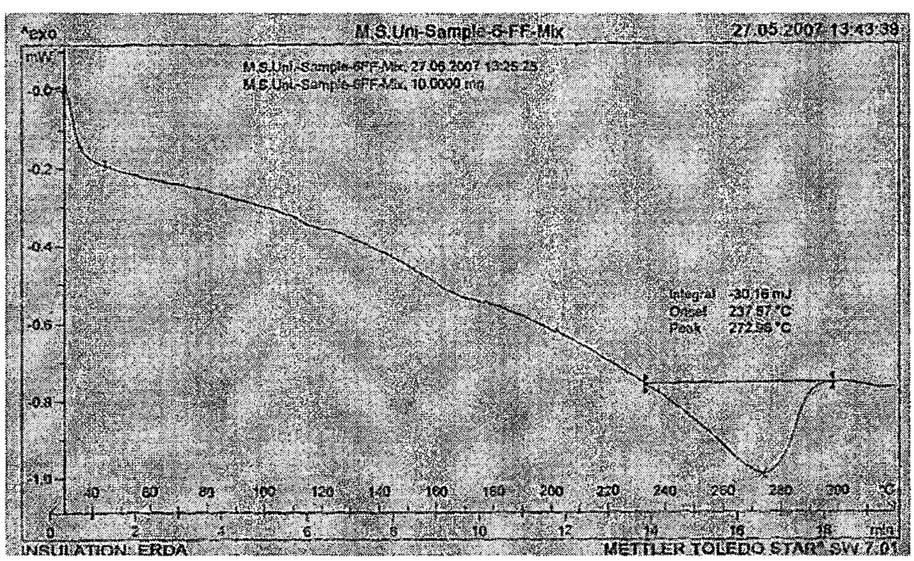

Fig. 5.9b. DSC Thermogram of FU patch dry mix

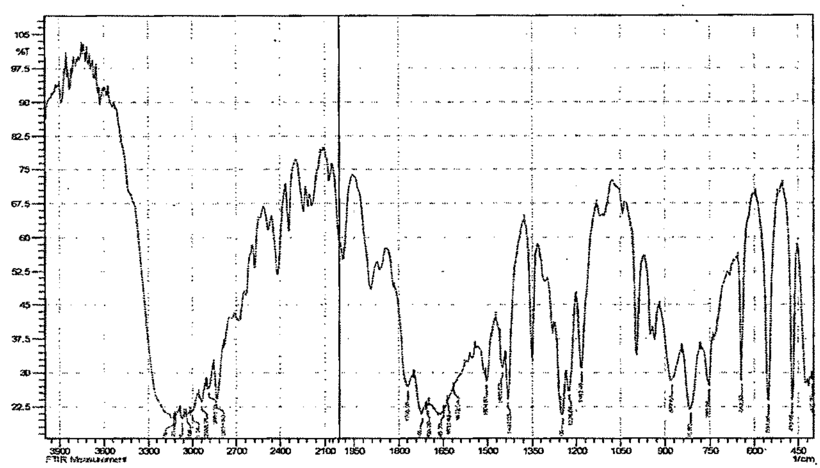

Fig. 5.10 a FTIR spectra of FU

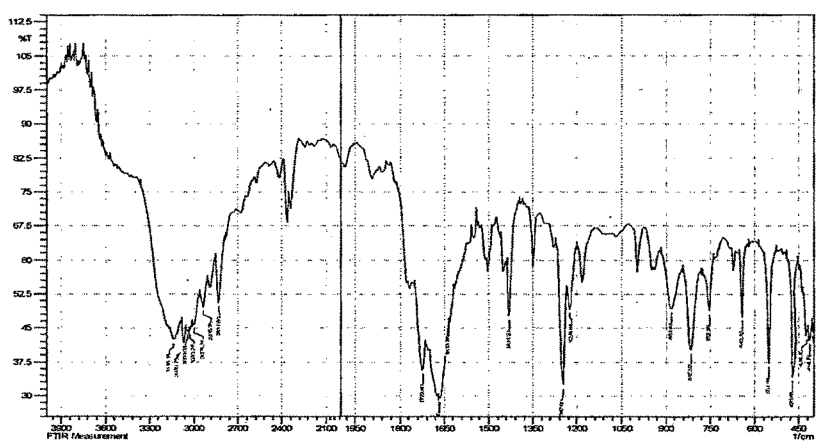

Fig.5.10b. FTIR spectra of FU patch dry mix

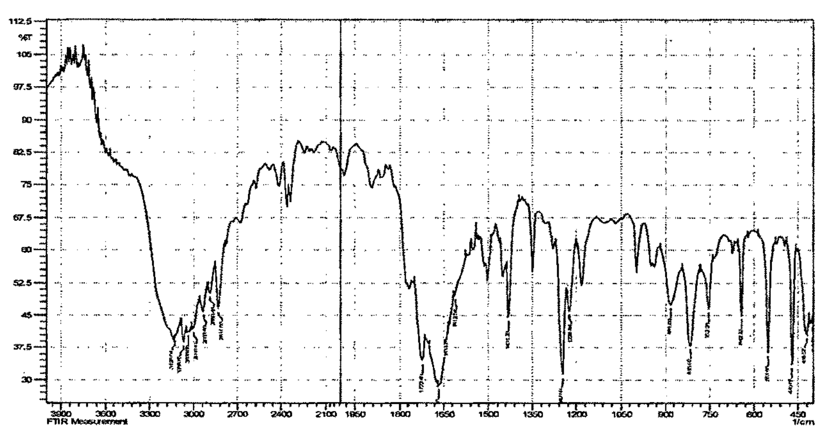

Fig. 5.10c. FTIR spectra of FU patch

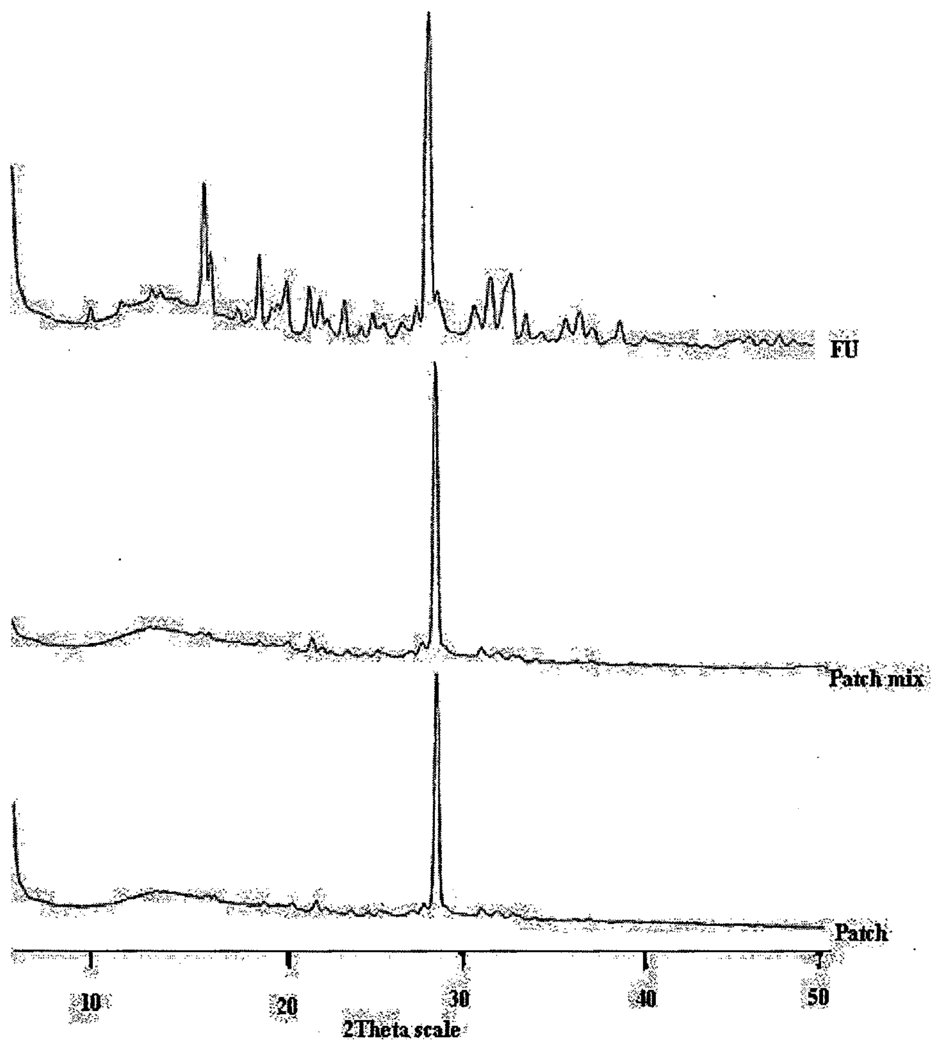

Fig. 5.11. X-ray diffractograms of test materials (FU, FU patch physical mixture and FU patch);
The graphs were replaced vertically for better visualization.

5.3 FORMULATION OF TABLETS:

5.3.1. *Preliminary studies:*

During preliminary studies, polymer combinations of Gantrez MS-955, HPMC K4M and HPMC K 15M were explored and the dissolution results of same are enlisted in (Table 5.7, and Fig. 5.12). Physical parameters of all the preoptimization batches were evaluated and the results are shown in table 5.7. On the basis of dissolution profile and bioadhesion, Gantrez MS 955 and combination of HPMC K4M and HPMC K15M were finally chosen for further optimization studies.

Table 5.7. Mean values of percent cumulative drug released for the FU bioadhesive formulations prepared during pre-optimization studies

Time (Hours)	Cumulative percent drug released ± SEM				
	PFT1	PFT2	PFT3	PFT4	PFT5
1	45.20±0.32	33.4±30.52	30.12±0.51	27.60±0.31	35.19±0.63
2	57.95±0.24	47.64±0.43	43.14±0.35	40.02±0.38	49.43±0.36
3	62.64±1.45	58.37±0.09	51.60±0.21	57.16±0.94	67.04±1.14
4	72.19±0.31	67.48±0.12	60.45±0.67	73.39±0.52	76.31±0.42
6	94.23±0.17	81.61±0.03	72.32±0.11	88.06±0.18	92.24±1.07
8	103.83±0.61	97.23±0.10	89.28±0.36	102.54±0.36	102.88±0.89

Table 5. 8. Physical characterization and drug assay for preoptimization batches of FU tablets

Batch	Weight (mg)	Hardness (kg/cm^2)	Diameter (mm)	Thickness (mm)	Friability (%)	Assay (%)	Bioadhesion ($x10^3 dynes/cm^2$)
PFT1	226±3	4.7±0.2	9.55±0.04	2.1±0.1	0.24±0.05	101.25±2.1	8.6±0.98
PFT2	224±2	4.6±0.1	9.46±0.01	2.1±0.1	0.32±0.04	100.54±2.1	10.8±1.1
PFT3	223±2	4.6±0.4	9.44±0.02	2.2±0.1	0.19±0.01	101.32±1.4	11.4±0.67
PFT4	225±4	4.5±0.3	9.48±0.04	2.1±0.1	0.34±0.04	99.87±2.5	14.9±0.88
PFT5	225±3	4.8±0.3	9.48±0.02	2.2±0.1	0.12±0.02	99.85±0.98	18.2±1.3

Based on the criterion of maximum values of bioadhesion and complete release between 6-8 hours, 5 different formulations having varying polymer combinations were studied during pre-optimization studies. An appropriate range of the factor ingredients (polymers) was selected to obtain the desired response parameters for further optimization studies. A range between 10 to 30 mg for Gantrez MS-955 and 4 to 16 mg for HPMC K15M was finally selected on the basis of pre optimization studies for exploration during optimization.

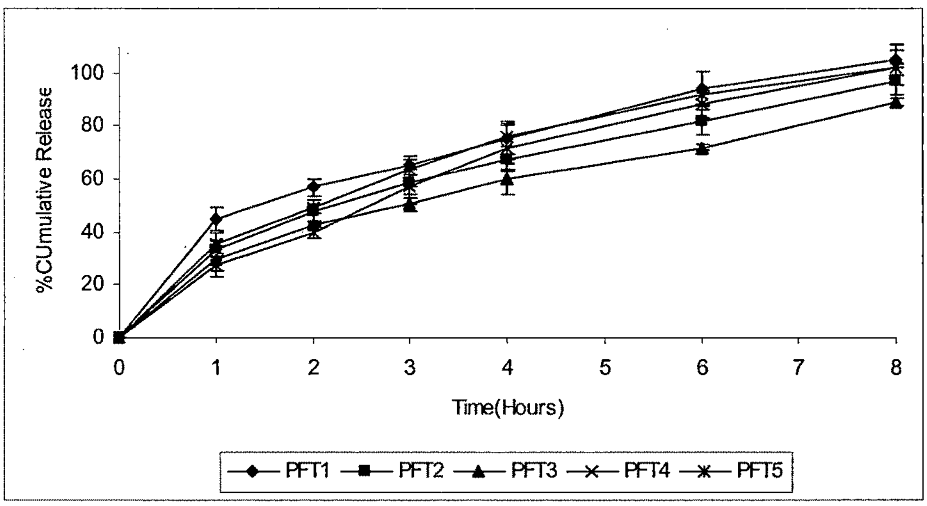

Fig. 5.12 Dissolution profile of preliminary batches of FU tablets

5.3.2 *Optimization studies:*

Nine formulations were prepared as per 3^2 Factorial Design (Section 4.4.2). All the formulations were evaluated for physical parameters and drug assay. Table 5.9 displays the values of physical parameters and drug assay for these formulations. The tablet weights ranged from 223±2 to 228±2mg, diameter 9.42±0.03 to 9.55±0.04 mm, thickness between 2.1±0.1 to 2.2±0.1 mm and hardness between 4.3±0.2 to 4.8±0.3 kg/cm^2. The FU content varied between 98.14±1.2 to 102.00±1.4 % and friability ranged between 0.12±0.02 to 0.44±0.03%. Thus, all the formulations were found to comply with the compendial requirements. pH of all the formulations was in the range 6.01±0.020 to 6.44±0.022. Table 5.10 enlists the response parameters of all the nine formulations prepared as per 3^2 Factorial

Design, while Fig. 5.13.a, b and c portrays their individual drug release profiles. The best release performance is unambiguously due to the formulation FT 5, i.e., prepared using the intermediate levels of Gantrez MS-955 and HPMC K 15M.

Table 5.9. Physical characterization and drug assay for FU tablets

Batch	Weight (mg)	Hardness (kg/cm^2)	Diameter (mm)	Thickness (mm)	Friability (%)	Assay (%)	pH
FT1	228±2	4.4±0.2	9.51±0.02	2.1±0.1	0.42±0.03	98.14±1.2	6.15±0.028
FT2	224±2	4.6±0.1	9.46±0.01	2.1±0.1	0.32±0.04	98.48±1.8	6.44±0.022
FT3	226±3	4.7±0.2	9.55±0.04	2.1±0.1	0.24±0.05	101.25±2.1	6.18±0.026
FT4	226±3	4.5±0.1	9.42±0.03	2.1±0.1	0.44±0.03	102.00±1.4	6.01±0.020
FT5	225±3	4.8±0.3	9.48±0.02	2.2±0.1	0.12±0.02	99.85±0.98	6.35±0.014
FT6	226±4	4.3±0.2	9.53±0.03	2.1±0.1	0.29±0.04	99.25±1.4	6.22±0.010
FT7	223±2	4.6±0.4	9.44±0.02	2.2±0.1	0.19±0.01	99.14±1.12	6.34±0.06
FT8	227±3	4.5±0.3	9.50±0.01	2.1±0.1	0.30±0.03	99.07±1.5	6.06±0.012
FT9	225±4	4.8±0.2	9.46±0.03	2.2±0.1	0.36±0.04	100.54±2.6	6.26±0.04

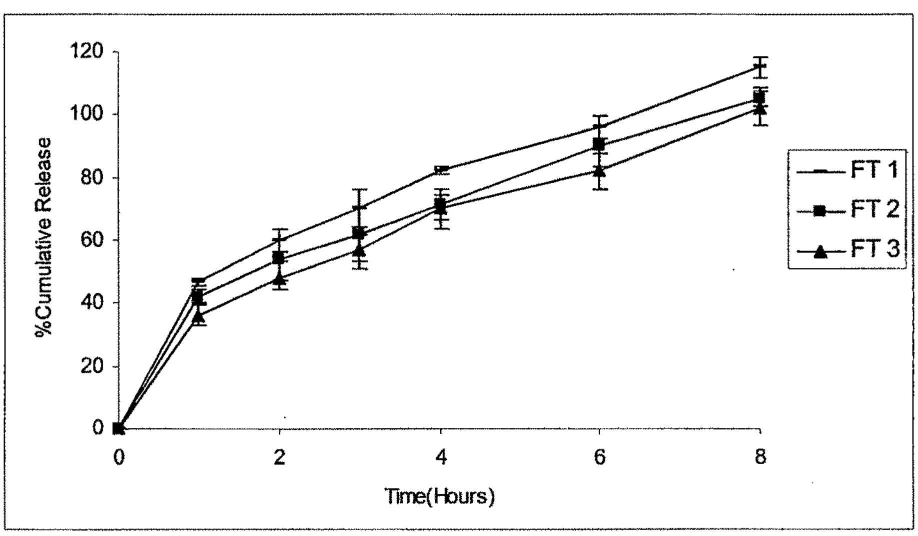

Fig. 5.13a. Dissolution profile of FU bioadhesive tablets (FT1-FT3).

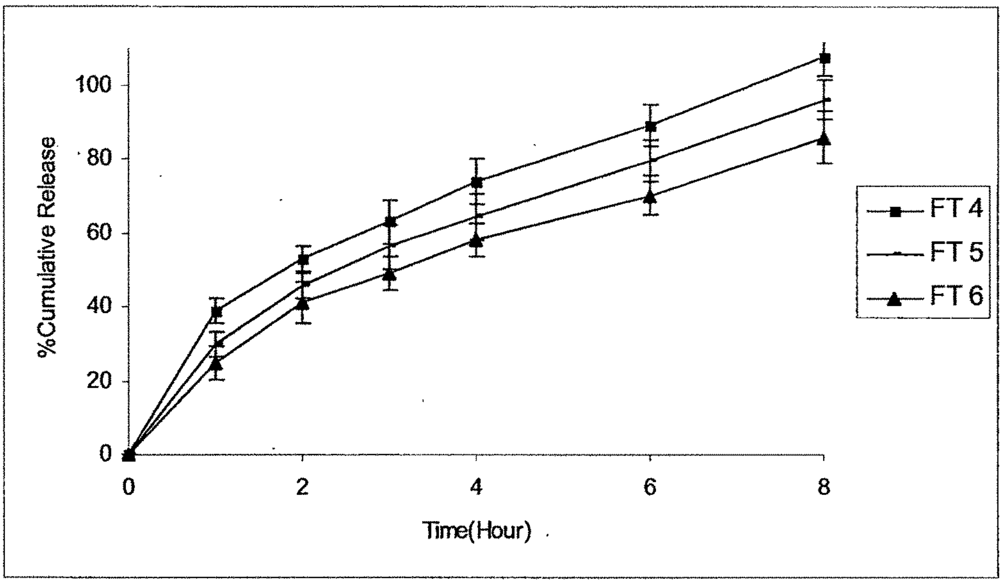

Fig.5.13b Dissolution profile of FU bioadhesive tablets (FT4-FT6).

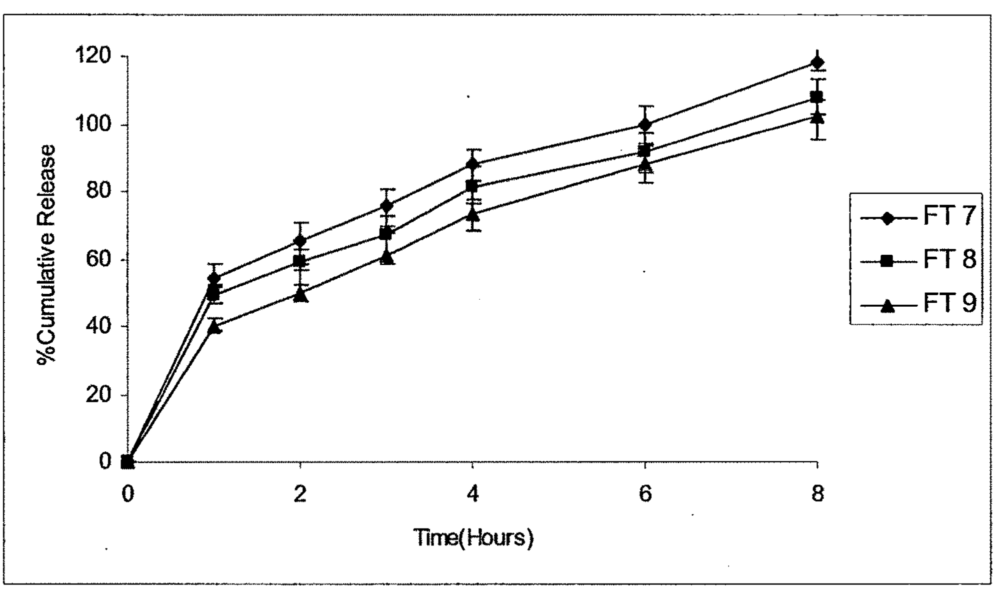

Fig. 5.13c. Dissolution profile of FU bioadhesive tablets (FT7-FT9).

Table 5.10 Response parameters for formulations of bilayered buccal tablets prepared as per 3^2 factorial design.

Formulation code	Formulation composition		Bioadhesion ($x10^3$dynes x cm^2) [Y_1]	Release till 8 hrs (rel_{8hr}) [Y_2]	$t_{50\%}$ (min) [Y_3]	n [Y_4]
	Gantrez(mg)	HPMCK15M (mg)				
FT1 (-1,-1)	10	4	8.86	99.60	72.00	0.3807
FT2 (-1,0)	10	10	9.63	99.22	96.00	0.4225
FT 3 (-1, 1)	10	16	11.36	97.17	156.00	0.4795
FT 4 (0, -1)	20	4	9.82	98.83	114.00	0.4546
FT 5 (0, 0)	20	10	10.78	96.44	168.00	0.5516
FT 6 (0, 1)	20	16	12.52	86.56	186.00	0.5753
FT7 (1,-1)	30	4	12.72	100.01	60.00	0.3234
FT 8 (1, 0)	30	10	13.58	99.12	74.00	0.3560
FT 9 (1, 1)	30	16	15.22	98.14	120.00	0.4493

Table 5.11. Observed and Predicted values of response parameters

Batch	Response parameters											
	Y1			Y2			Y3			Y4		
	Observed	Predicted	%RE	Observed	Predicted	%RE	Observed	Predicted	%RE	Observed	Predicted	%RE
FT1	8.86	8.86	0.00	99.60	101.06	1.44	72.00	67.78	6.23	0.38	0.38	0.00
FT2	9.63	9.64	0.10	99.20	99.66	0.46	96.00	104.44	8.08	0.42	0.43	2.325
FT3	11.36	11.37	0.088	97.10	95.18	2.02	156.00	151.781	2.78	0.48	0.48	0.00
FT4	9.82	9.84	0.203	98.80	96.16	2.76	114.00	121.78	6.388	0.45	0.47	4.25
FT5	10.78	10.77	0.185	96.40	94.93	1.55	168.00	152.44	10.2	0.55	0.53	3.77
FT6	12.52	12.53	0.079	86.50	90.61	4.54	186.00	193.78	4.01	0.58	0.58	0.00
FT7	12.72	12.71	0.0786	100.01	101.18	1.17	60.00	56.44	6.30	0.32	0.31	3.22
FT8	13.58	13.59	0.0735	99.10	100.11	1.00	74.00	81.11	8.76	0.36	0.38	5.26
FT9	15.22	15.21	0.0657	98.14	95.95	2.28	120.00	116.44	3.05	0.45	0.44	2.27

% RE= % Relative Error

5.3.3. *Effect of formulation variables on the response parameters:*

On analyzing the data of all the 9 formulations prepared as per 3^2 Factorial design using Design Expert® software, various polynomial equations, response surface and contour plots were generated (section 4.4.2 and 4.4.3.8). The information obtained from the software is discussed in the following sections, depicting the effects of various formulation variables on the respective response parameters (Y_1, Y_2, Y_3 and Y_4).

5.3.3. (a). Bioadhesion force (Y_1)

The polynomial equation and regression coefficient for Y_1 (bioadhesion force) are as follows:

$$Y_1\ (\text{Bioadhesion}) = +10.766 + 1.975 * X_1 + 1.348 * X_2 - 1.11\text{E-}016 * X_1 * X_2 + 0.851 * X_1^2 + 0.4175 * X_2^2 - 0.0965 * X_1^2 * X_2 - 0.0485 * X_1 * X_2^2 \quad \text{-----(5.1)}$$

R-Squared = 0.9998

The polynomial model (equation 5.1) was found to be significant with an F value of 4792.24 (*p=0.0111*). The value of correlation coefficient was found to be 0.9998, indicating a good fit. The data from equation 5.1 reveals that both the factors (X_1 and X_2) affect the parameter Y_1. The low value of X_1X_2 coefficient also suggests that the interaction between X_1 and X_2 is not significant.

The combined effect of factors X_1 and X_2 can further be elucidated with the help of response surface and contour plots (Fig. 5.14a and 5.14b respectively) which demonstrates that Y_1 varies in a linear fashion with the amount of both the polymers. However, the steeper ascent in the response surface with Gantrez (X_1) than with HPMC K15M (X_2) is clearly discernible from both the plots, indicating that the effect of Gantrez is comparatively more pronounced than that of HPMC K15M. From this discussion, one can conclude that appropriate selection of the levels of X_1 and X_2 yields optimum values of bioadhesion. The predicted and observed values of response parameters are shown in table 5.11. Low values of the relative error showed that there was a reasonable agreement of predicted values and experimental values.

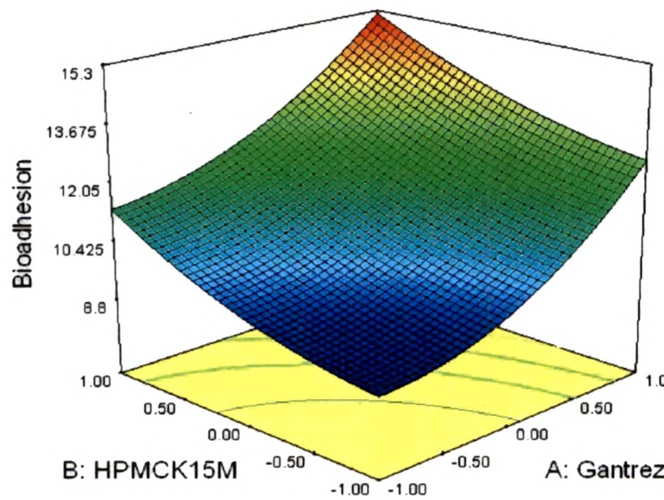

Fig 5.14a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on Bioadhesive force.

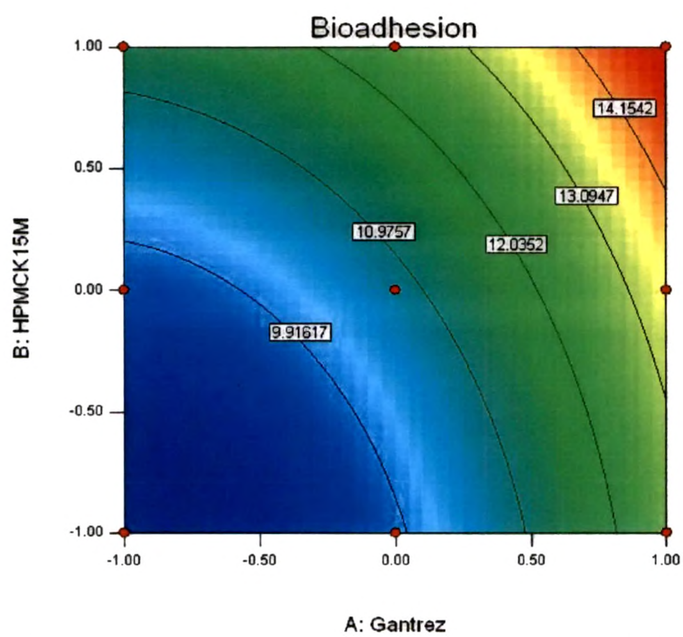

Fig 5.14b: Corresponding contour plot showing the relationship between Various levels of both polymers.

5.3.3 (b). Percentage release at 8 h (Rel_{8hr} or Y_2)

The model terms for Y_2 (Rel_{8hr}) were found to be non significant (p=0.3693) on using polynomial model with an F value of 3.96 and R^2 value of 0.9652.

However, on reducing the model to a quadratic one, F value was 1.54 (p= 0.3839) and all the model terms were found to be non significant and the R^2 value reduced significantly to 0.7194. The quadratic model (equation 5.2) describing the percentage FU release at 8 h can be written as:

$$Y_2 (Rel_{8hr}) = +94.92 + 0.223 * X_1 - 2.77 * X_2 + 0.16 * X_1 * X_2 + 4.956 * X_1^2 - 1.543 * X_2^2 \qquad \text{.......................(5.2)}$$

R^2 =0.7194

The PRESS value (Predicted Residual Sum of Square) for the polynomial model was 887.44 whereas, for quadratic model it was found to be 455.14. As low PRESS values indicates adequate fitting of model (Huang et al, 2005).The quadratic model with the lower PRESS value of 455.14 was selected.

Response surface plot of Rel_{8hr} displays a non-linear twisted relationship at intermediate and high levels of the polymers (Fig.5.15a). This can be attributed to the potential occurrence of interaction between the two polymers at the corresponding factor levels, construing that each polymer is tending to modify the effect of another towards the release of drug. The contour plot (Fig.5.15b) of the same shows that Gantrez has a comparatively greater influence on the response variable than HPMC K15M. High value of percentage FU release at 8 h was observed in formulation having low value of either of the independent variables (Table 5.10), which may be due to low polymer concentration, thus weakening the gel strength of the matrix. High level of X_2 gave low value of percentage FU release at 8 h at all the levels of X_1 which might be due to increased thickness of the gel layer formed upon contact with dissolution medium and thus retarding the drug diffusion.

At higher levels of X_1 increase in FU Rel_{8hr} was observed at all corresponding levels of X_2. As Gantrez MS 955 is a water soluble polymer, the above outcome can be explained by the phenomenon that when a soluble polymer is used, the void formed in the matrix by dissolution initially contain a rather viscous solution, characteristic of dissolved polymers. The high viscosity in the pores serves to retard the diffusion of drug at the early stages of release. At the later stages, the polymer solution becomes dissipated and resistance to diffusion is decreased (Korsemeyer, et al., 1983). It was also observed that tablets with high percentage of Gantrez had eroded by the end of release study. From the results, it can be concluded that both the independent variables have pronounced effect on percentage FU release at 8 h.

Low values of the relative error (Table 5.11) showed that there was a reasonable agreement of predicted values and experimental values indicating the correctness of the model.

5.3.3. (c). $t_{50\%}$ (Y_3):

The quadratic model for (Y_3) was found to be significant (*p=0.0199*) with an F value of 17.49. In this case, factors X_1, X_2 along with interaction factor X_1X_2 were found to be significant. Thus, model becomes:

$$Y_3\,(t_{50\%}) = +152.44 - 11.666{*}X_1 + 36.00 * X_2 - 6.00{*}\, X_1{*}X_2 - 59.66{*}X_1^2 + 5.33 * X_2^2 \qquad \text{..................(5.3)}$$

R^2 =0.9668

Response surface (Fig.5.16a) and contour plots (Fig. 5.16b) shows a "region of maximum" for $t_{50\%}$, lying between intermediate to high levels of both the polymers. The value of $t_{50\%}$ varies from 60 min to 186 min indicating that the variables significantly affect (p<0.05) this parameter (Table 5.10). Linear relationship was obtained between the fraction of HPMC K 15M and $t_{50\%}$, and it was observed from response surface and contour plots that as the fraction of HPMC K15M(X_2) increased, the value of $t_{50\%}$ increased, at all the 3 levels of Gantrez (Fig 5.16a and 5.16b). On increasing the amount of Gantrez(X_1), nonlinear trend in the response curve was observed i.e. "region of maximum" for $t_{50\%}$ at intermediate levels of

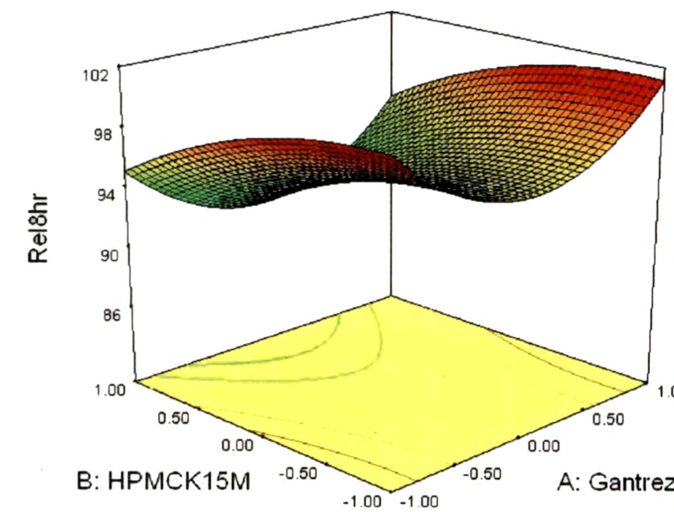

Fig 5.15a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on Rel_{8hrs}.

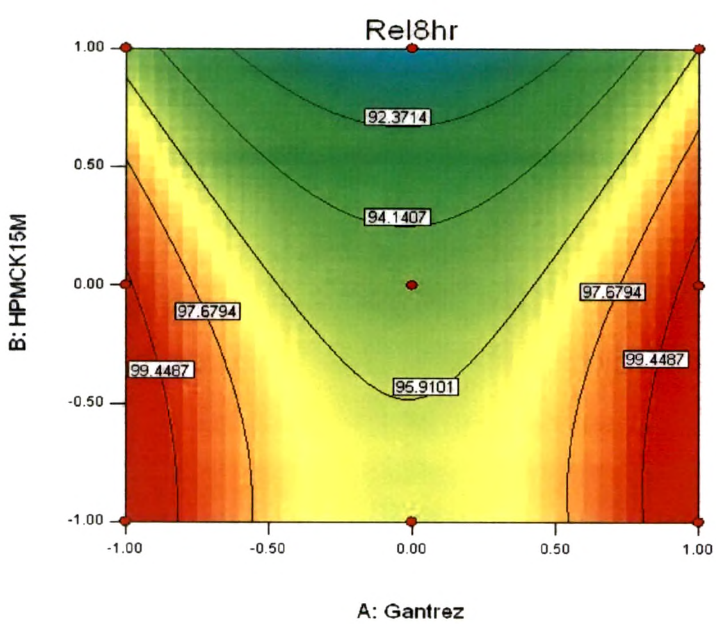

Fig 5.15b: Corresponding contour plot showing the relationship between various levels of both polymers.

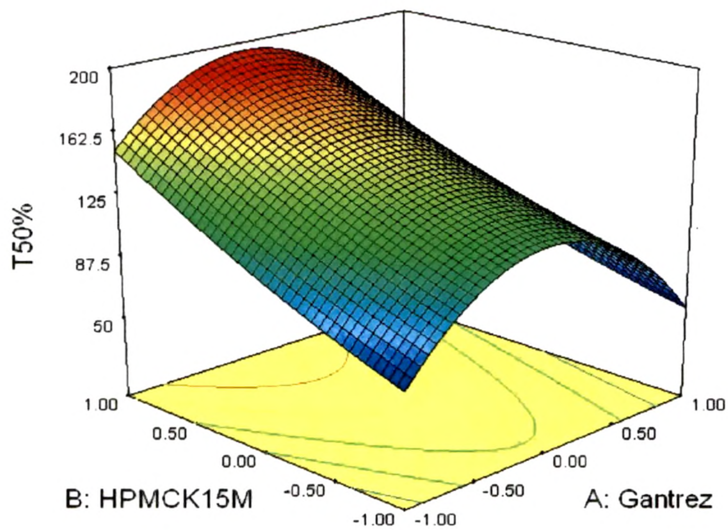

Fig 5.16a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on $t_{50\%}$.

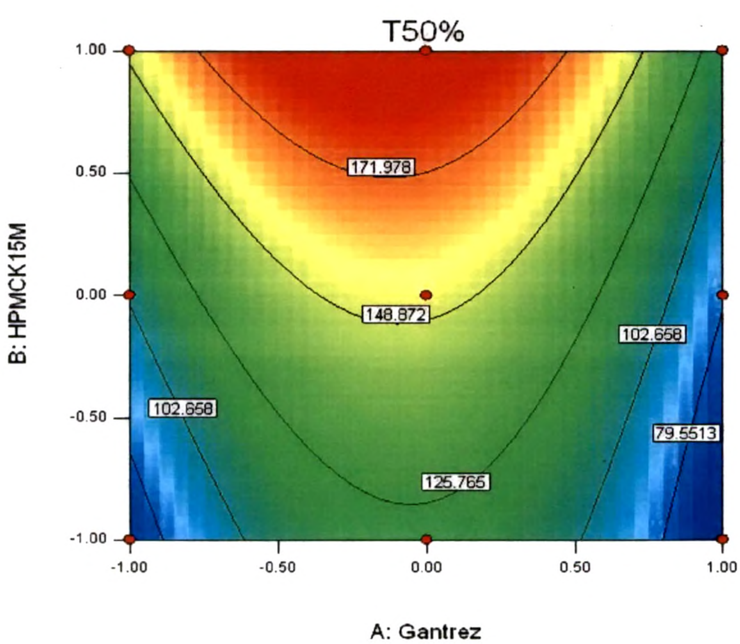

Fig 5.16b: Corresponding contour plot showing the relationship between various levels of both polymers.

Gantrez (X_1) at all the 3 levels of X_2. The contour lines (Fig.5.16b) depict a nonlinear relationship between the factors with region of maximum for $t_{50\%}$. It may be because high level of HPMC K15M will form a highly viscous gel which will increase the diffusion barrier and thereby decrease the release rate and in turn increase the $t_{50\%}$. Nonlinear effect of Gantrez on $t_{50\%}$ could be due to the erosion of the polymer at higher concentrations. Similar results have been reported by Owens et al (2005) where, tablets containing high percentage of Gantrez MS visually exhibited erosion and faster release. Low values of the relative error (Table 5.11) showed that there was a reasonable agreement of predicted values and experimental values indicating the correctness of the model.

5.3.3. (d). Diffusion exponent 'n' (Y_4):

Table 5.10 lists diffusional exponent (n) values calculated by fitting dissolution data to the power law equation (Korsmeyer et al, 1983; Peppas, 1985). In the current study, the values of diffusion exponent (n) ranged between 0.32 and 0.58. Using an aspect ratio of 4.41(calculated from diameter and thickness of tablets), the critical values of n for declaring Fickian diffusion, non-Fickian difusion and zero-order release were found to be 0.45, $0.45 < n < 0.89$ and 0.89, respectively (Peppas, 1985).

The quadratic model for 'n' was found to be significant with an F value of 21.16 ($p=0.0152$). In this case, factors X_1, X_2 along with interaction factor X_1X_2 were found to be significant. Thus, model equation is:

$$Y3\ (n) = +0.5268-0.025*X_1+0.0575*X_2+0.0067*X_1*X_2-0.125*X_1^2+0.00043*X_2^2 \quad \ldots\ldots\ldots\ldots\ldots (5.4)$$

$R^2=0.9724$

Results from equation 5.4 show that both the factors X_1 and X_2 affected the values of release exponent 'n'. The value of correlation coefficient was 0.9724 indicating a good fit.

Response surface (Fig 5.17a) and contour plot (Fig 5.17b) show a "region of maximum" for n, lying between intermediate to high levels of both the polymers. Diffusion exponent ranged from 0.32 to 0.58, which indicates that independent variables had a significant effect on mechanism and kinetics of drug release. Linear relationship was obtained between the fraction of HPMC K 15M and the diffusion exponent, and it was observed that as the fraction of HPMC K15M increased, the value of the diffusion exponent increased, at all 3 levels of Gantrez (Fig 5.17a and 5.17b).

The response values of diffusion exponent (n) varied analogously to $t_{50\%}$ values with respect to variation in the levels of Gantrez. On increasing the amount of Gantrez, nonlinear trend in the response curve was observed i.e. "region of maximum" for n was obtained at intermediate levels of Gantrez (X_1) at all the 3 levels of X_2. The release mechanism changed from Fickian to anomalous (Non Fickian) upto maxima at all 3 levels of X_2 and then again changed to Fickian diffusion on increasing the amount of Gantrez to the maximum level. The above outcome due to dominance of chain disentanglement leads to chain relaxation and subsequent dissolution of polymer (Patel et al, 2006). Low values of the relative error (Table 5.11) showed that there was a reasonable agreement of predicted values and experimental values indicating the correctness of the model.

Regression analysis was carried out to obtain the regression coefficients (Table 5.12). The results of ANOVA in Table 5.13 for the dependent variables demonstrate that the model was significant for all response variables except for Y_2 which was further analyzed by PRESS analysis for appropriate model fitting.

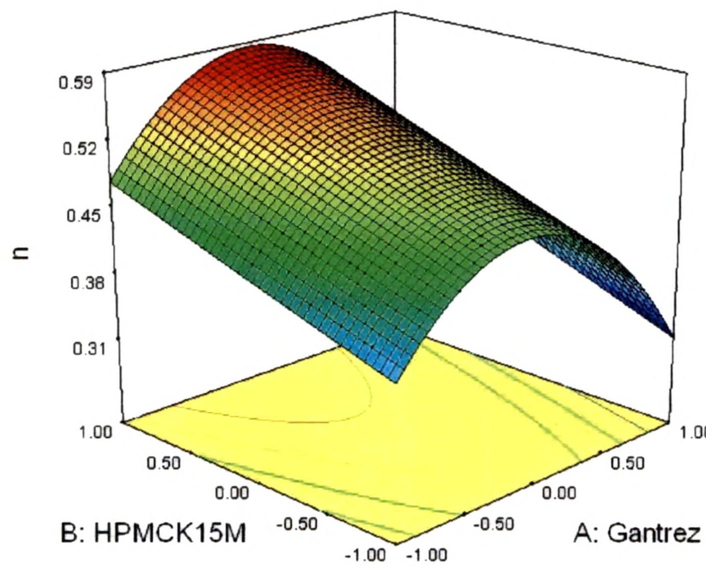

Fig 5.17a: **Response surface plot showing the influence of Gantrez and HPMC K15M polymers on diffusion coefficient (n).**

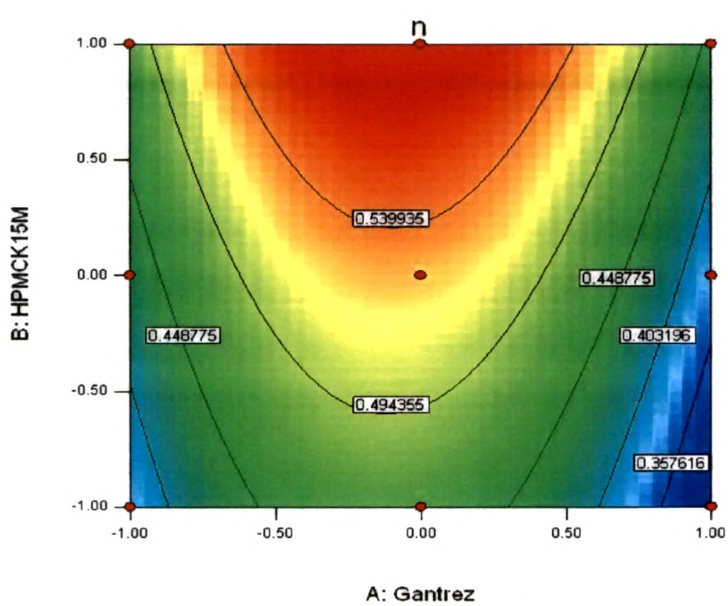

Fig 5.17b: **Corresponding contour plot showing the relationship between various levels of both polymers.**

Table 5.12. Multiple Regression Output for Dependent Variables*

	Coefficient of regression parameters									
Parameters	b_0	b_1	b_2	b_3	**b4**	**b5**	**b6**	**b7**	r^2	***P***
Bioadhesion	10.766	1.975	1.348	-1.11E-016	0.851	0.4175	-0.0965	-0.0485	0.9998	0.0111
Rel_{8hr}	94.92	0.223	-2.77	0.16	4.956	-1.543	--	--	0.7194	0.3839
$t_{50\%}$	152.44	-11.666	36.00	-6.00	-59.66	5.33	--	--	0.9668	0.0199
n	0.5268	-0.025	0.0575	0.0067	-0.1252	0.00043	--	--	0.9724	0.0152

* $t_{50\%}$ indicates the time required for 50% of drug release; **Rel_{8hr}** percentage drug release at 8 hrs; **n** is the release exponent

Table 5.13 Results of Analysis of Variance (ANOVA) for measured responses

Parameters	df	SS	MS	F	Significance F
			$t_{50\%}$		
Model	5	15913.78	3182.76	17.49	0.0199
Residual	3	545.78	181.93	--	--
Total	8	16459	--	--	--
			Rel_{8hr}		
Model	5	100.56	20.11	1.54	0.3839
Residual	3	39.23	13.08	--	--
Total	8	139.79	--	--	--
			n		
Model	5	0.055	0.011	21.16	0.0152
Residual	3	1571E-003	5.236E-004	--	--
Total	8	0.057	--	--	--
			Bioadhesion		
Model	7	34.35	4.91	4792.24	0.0111
Residual	1	0.001	0.001	--	--
Total	8	34.35	--	--	--

*df indicates degrees of freedom; SS sum of square; MS mean sum of square and F is Fischer's ratio.

5.3.4 *Optimum formulation*

A numerical optimization technique by the desirability approach was used to generate the optimum conditions for the formulation. The process was optimized for the dependent (response) variables Y1–Y4 and the optimized formula was arrived by keeping the bioadhesion force >10 dynes/cm^2. The FU release at 8 h was targeted to >90% with release exponent (n)>0.5 and time required for 50% of drug release ($t_{50\%}$) was kept at a range of 150 to 175 min. Formulation FT5 (containing intermediate levels of both variables, X_1 and X_2) fulfilled all the criteria set from desirability search (Narendra et al, 2005). To gainsay the reliability of the response surface model, new optimized formulation (as per formula FT5) was prepared according to the predicted model and evaluated for the responses (Y_1, Y_2, Y_3 and Y_4). The result in Table 5.14 illustrates a good relationship between the experimental and predicted values, which confirms the practicability and validity of the model. The predicted error of all the response variables was below 6% indicating that the Response Surface Methodology (RSM) optimization technique was appropriate for optimizing the FU bioadhesive buccal tablets.

Table 5.14. The predicted and observed response variables of the optimal FU buccal bioadhesive tablets

	Y_1	Y_2	Y_3	Y_4
Predicted	10.77	94.93	0.53	152.44
Observed	10.31±0.89	98.21±1.5	0.55±0.09	161.22±4.6
Predicted Error (%)	-4.27	3.45	3.77	5.75

Predicted Error (%) = (Observed value – Predicted value)/ Predicted value x 100%

5.3.5 *In vitro diffusion of FU from tablets across buccal mucosa:*

The penetration of FU from tablet formulation (FT5) through buccal mucosa (Fig. 5.18) was found very low (Kp=1.750 (±0.67) x10^{-5} cm/sec) when compared with that of FU solution (0.799 (±0.31) x 10^{-4} cm/sec) (Fig.5.1a; Table 5.1) and the difference was statistically significant ($p < 0.05$). This could be attributed to the fact that the complexity of the composition of the mucosa offered more resistance to the penetrating drug molecules during the diffusion process.

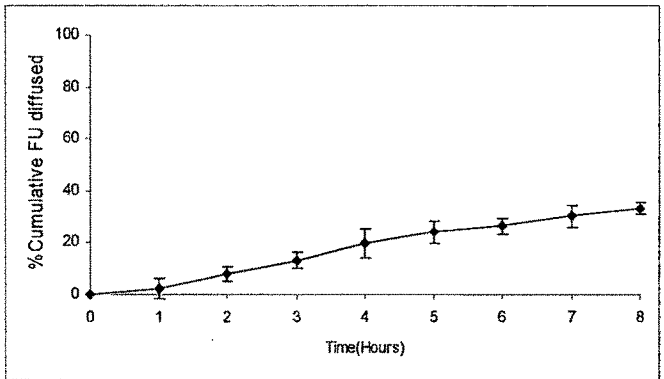

Fig. 5.18. ***In vitro*** **diffusion of FU from tablets across buccal mucosal membrane.** Data are expressed as mean ± S.E.M. for *n*=3.

The formulation (FT5) was prepared further using STGC as a permeation enhancer (0.05M) for *in vivo* studies (Section 5.1). The bioadhesive strength of these tablets was 9.92±0.95 dynes/cm^2 while hardness was 4.6 ± 0.2 kg/cm^2. No significant difference in adhesive bond strength and hardness was observed between STGC containing and STGC free tablets. Addition of STGC to tablet formulations resulted in an increase in release rate (Fig5.19). 51.12 ± 4.6%, 72.58 ± 5.9 % and 102.13± 3.7% FU was released from FU tablet formulations containing STGC as compared to 45.61 ± 4.15%, 64.48 ± 3.5% and 96.28 ± 4.8% from STGC free tablets after 2, 4 and 8 hours respectively. These results are in agreement with those given in literature (Ikinci, G. et al, 2000).

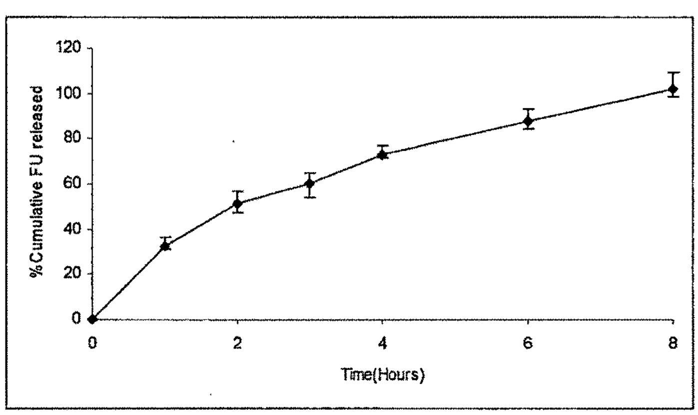

Fig. 5.19. ***In vitro*** **release profile of FU from buccoadhesive tablets containing STGC in phosphate buffer (pH 6.8). (Mean±SEM; n=3).**

5.3.6. *Investigations of drug–excipient interactions*

DSC thermogram gives information regarding the physical properties like crystalline or amorphous nature of the samples (Clas et al, 1999). The DSC thermogram of FU shows an endothermic peak at 283.5°C corresponding to its melting temperature, which was also detected at 281°C in the thermogram of the physical mixture of tablet components (FU, Gantrez, HPMC, Tablettose-100, STGC, Magnesium stearate in 1:2:2:2:1:0.1 proportion) signifying no interaction between FU and other tablet components (Fig. 5.20).

FTIR spectra of FU showed (Fig. 5.21) sharp characteristic peaks at 812 (CH out of plane), 1243 (CH in plane), 1425, 1655 and 1718 (C=O, C=N- stretch), and 3122 cm–1 (NH stretch). All the above characteristic peaks appeared in the spectra of the physical mixture of tablet components at same wavenumber indicating no modification or interaction between the drug and polymers.

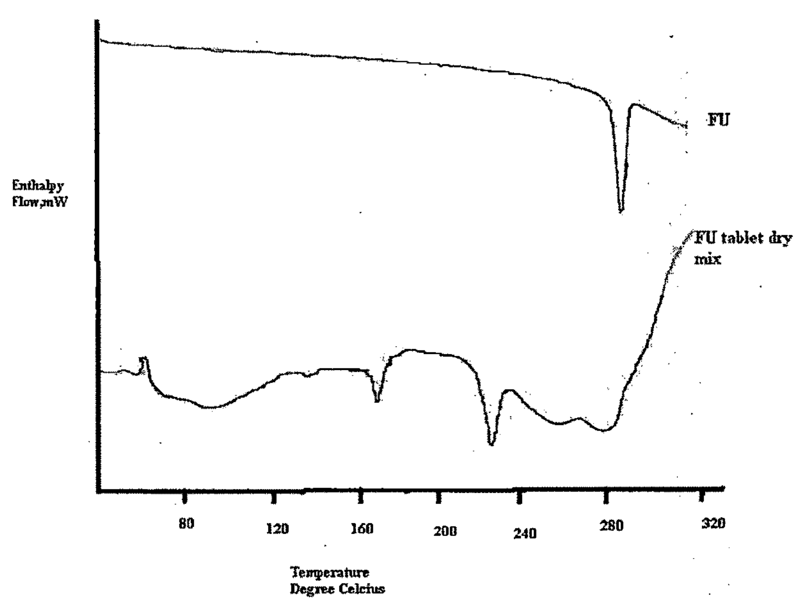

Fig 5.20 DSC thermograms of FU and FU tablet dry mixture.

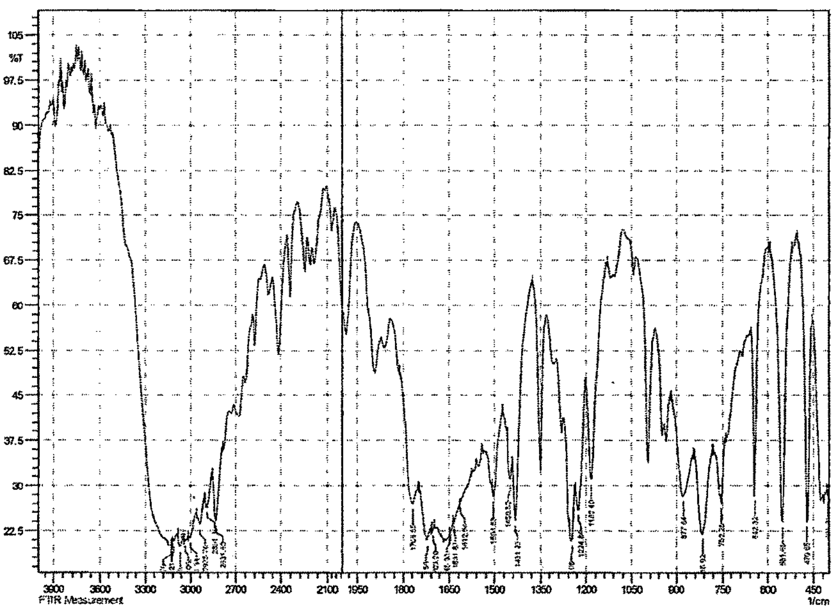

Fig. 5.21a.

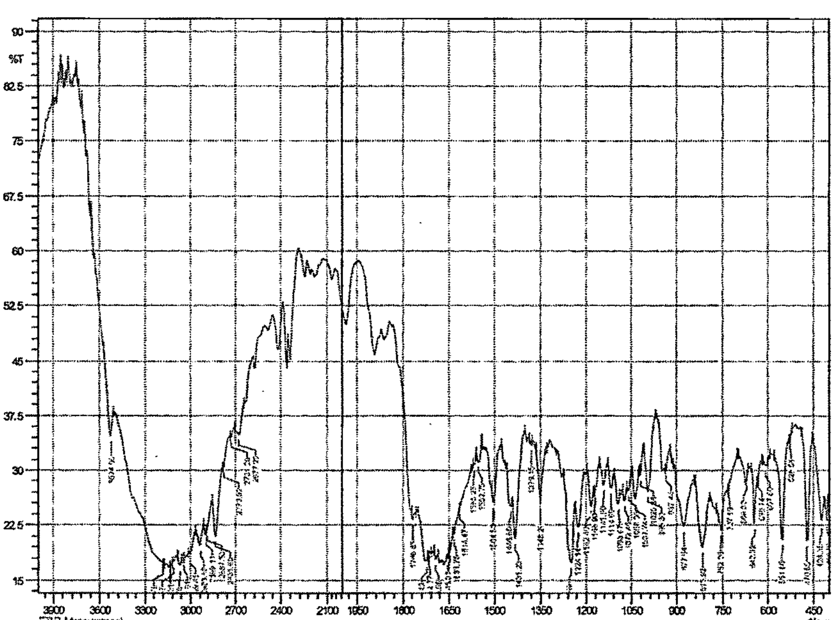

Fig. 5.21 b.

Fig. 5.21. **FTIR spectra of FU (a) and FU tablet dry mixture (b).**

5.3.7 *In vivo bioadhesion test:*

The γ- scintigraphic images of the rabbits taken after 4 h post administration of buccoadhesive tablets, showed the presence of tablets in the same place in buccal cavity (Fig.5.22) indicating that the tablets prepared using optimized combination of HPMC and Gantrez remained adhered to the buccal cavity at least for 4 hours.

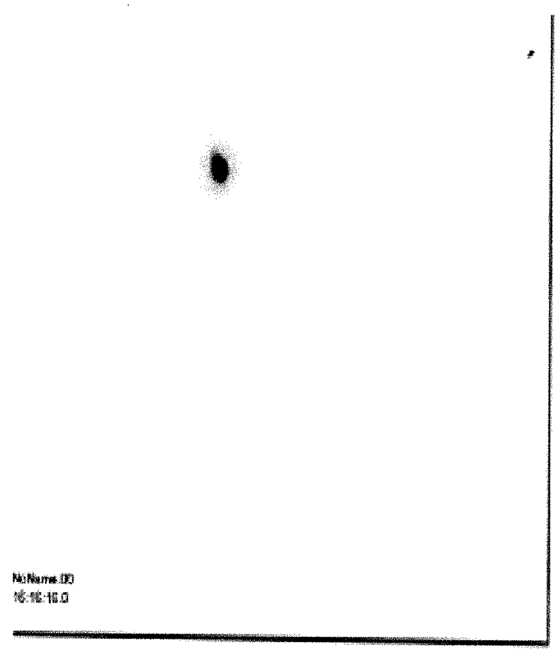

Fig. 5.22 Scintigraphic Image showing bioadhesive tablet in buccal cavity of rabbit

5.3.8 *Stability study of FU bioadhesive tablets:*

FU bioadhesive tablets depicted good stability. No physical changes were observed during storage. Drug content, hardness, friability and release values of the formulations carried out at 3 months showed no significant differences (*$p>0.05$*) (Table 5.15) indicating a stable formulation.

Table 5.15. Stability results for FU bioadhesive tablets (n=3)

Parameter	Initial	3 Months	3 Months
		30 ± 2°C/ 65% ± 5% RH	40 ± 2°C/ 75% ± 5% RH
Hardness (kg/cm^2)	4.6(±0.20)	4.6 (±0.18)	4.42(±0.14)
Friability (%)	0.25	0.32	0.18
Assay(%w/w)	99.74 (± 0.225)	98.75 (±0.164)	97.25 (± 0.095)
%Cumulative Release (8 h)	102.13(± 3.7)	98.14 (± 6.15)	99.65 (± 5.64)

5.4. FORMULATION OF FU BUCCOADHESIVE GELS

5.4.1 *Preparation of gel formulations of FU*

Bioadhesive gels containing FU were formulated using Poloxamer- 407, HPMC K15M and Gantrez S 97 in various combinations. All formulated gels were found to be viscous and clear indicating that drug remained completely dissolved in the gel.

5.4.2. *Evaluation of Gel formulations:*

5.4.2.1. Bioadhesive Force

Assessment of the bioadhesive strength in terms of detachment force is shown in **Fig 5.23**. The detachment force of only Poloxamer- 407 gels (B1) was less than that of Poloxamer- 407 and HPMC containing formulations (B2-B5). An increasing trend in the bioadhesive strength with increase in the amount of HPMC content (B2, B3, B4 and B5) was observed, but the increase was not statistically significant ($p > 0.05$). However, detachment force increased significantly ($p < 0.001$) when Gantrez S-97 was added to the gels (B6-B11). Maximum bioadhesive force (18.33±2.6 x 10^3 dyne/cm^2) was found in the formulation B 11, containing 4% Gantrez. Thus, though incorporation of HPMC to poloxamer gels improved its bioadhesion strength by 3.5 times (2.18± 0.77 x 10^3 dyne/cm^2; B1 and 7.80 ± 1.2 x 10^3 dyne/cm^2; B5), incorporation of GantrezS-97 improved adhesion by 8 times (18.33±2.6 x 10^3 dyne/cm^2; B11).

The significant enhancement ($p < 0.05$) in bioadhesion due to Gantrez can be attributed to its carboxylic groups that gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus membrane. Thus, Gantrez having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. In addition, Gantrez may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of the delivery system may lead to the prolonged retention and increased absorption across mucosal tissues. HPMC contains neutral cellulose groups and hence has got less affinity for mucin glycoproteins (Yong et.al, 2001).

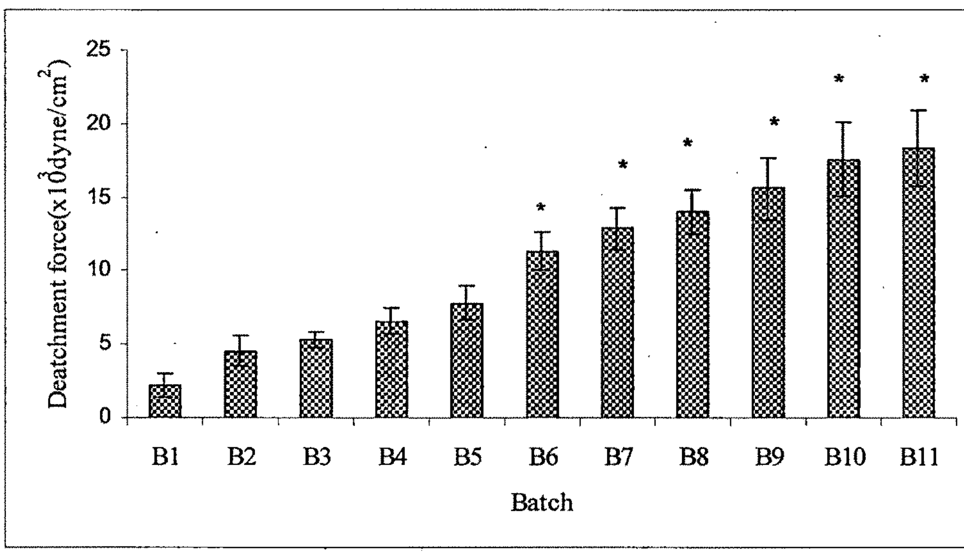

Fig 5.23. The bioadhesive strength between the mucosa and FU gels.
Values are expressed as mean ± S.E.M. (n = 3).
*p<0.001versus B1.

5.4.2.2 Assay

All the formulations were found to exhibit assay values in the range 95-105 % depicting good content uniformity (Table 5.15).

5.4.2.3 pH measurements

Table 5.16 displays pH value of the formulations. The pH was measured in each gel and found to be within the satisfactory limit of 5.5 – 7.5 which implies that the gel would not cause irritation in the buccal cavity.

Table 5.16. pH and Assay of FU bioadhesive gel formulations

Batch No	pH	Assay (%)
B1	5.76 (±0.061)	98.24 (±0.146)
B2	5.82 (±0.051)	99.75 (±0.099)
B3	6.10 (±0.024)	98.75 (±0.164)
B4	6 .39 (±0.046)	98.24 (± 0.218)
B5	6.18 (±0.026)	98.75 (±0.099)
B6	6.04 (±0.082)	99.03 (±0.103)
B7	6.12 (±0.028)	99.74 (± 0.225)
B8	6.22 (±0.014)	98.51 (±0.208)
B9	6.47 (±0.022)	99.43 (±0.198)
B10	6.25 (±0.011)	98.33 (±0.152)
B11	5.98 (±0.033)	97.25 (± 0.095)

(±SEM): Standard Error Mean.
Note: Each reading is an average of three determinations.

5.4.2.4 Viscosity

The apparent viscosity of the Formulation B7 at 40 RPM (at 25±1°C) was found to be 544.49 ± 5.9 cps. However, when it was subjected to variable shearing stress, the behavior indicated plastic flow (Fig. 5.24). Shear rate was found to reduce the viscosity initially after which it had no pronounced effect on the viscosity of the gel, producing a linear curve after the yield value – a characteristic of a plastic system (Fig 5.24 insert). This means that consistency is imparted to the gel due to three dimensional structures of the polymers. Upon shearing, once the gel structure is disturbed, the polymer chains will align in the direction of stress. Hence, the resistance to flow decreases after the shearing rate sufficient to disrupt the gel structure is applied and thereafter it behaves like a Newtonian system. The extrapolation of the graph in Fig 5.24 gives yield value (390.01 dynes/cm^2) which is used to find plastic viscosity. The plastic viscosity of the gel was calculated to be 464 cps by using the following formula. (Martin, 1999).

U= (F-f)/G;

U= Plastic viscosity,
F= Shearing stress
f = Yield value, or the intercept on shear stress axis (dynes/cm^2)

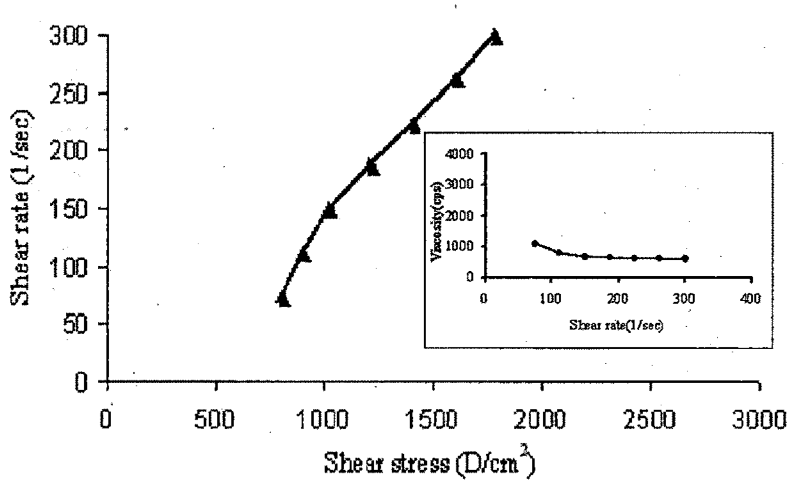

Fig. 5.24. Effect of Shear rate on shear stress.
The insert represents the effect of shear rate on viscosity of gel.

5.4.2.5. *In vitro* diffusion study

5.4.2.5 (a). *In vitro* diffusion across cellulosic membrane

The *in vitro* release profiles from various formulations are depicted in Fig 5.25a, 5.25b and 5.25c. Batch B1 contained only FU and Poloxamer-407 while B2, B3 and B4 were prepared with Poloxamer- 407 at 16%w/w concentration and varying proportions of HPMC (1%, 1.5%, 2 %). Formulations B5-B8 contained 17% Poloxamer-407 and 2% of HPMC while B9-B11 contained 18% Poloxamer-407 and 2% of HPMC. Increase in HPMC content from 1 to 2%, resulted in retardation of release (Fig 5.25a). But in all these batches (B2-B4), drug release was accompanied by burst release and 50% of the drug was released in first 2 hours, so the amount of Poloxamer-407 was increased from 16% to 17%. Batches with higher Poloxamer- 407 composition displayed lower burst in release than those batches with its low content. In B5, the burst release at first hour was reduced to 32.02±2.31% and after 7 hours, 100% of FU was released. In B6, 2 % Gantrez S - 97 was added as bioadhesive polymer, which was found to affect the release. As the content of Gantrez was increased, decrease in release was evident from the release profile data (Fig. 5.25b). Increasing the concentration of Poloxamer -407 from 17% to 18% (Fig 5.25c) significantly decreased the cumulative percent drug released after 8 h from 98% (B7) to 69% (B 11) ($p<0.05$).

The Poloxamer system is one of the swelling-controlled systems that functions by a process of continuous swelling of the polymer carrier. Drug diffusion from a Poloxamer gel is through extramicellar aqueous channels. The microviscosity of the water channels controls drug diffusion and release by this mechanism. The decrease in the rate of release with an increase in the Poloxamer 407 concentration could be attributed to the increase in the number and size of the micelles formed at higher polymer concentration. This could cause a greater tortuosity in the aqueous phase of the gel structure and a slower rate of drug release (Gilbert et al 1986). In addition, higher Poloxamer concentrations results in a shorter inter-micellar distance, leading to greater number of cross-links between neighboring micelles and a greater number of micelles per unit volume (Bhardwaj and Blanchard, 1996). Combining it with another inert, hydrophilic polymer would decrease the amount of free water in the water channels, and affect molecular orientation of the gel matrix. Thus both HPMC and Gantrez decreased the rate of FU release from the gel. Similar results have also been obtained in a previous study, where inclusion of PVP, CMC, and HPMC in the Poloxamer -407 gel decreased the rate of release of ceftiofur (Zhang et al 2002). Formulation B 7 was considered for further studies as it gave complete release of FU (98.6 ± 1.88%) in 8 hours.

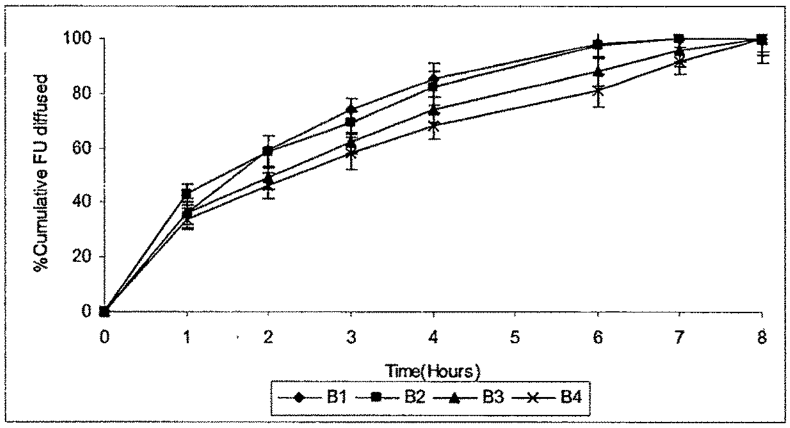

Fig.5.25a. ***In vitro* diffusion of FU across cellulose membrane.**
Data are expressed as mean ± S.E.M. for *n=3*.

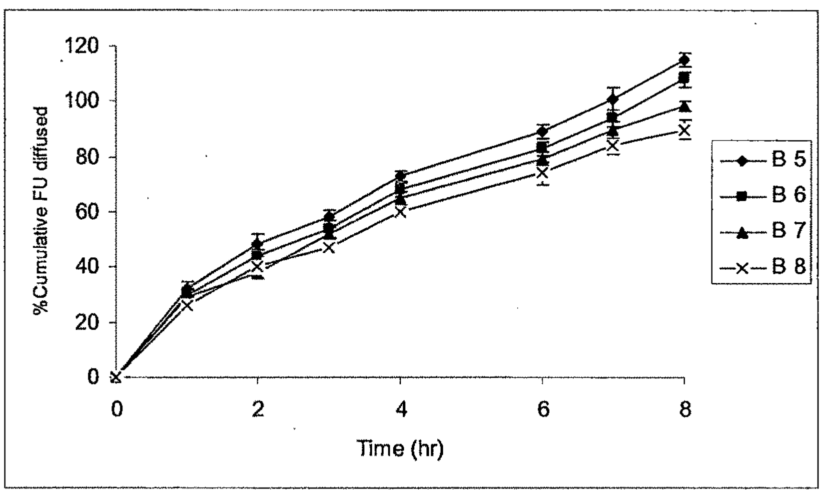

Fig. 5.25b. ***In vitro* diffusion of FU across a cellulose membrane.**
Data are expressed as mean ± S.E.M. for *n=3*.

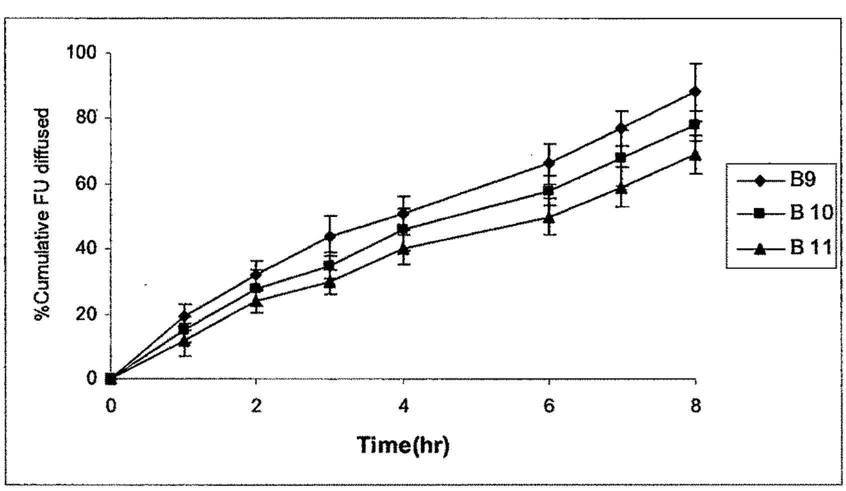

Fig.5.25c ***In vitro* diffusion of FU across a cellulose membrane.**
Data are expressed as mean ± S.E.M. for *n=3*.

5.4.2.5. (b). Drug release kinetics

Table 5.17. enlists the regression parameters obtained after fitting various release kinetic models to the *in vitro* dissolution data. The rate constants were calculated from the slope of the respective plots.

The goodness of fit for various models investigated for gel formulations ranked in the order of Higuchi $\approx$ Peppas > zero order > first-order. All the kinetic models, other than the first order, fitted well. The values of diffusional exponent 'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.4486±0.025 and 0.7244±0.060. Using an aspect ratio (width/thickness) of 10.52, the critical values of n for declaring Fickian diffusion and zero-order release were found to be 0.5 and 1.0, respectively. Only three formulations B2, B3 and B4 tended to exhibit Fickian diffusional characteristics, as the corresponding values of 'n' were lower than the standard value for declaring Fickian release behaviour, i.e., 0.5. Rest of the formulations B5 - B11 has 'n' value in the range 0.5 - 1.0 indicating anomalous (non-Fickian diffusion). Statistically significant ($p<0.05$) differences in 'n' values were found between B2 vs B9, B3 vs B9, B4 vs B9 on application of ANOVA followed by Newman-Keuls Multiple Comparison Test.

The non-Fickian behaviour (n=0.5-1.0) of the formulations may be attributed due to the gelling property of Poloxamer-407 (as it is present in higher proportions in B5-B11 than in B2-B4). For B7 (the optimized batch), value of 'n' was observed to be 0.5973 ($r^2 = 0.9856$) and other kinetic parameters were Higuchi ($r^2 = 0.988$), Zero order ($r^2 = 0.983$), First order ($r^2 = 0.9457$). Also, the formulations were observed to yield statistically valid correlations with the Higuchi and Zero-order models too (Table 5.17.). The results point out the prevalence of diffusional mechanistic phenomena, in consonance with the results obtained while fitting Korsemeyer–Peppas Model (Korsemeyer et al., 1983). According to the model developed by Higuchi, this harmony among kinetics is seen in case of homogenous distribution of the active agent in the formulations (Tas et. al, 2004).

Table 5.17. Release Kinetics of 5-Fluorouracil containing bioadhesive gels

Batch No.	Zero order		First order		Higuchi		Peppas	
	r^2	k_0	r^2	k_1	r^2	k_h	r^2	n**
B2	0.9914	10.42	0.9483	0.062	0.9969	38.89	0.9988	0.4486±0.025
B3	0.9790	9.81	0.9273	0.0671	0.9974	36.85	0.9936	0.4896±0.010
B4	0.9867	9.28	0.9398	0.0672	0.9949	34.72	0.9976	0.4879±0.011
B5	0.9880	11.09	0.9332	0.077	0.9940	41.41	0.9960	0.5606±0.030
B6	0.9900	10.36	0.9375	0.0771	0.9942	38.65	0.9946	0.5987±0.050
B7	0.9830	10.06	0.9457	0.0788	0.9880	37.44	0.9856	0.5973±0.065
B8	0.9899	9.31	0.9351	0.0781	0.9922	34.68	0.9958	0.5896±0.040
B9	0.9878	8.40	0.9009	0.098	0.9929	31.37	0.9909	0.7244±0.060
B10	0.9944	9.23	0.9313	0.0921	0.9891	34.27	0.9981	0.6724±0.050
B11	0.9974	10.23	0.9711	0.089	0.9781	37.69	0.9859	0.6322±0.080

r^2 = Correlation Coefficient ; n = Diffusion Coefficient (± SEM)

#k_0, release rate constant of zero order kinetic,

#k_1, release rate constant of first order kinetic,

#k_h, release rate constant of Higuchi's square root of time

**ANOVA followed by Newman-Keuls Multiple Comparison Test ;($P<0.05$)

B2 Vs B9, B3 Vs B9, B4 Vs B9.

Note: Each reading is an average of three determinations.

5.4.3 *Effect of formulation factors on in-vitro release of FU from P407 gel*

5.4.3.1 Effect of temperature on drug release from the gel formulation: As poloxamer-407 gels are thermoreversible, it is expected that their viscosity will increase upon application in the buccal mucosa, which might alter its release. Hence, the effect of temperature on FU release at room temperature (25 °C) and 37 °C was studied using B7 gel formulation as shown in Fig. 5.26. It can be seen that at room temperature the cumulative percentage of FU released after 6 h was less (68.54±6.1%) as compared to 79.09±4.1 % at 37 °C. However, statistical comparisons indicated that there was no significant difference ($p>0.05$) in the drug release profiles. The Poloxamer-407 containing gels are considered to consist of micelles and aqueous channels. The incorporated solute is directly available for release from aqueous channels. An increase in temperature increased the release rate of drug despite an increase in bulk viscosity (Zhang et al, 2002). This small increase in release rate is likely due to increase in drug diffusion at higher temperature.

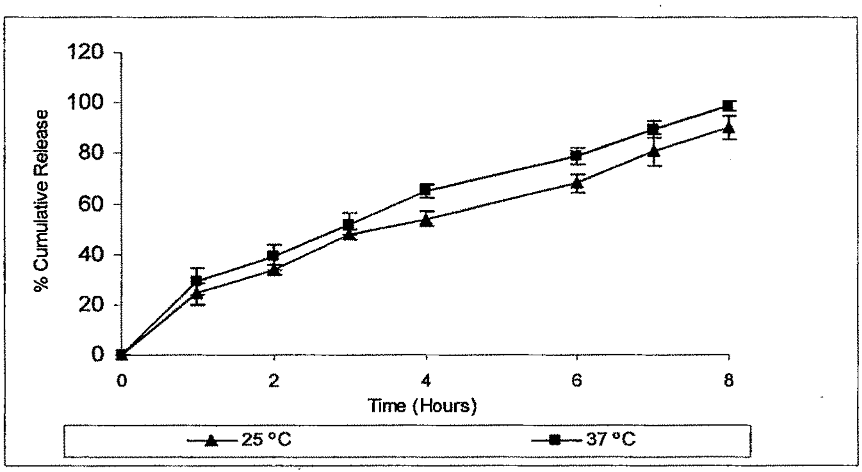

Fig. 5.26. Effect of temperature on drug release from the gel formulation. Data are expressed as mean ± S.E.M. for n=3.

5.4.3.2. Effect of pH on drug release from the gel formulation

The effect of pH on FU release was studied at release medium pH values of 5.5, 6.8, and 7.4 using B7 gel (Fig. 5.27). Cumulative percentage of FU released after 6 h was 86.51 ± 3.28% at pH 5.5, 77.09 ± 2.04% at pH 6.8 and 82.03 ± 4.10 % at pH 7.4 respectively. Thus, the results showed that there was no significant difference (*p>0.05*) due to pH in the release profiles of FU from the gels between pH 5.5, 6.8 and 7.4.

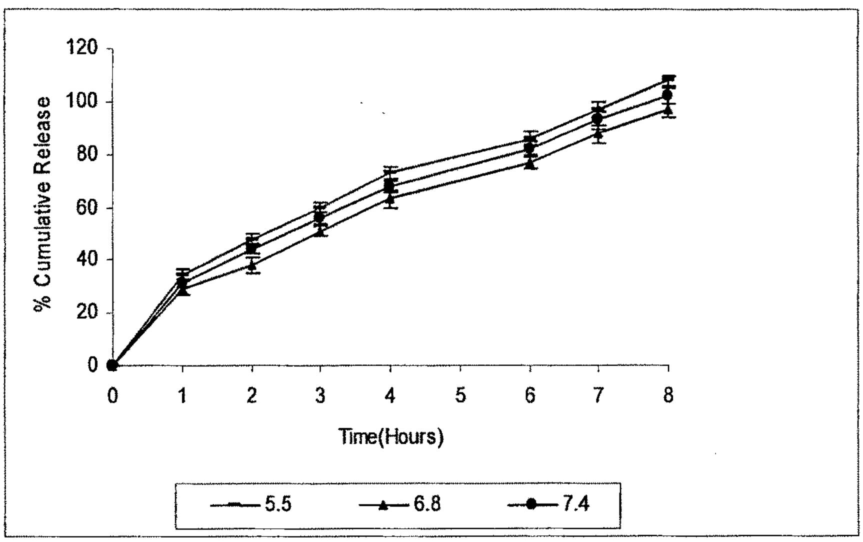

Fig. 5.27. Effect of pH on drug release from the gel formulation.
Data are expressed as mean ± S.E.M. for *n*=3.

5.4.3.3. *In vitro* diffusion across buccal mucosal membrane:

The permeability coefficients (Kp) of gel across cellulose membrane and buccal mucosal membrane were 1.06x 10^{-4}cm/sec and 3.94x10^{-5} cm/sec respectively.

The penetration of FU from gel formulation (B7) through buccal mucosa (Fig. 5.28) was found very low when compared with cellulose membrane (Fig. 5.25b) and the difference was statistically significant (*p < 0.05*). This could be attributed to the fact that the complexity of the composition of the mucosa offered more resistance to the penetrating drug molecules during the diffusion process.

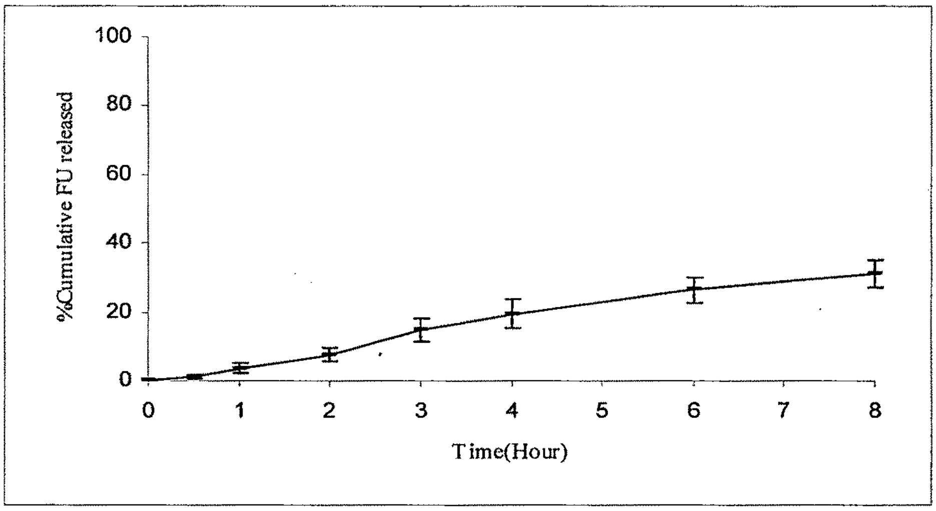

Fig. 5.28 ***In vitro* diffusion of FU from gels across buccal mucosal membrane.** Data are expressed as mean ± S.E.M. for *n*=3.

The final formulation (B 7) selected on the basis of *in vitro* release study and other characterization parameters was prepared further using STGC (0.05M) as a permeation enhancer (as discussed in section 5.1.) for *in vivo* studies. The bioadhesive strength of the gel formulation was 12.62±0.85 dynes/cm^2. No significant difference in adhesive bond strength was observed between STGC containing and STGC free gels.

Addition of STGC to gel formulations resulted in an increase in release rate. 43.2 ± 2.5 %, 71.45 ±3.7 % and 103.21 ±3.9 % FU was released from FU gel formulations containing STGC (Fig.5.29) as compared to 38.01 ± 4.15%, 65.12 ± 3.5% and 98.6 ± 4.8% after 2, 4 and 8 hours, respectively.

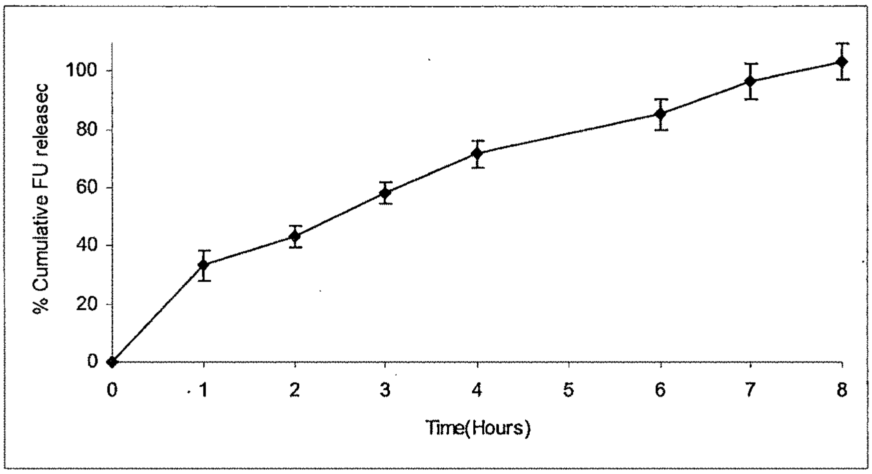

Fig. 5.29. ***In vitro* diffusion of FU from gel containing STGC across cellulose membrane.**

Data are expressed as mean ± S.E.M. for n=3.

5.4.4 Drug: Polymer compatibility studies:

5.4.4.1 Fourier Transform Infrared (FTIR) Spectroscopy

IR spectra of FU, its physical mixture with various polymers and solid dispersions with polymers are presented in Fig 5.30. Pure FU spectra showed sharp characteristic peaks at 812 (CH out of plane), 1243 (CH in plane), 1425, 1655 and 1718 (C=O, C=N- stretch), and 3122 cm^{-1} (NH stretch). All the above characteristic peaks appeared in the spectra of all physical mixtures and solid dispersions at same wavenumber indicating no modification or interaction between the drug and polymers. As the solid dispersions were prepared by coprecipitation technique from a common solvent, it can be reasonably expected that FU would be compatible with all these polymers when formulated in a gel (Shin et al, 2000b).

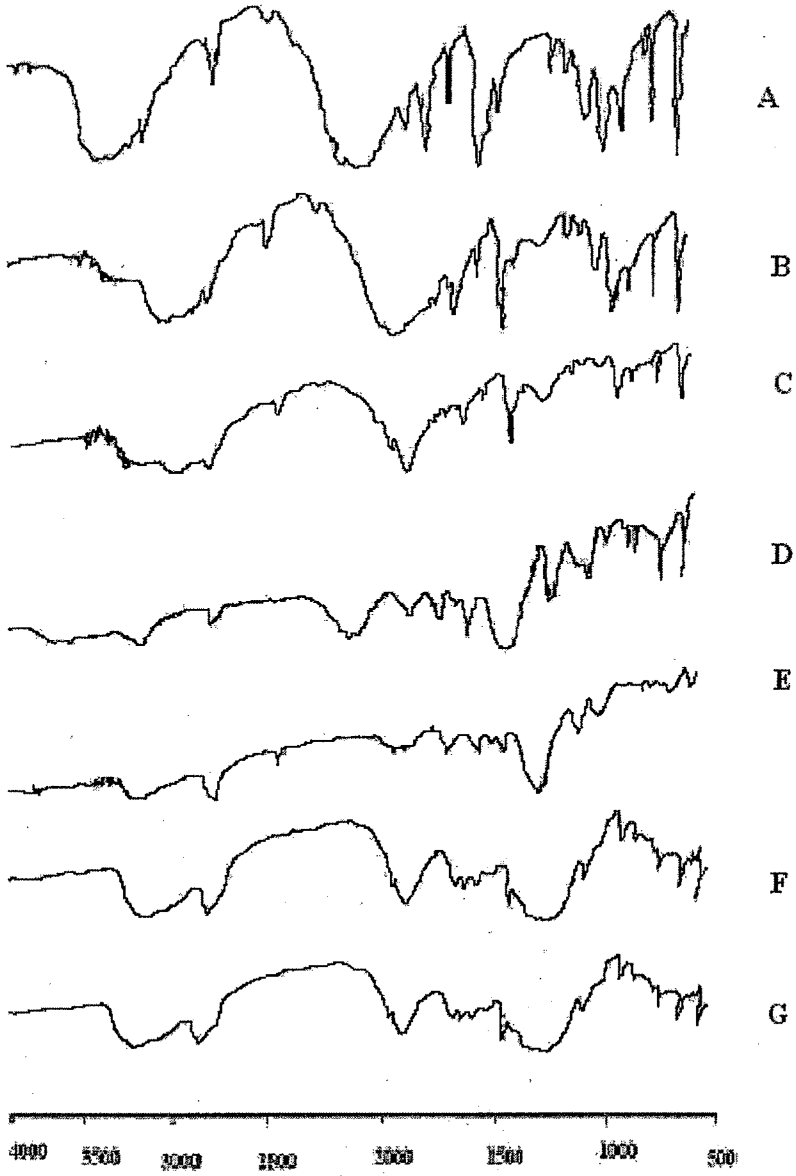

Fig.5.30. IR spectra of test materials;
(A) FU, (B) FU- Gantrez S 97 Physical mixture (1:4), (C) FU- Gantrez S 97 solid dispersion (1:4), (D) FU-Poloxamer 407 physical micture (1:4), (E) FU - Poloxamer 407 solid dispersions (1:4), (F) FU- HPMC K15M Physical mixture (1:4), (G) FU- HPMC K 15M Solid dispersion (1:4).
The x-axis represents wavenumber (cm^{-1}). The graphs were overlapped vertically for better visualization.

5.4.4.2. X ray Diffraction:

X-ray diffraction (XRD) spectra of pure compound and binary systems with carriers are presented in Fig 5.31. The X-ray diffractogram of FU has sharp peaks at diffraction angles (2θ) 16.16°, 18.92°, 20.51°, 21.78°, and 28.49° showing a typical crystalline pattern. All major characteristic crystalline peaks also appeared in the diffractogram of both physical mixtures and solid dispersions. Moreover, the relative intensity and 2θ angle of these peaks remained unchanged (Table 5.18), clearly suggesting that there is no amorphization of FU and it is still in its original crystalline form.

Table 5.18. Characteristic XRD peaks of FU and its physical mixture and solid dispersions with polymers

S.No.	Formulation	Characteristic peaks at 2θ (degrees) *Intensity in Parentheses
1	FU	16.16°(21.32),18.92°(12.69),20.51°(11.79),21.78°(13.35), and 28.49°(100.00)
2	FU- Gantrez S 97 Physical mixture (1:4)	16.20°(1.86), 18.96°(1.53), 20.54°(1.84), 21.78°(2.28), and 28.55°(100.00)
3	FU- Gantrez S 97 solid dispersion (1:4)	16.20°(3.94), 18.96°(4.30), 20.55°(3.43), 21.79°(3.08), and 28.57°(100.00)
4	FU-Poloxamer 407 physical mixture (1:4)	16.18°(7.29), 19.01°(100), 20.46°(13.89), 22.05°(3.35), and 28.41°(53.21)
5	FU- Poloxamer 407 solid dispersions (1:4)	16.18°(3.58), 19.02°(31.76), 20.52°(3.99), 21.78°(5.36), and 28.54°(100.00)
6	FU- HPMC K15M Physical mixture(1:4)	16.16°(4.47),18.94°(8.67),20.47°(9.72),22.34°(7.49)and 28.54°(100.00)
7	FU- HPMC K 15M Solid dispersion (1:4).	16.16°(4.47),18.94°(8.67),20.47°(9.72),22.34°(7.49)and 28.54°(100.00)

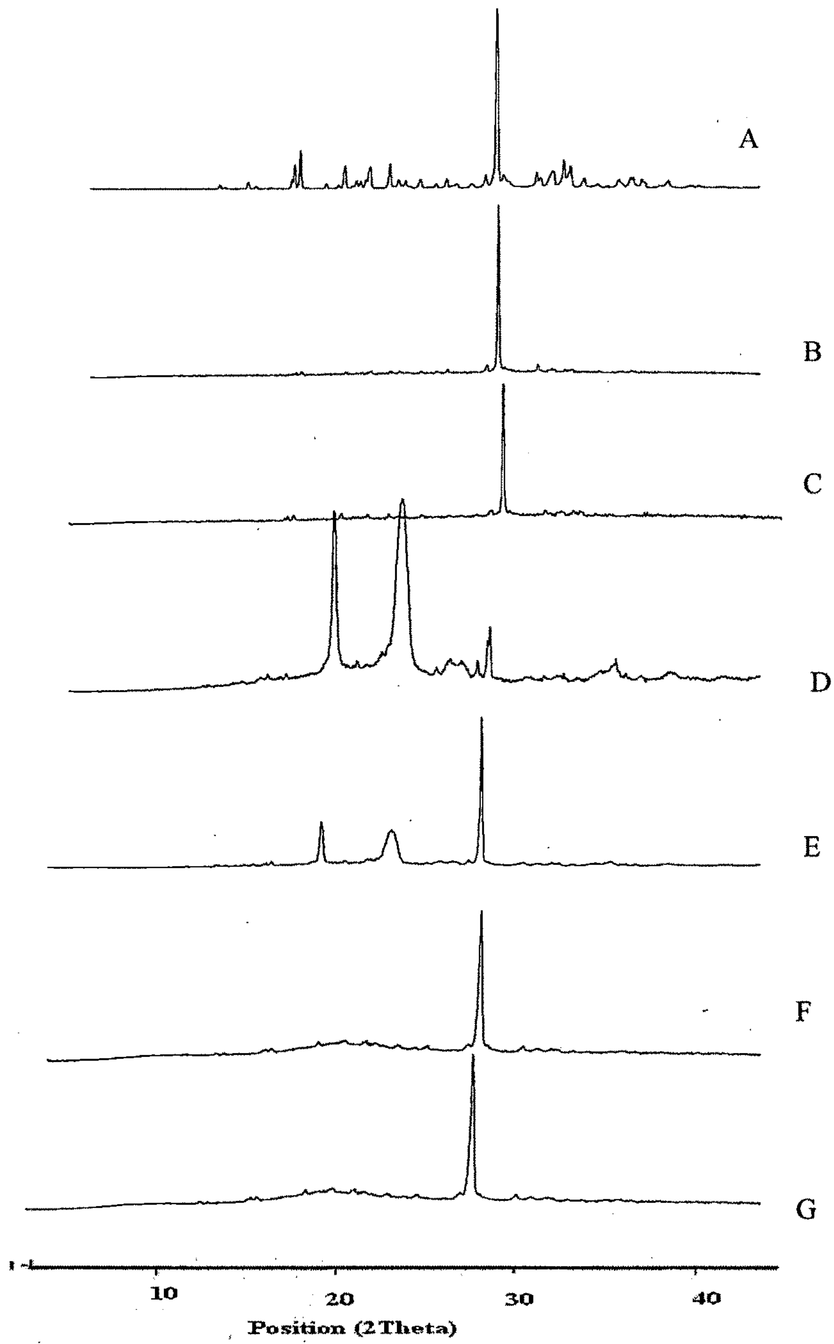

Fig.5.31. X-ray diffractograms of test materials;
(A) FU, (B) FU- Gantrez S 97 Physical mixture (1:4), (C) FU- Gantrez S 97 solid dispersion (1:4), (D) FU-Poloxamer 407 physical micture (1:4), (E) FU-Poloxamer 407 solid dispersions (1:4), (F) FU- HPMC K15M Physical mixture (1:4), (G) FU- HPMC K 15M Solid dispersion (1:4).
The graphs were overlapped vertically for better visualization.

5.4.4.3 .Differential Scanning Calorimetry (DSC)

DSC thermogram of FU, its physical mixture with various polymers and solid dispersions are presented in Fig 5.32. The DSC thermogram of FU (Fig. 5.32a) shows an endothermic peak at 283.5°C corresponding to its melting temperature, which was also detected in physical mixtures and solid dispersions of FU with various polymers (Gantrez® S – 97, HPMC K15M and Poloxamer-407) at the same temperature (Table 5.19). This indicated absence of any kind of interaction between drug and polymers.

Table 5.19. Characteristic DSC peaks of FU and its physical mixture and solid dispersions with polymers

S.No.	Formulation	Characteristic peaks (°C)
1	FU	283.54
2	FU- Gantrez S 97 Physical mixture (1:4)	285.27
3	FU- Gantrez S 97 solid dispersion (1:4)	282.77
4	FU-Poloxamer 407 physical mixture (1:4)	280.50
5	FU- Poloxamer 407 solid dispersions (1:4)	282.77
6	FU- HPMC K15M Physical mixture(1:4)	284.55
7	FU- HPMC K 15M Solid dispersion (1:4).	286.13

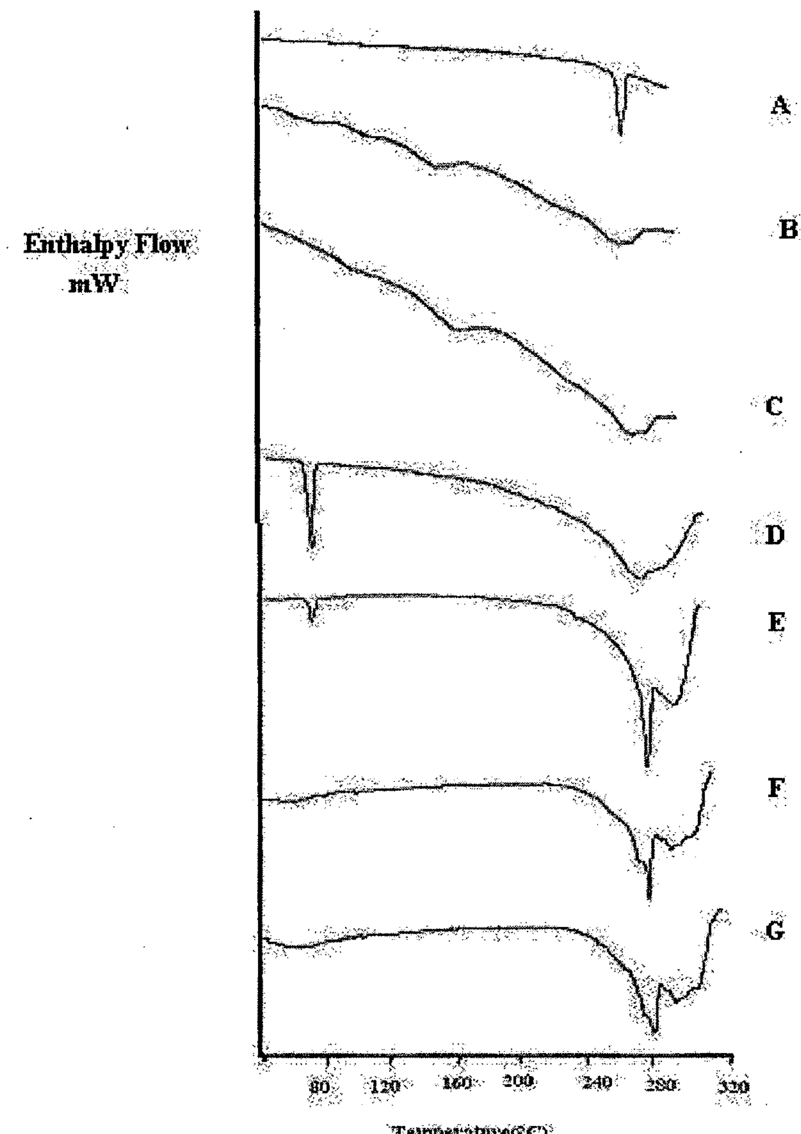

Fig 5.32. DSC thermograms of test materials;
(A) FU, (B) FU- Gantrez S 97 Physical mixture (1:4), (C) FU- Gantrez S 97 solid dispersion (1:4), (D) FU-Poloxamer 407 physical micture (1:4), (E) FU-Poloxamer 407 solid dispersions (1:4), (F) FU- HPMC K15M Physical mixture (1:4), (G) FU- HPMC K 15M Solid dispersion (1:4)
The graphs were overlapped vertically for better visualization.

5.4.5 *In vivo bioadhesion:*

The γ- scintigraphic images of the rabbits taken after 4 h post administration of buccoadhesive gels, showed the presence of gels in the buccal cavity (Fig.5.33). Though there was ingestion of the gel observed in the images, but the drug ingested in such a way will probably be available for local action at the oropharynx. It can be seen that major portion of the gel remains in the buccal cavity only.

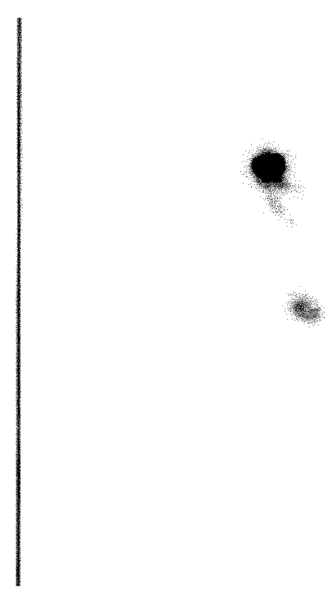

Fig.5.33 Scintigraphic Image showing bioadhesive gel in buccal cavity of rabbit

5.4.6. *Stability studies of bioadhesive gels:*

Table 5.20 depicts results of stability study of FU bioadhesive gels. No macroscopical physical changes were observed during storage. Drug content, pH, and release values of the formulations carried out at 3 months showed no significant differences. Hence, FU bioadhesive gels presented good stability.

Table 5.20: Stability results of FU bioadhesive gels

Parameter	Initial	2 Months	3 Months
		25 ± 2°C	25 ± 2°C
pH	6.12 (±0.028)	6.08(±0.112)	6.23(±0.217)
Assay(%w/w)	99.74 (± 0.225)	99.05 (±0.201)	97.05 (± 0.095)
Release (8 h)	103.21(±3.78)	99.14(± 6.15)	98.65(± 5.64)

5.4.7. Histology studies on buccal mucosa:

Fig 5.34a shows photo micrograph of buccal mucosa depicting the outermost layer of stratified squamous epithelium, below which can be seen the basement membrane, lamina propria and submucosa. The boundary between the buccal epithelium and the connective tissue is delineated by the basement membrane.

Typical photo micrographs of buccal epithelium after 8 hour treatment with different formulations of FU (patch, tablet and gel) containing 0.05M STGC are shown in Fig 5.34 b, c and d where epithelial layer is intact. No significant loss of superficial cell layers was observed, but there was formation of vacuoles especially in the prickle cell layers. In addition, an increase in intercellular space and swelling, especially inside the cells was observed. This can be collaborated by the reports that bile salts (like STGC and SDC) accumulate in the tissue after penetration into the tissue without causing a loss of superficial cell layers, and then interact with the intercellular or membrane lipids thus increasing the permeability of the permeant through the epithelium (Senel et al, 1997).Thus, it can be concluded that STGC altered the histology of the buccal epithelium. These histological findings explain well the permeation results where the permeation of FU through the buccal mucosa increased significantly ($p<0.05$) in presence of STGC (Fig. 5.2).

However, the effect of bile salts on the permeability barrier has been reported to be reversible (Zhang et al., 1994; Hoogstraate et al., 1990). Hence it is expected that the buccal mucosa would recover once the treatment is interrupted or stopped.

Fig.5.34.a) Photmicrograph (40X) of Buccal mucosa (Control)

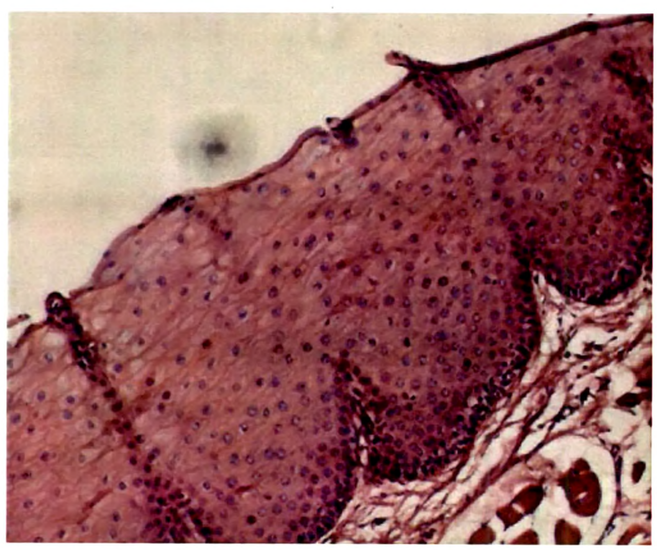

Fig.5.34.b) Photmicrograph (40X) of Buccal mucosa after 8 hour treatment with FU bioadhesive patches

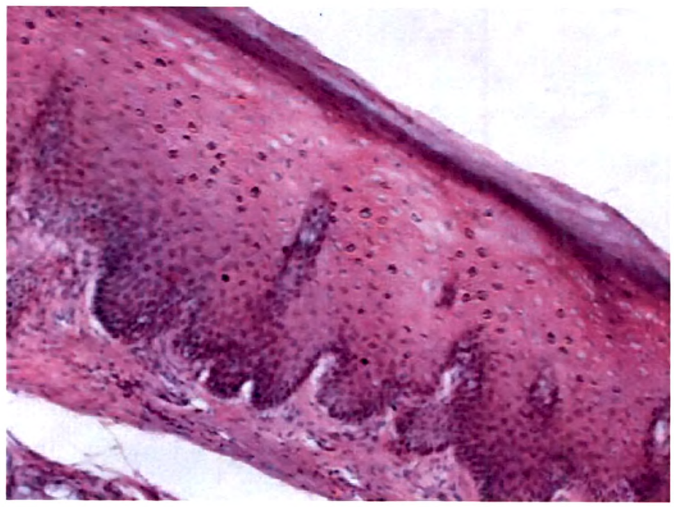

Fig 5.34.c) Photmicrograph(40X) of Buccal mucosa after 8 hour treatment with FU bioadhesive tablets

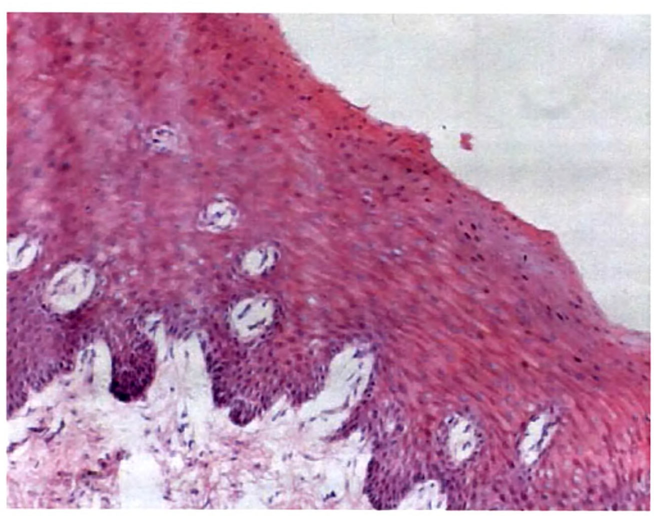

Fig.5.34.d) Photmicrograph(40X) of Buccal mucosa after 8 hour treatment with FU bioadhesive gels

S - Superficial cells; P- Prickle cells, B- Basal cells, C- Connective tissue.

5.5 *IN VIVO* STUDIES ON FU BIOADHESIVE PATCHES, TABLETS AND GELS:

The plasma concentration - time curve for FU after the oral conventional tablet (prepared by direct compression of FU, Lactose, microcrystalline cellulose and Magnesium stearate) and I.V. administration to rabbits of a single 20 mg dose {Marketed FU injection,I.P. (Fluracil®, Biochem pharmaceutical Industries, Mumbai.} is shown in Fig. 5.35 while Fig 5.36 depicts plasma concentration – time profile of FU containing bioadhesive dosage forms developed in our lab (patch, tablet and gel). Table 5.21 gives Mean plasma concentration (ng/ml) of FU from the oral, IV and buccal mucoadhesive administration of FU formulations. The pharmacokinetic parameters of FU following administration of oral conventional tablet, I.V. injection, and application of buccal bioadhesive formulations are summarized in Table 5.22.

The AUC for the intravenous administration and oral conventional tablets were about 20404.70±2064 hng/ml and 6562.73 ± 854 hng/ml respectively which was statistically significant (*$p<0.001$*; ANOVA followed by Dunnett's multiple comparison test). The absolute bioavailability of oral formulation is 32.16% which is as per reports (Collin, 2001; Umejima et al, 1995.).

Following buccal administration of bioadhesive patch, tablet and gel formulations to rabbits, the AUC values were 8624.90 ± 1094, 14779.48 ± 1641 and 12653.65±1405 hng/ml, respectively which were significantly different ($p<0.001$) from AUC obtained on IV administration. The absolute bioavailability for bioadhesive patch, tablet and gel were 42.27%, 72.4% and 62.01% respectively which indicate improvement in bioavailability of FU when given by buccal route. Highest C_{max} (2058.25±214 ng/ml) amongst all tested formulations was observed with oral conventional tablet and amongst buccoadhesive formulations it was found with tablet (1468.25 ± 203 ng/ml) followed by gel (1189.75±362 ng/ml) and patch (1042.4±284 ng/ml).

The conventional tablets of FU might have disintegrated very fast in GI tract and resulted in the quick absorption of the drug from stomach and small intestine thereby producing peak plasma concentration(C_{max} =2058.25±214 ng/ml) within 2.0±0.75h (T_{max}).

Tmax values were 4.0 ± 0.88h, 4.0 ± 1.10h and 3.0 ± 1.25h for buccal administration of patch, tablet and gel. The average $T_{1/2}$ values were 5.09±1.68, 3.31± 0.924 and 5.47± 2.04h, for patch, tablet and gel formulations, respectively, as compared to 1.417 ± 0.72 and 0.54±0.057h following oral tablet and I.V. administration.

The average K_{el} was 1.27±0.80 h^{-1} in the intravenous administration group and 0.488 ± 0.064 in the oral conventional tablets while buccoadhesive formulations depicted lower values 0.136 ± 0.058, 0.21 ± 0.045 and 0.126 ± 0.068 h^{-1} for patch, tablet and gel, respectively.

The sustained-release characteristics of the bioadhesive formulations were also reflected in the MRT and MAT of FU in the body. Both these parameters were considerably increased following buccal administration of the bioadhesive formulations as compared to I.V. administration. The average MRT after buccal administration of patch, tablet and gel were 8.32 ± 2.11, 6.43 ± 1.02 and 8.08± 1.85h, respectively, as compared to 1.07 ± 0.68h after I.V. administration and they were significantly different (*$p<0.001$*, ANOVA followed by Dunnett's multiple comparison test), while the MAT values were 7.25 ± 1.54h, 5.36 ± 1.34h and 7.01± 1.22, for buccoadhesive patch, tablet and gels. Between the subjects the difference was non significant (*$p>0.05$*) indicating that there was less intersubject variation.

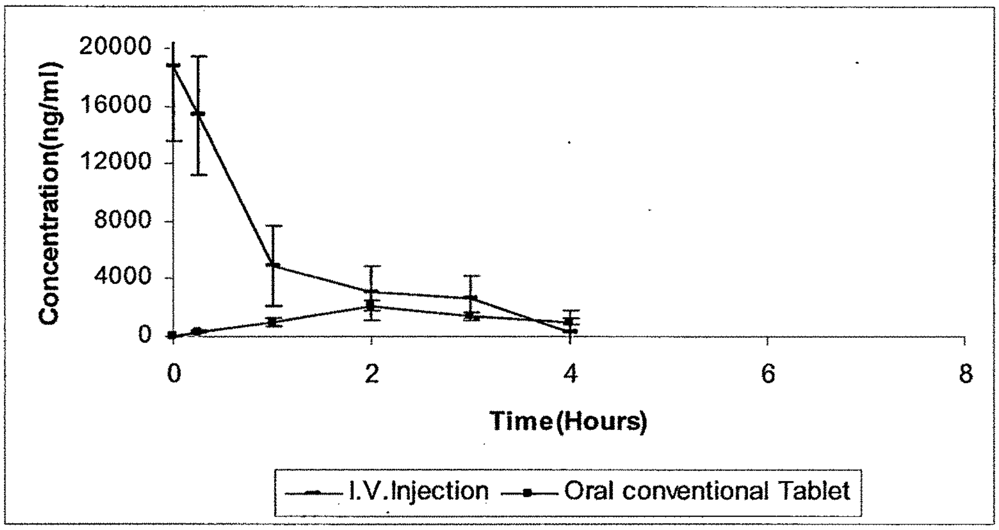

Fig.5.35. Plasma profiles of FU in rabbits following i.v. administration of injection and oral administration of conventional tablets #.

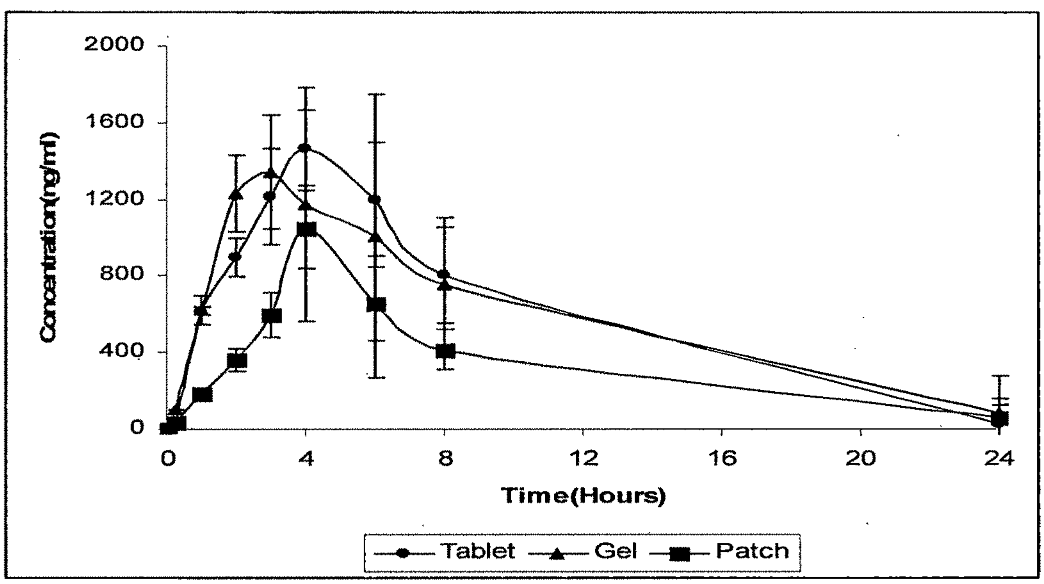

Fig.5.36. Plasma profiles of FU in rabbits following buccal administration of patches, tablets and gels.

Each value represents the mean ± SEM. of six determinations.

Table 5.21. Mean plasma concentration (ng/ml) of FU from the oral, IV and buccal mucoadhesive administration of FU formulations.

Time(Hours)	I.V.	Oral Conventional Tablet	Patch	Tablet	Gel
0.25	4380.25±1003.7	302.75±85.4	35±11.4	28±14.2	100±32.2
1	1412.75±314.3	952.75±154.05	185.25±25.8	615±189.9	560.75±124.5
2	867.75±147.8	2058.25±378.1	360.25±49.5	895±203.54	1029±212.84
3	777.75±107.5	1374±184.9	595.25±94.8	1212±312.04	1189±354.1
4	67.75±26.12	842.75±104.7	1042.4±187.2	1468±240.12	1054±380.24
6	--	--	652.75±102.98	1200.23±321.23	898±168.2
8	--	--	411.02±78.98	800±187.76	574±142.02
24	--	--	60±18.4	25±11.32	85±27.98

(n = 6, Mean ± SEM)

Table5.22. Pharmacokinetic Characteristics of FU after Oral Administration of Conventional Tablet, Intravenous injection and Application of Buccoadhesive formulations (Patch, Tablet, Gel) in Rabbits.

Parameters#	I.V.	Oral Conventional Tablet	Patch	Tablet	Gel
AUC((ng.h/ml)	20404.79 ± 2064	6562.73 ± 854	8624.90 ± 1094	14779.48 ± 1641	12653.65 ± 1405
C_{max} (ng/ml)	--	2058.25±214	1042.4±284	1468.25 ± 203	1189.75±362
T_{max} (h)	--	2.0 ± 0.75	4.0 ± 0.88	4.0 ± 1.10	3.0 ± 1.25
MRT(h)	1.07 ± 0.68	3.23 ± 1.05	8.32 ± 2.11	6.43 ± 1.02	8.08 ± 1.85
MAT(h)	--	1.96 ± 0.47	7.25 ± 1.54	5.36 ± 1.34	7.01± 1.22
K_{el} (h^{-1})	1.27±0.80	0.488 ± 0.064	0.136 ± 0.058	0.21 ± 0.045	0.126 ± 0.068
$T_{1/2}$(h)	0.54±0.057	1.417 ± 0.72	5.09±1.68	3.31± 0.924	5.47± 2.04
A. B. (%)	100	32.16	42.27	72.4	62.01

(n = 6, Mean ± SEM)

REFERENCES

Agarwal,V. and Mishra, B., Design, development, and biopharmaceutical properties of buccoadhesive compacts of pentazocine. *Drug Dev. Ind. Pharm.*, 25 (1999) 701–709.

Bhardwaj, R and Blanchard, J., Controlled-Release Delivery System for the r-MSH Analog Melanotan-I Using Poloxamer 407. *J. Pharm. Sci.*, 85 (1996) 915-919.

Clas, D.S., Dalton, C. R.,Hancock, B.C., Differential scanning calorimetry: applications in drug development. *PSTT*, 2 (1999)311-20.

Collin, Dollery. (ed.), Therapeutic Drugs. Vol 1, 2nd edition, Churchill Livingstone, Edinburgh., 1999; F 103-108.

Ganem-Quintanar, A., Quintanar-Guerrer, D., Falson-Rieg, F., Buri, P., Ex vivo oral mucosal permeation of lidocaine hydrochloride with sucrose fatty acid esters as absorption enhancers. *Int J Pharm.*, 173 (1998) 203–210.

Gilbert, J. C., Hadgraft, J., Bye, A., Brookes, L.G., Drug release from Pluronic F-127 gels. *Int. J. Pharm.*,32 (1986)223–228.

Hoogstraate, A. J., Senel, S., Cullander, C., Verhoef, J., Junginger, H. E., Bodde, H. E., Effects of bile salts on transport rates and routes of FITC-labelled compounds across porcine buccal epithelium in vitro. *J. Control Rel.*, 40 (1990) 211-221.

Huang,Y.B., Tsai, Y.H., Lee, S. H., Chang, J.S., Wu, P.C., Optimization of pH-independent release of nicardipine hydrochloride extended-release matrix tablets using response surface methodology. *Int. J. Pharm.*, 289 (2005) 87-95.

Ikinci, G., Capan, Y., Senel, S., Alaaddinoglu, E., Dalkara, T., Hincal, A.A., In vitro/in vivo studies on a buccal bioadhesive tablet formulation of carbamazepine. *Pharmazie.*, 55(2000)762-765.

Khamanga, S. M., and Walker, R. B., Evaluation of Rate of Swelling and Erosion of Verapamil Sustained Release Matrix Tablets. *Drug Dev. Ind. Pharm.*, 32 (2006)1139–1148.

Korsmeyer, R. W., Gurny, R., Doelker, E., Buri, P., Peppas, N. A., Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.*, 15(1983) 25-35.

Martin, Alfred, Ed., Physical Pharmacy, 4th Edition, B.I.Waverly Pvt.Ltd, New Delhi, 1999, pp 453-460.

Narendra, C., Srinath, M.S., Prakash Rao, B., Development of three layered buccal compact containing metoprolol tartrate by statistical optimization technique. *Int. J. Pharm.*, 304 (2005) 102-114.

Owens, T.S., Dansereau, R.J., Sakr, A., Development and evaluation of extended release bioadhesive sodium fluoride tablets. *Int J Pharm.*, 288(2005)109-22.

Patel, V. F. and Patel, N. M., Intragastric floating drug delivery system of cefuroxime axetil: in vitro evaluation. *AAPS Pharm. Sci. Tech.*, 7 (2006) E17.

Peppas, N.A., Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta. Helv.*, 60 (1985)110–111.

Senel, S., Çapan, Y., Sargon, M.F., Ikinci, G., Solpan, D., Güven, O., Boddé, H.E., Hincal, A.A., Enhancement of transbuccal permeation of morphine sulfate by sodium glycodeoxycholate in vitro. *J. Control Rel.*, 45 (1997)153-162.

Senel, S., Duchene, D., Hincal, A.A., Capan, Y., Ponchel, G., In vitro studies on enhancing effect of sodium glycocholate on transbuccal permeation of morphine hydrochloride. *J.Control Rel.*, 51(1998)107-13.

Shin, S. C., Bum, J. P., Choi, J.S., Enhanced bioavailability by buccal administration of triamcinolone acetonide from the bioadhesive gels in rabbits. *Int. J. Pharm.*, 209 (2000a) 37–43.

Shin, S.C., Kim, J. Y., Oh, I. J., Mucoadhesive and physicochemical characterization of carbopol-poloxamer gels containing triamcinolone acetonide. *Drug Dev. Ind. Pharm.*, 26 **(2000b)** 307-312.

Tas, C., Ozkan, Y., Savaser, A., Baykara, T., In vitro and ex vivo permeation studies of chlorpheniramine maleate gels prepared by carbomer derivatives. *Drug Dev.Ind. Pharm.*, 30(2004) 637–647.

Tur, K. M. and Chang, H. S., Evaluation of possible mechanism(s) of bioadhesion, *Int. J. Pharm.*, 160 (1998) 61–74.

Turunen, T.M., Urtti, A., Paronen, P., Audus, K.L., Rytting, J.H..,Effect of some penetration enhancers on epithelial membrane lipid domains: evidence from fluorescence spectroscopy studies. *Pharm Res.*, 11 (1994)288-94.

Umejima, H., Kikuchi, A., Kim, N.S., Uchida, T., Goto, S., Preparation and evaluation of Eudragit gels. VIII. Rectal absorption of 5-fluorouracil from Eudispert hv gels in rats. *J. Pharm. Sci.*, 84(2) (1995)199-202.

Woolfson, A. D., McCafferty, D. F., McCarron, P. A., Price, J. H., A bioadhesive patch cervical drug delivery system for the administration of 5-fluorouracil to cervical tissue. *J. Control. Rel.*, 35 (1995) 49-58.

Yong, C. S., Jung, J. H., Rhee J. D., Kim, C. K., Choi H. G., Physicochemical Characterization and Evaluation of Buccal Adhesive Tablets Containing Omeprazole . *Drug Dev. Ind. Pharm.*, 27(2001) 447–455.

Zhang, J., Niu, S., Ebert, C., Stanley, T.H., An in vivo dog model for studying recovery kinetics of the buccal mucosa permeation barrier after exposure to permeation enhancers: apparent evidence of effective enhancement without tissue damage. *Int. J. Pharm.*, 101(1994)15-22.

Zhang, L., Parsons, D. L., Navarre, Christine, Kompella, U. B., Development and in-vitro evaluation of sustained release Poloxamer 407 gel formulations of ceftiofur. *J. Control. Rel.*, 85 (2002) 73–81.