Chapter 7
Results & Discussion
(Methotrexate)

7.1 PERMEATION STUDIES OF METHOTREXATE (MTX) ACROSS BUCCAL MUCOSA

18.32 ± 8.6% MTX permeated through the buccal mucosa in 8 hours with permeability coefficient (Kp) of 4.69(±0.15) x 10^{-5} cm/s (Table 7.1). As the permeation of MTX is very poor hence there is a need of permeation enhancers.

7.1.1 *Effect of Permeation Enhancers on buccal permeation of MTX*

Penetration enhancers were utilized to enhance the intrinsic buccal permeation and hence absorption of MTX. Various permeation enhancers such as EDTA, β-CD, SDC, STGC, GMS, PEG-200(5%) +EtOH (10%), PG-200(5%)+EtOH (10%), Oleic acid (3%) in PEG-200 (5%) and ethanol (10%), Oleic acid (3%) in PG (5%) and EtOH (10%) were used and their enhancement factors (EF) were calculated. Table 7.1 summarizes the buccal permeation parameters in presence of various penetration enhancers.

Permeability coefficient (Kp) of control is 4.69 (±0.15) x 10^{-5} cm/s. EDTA and β-CD displayed Kp values of 5.49 (±0.60) x 10^{-5} and 6.17 (±0.52) x 10^{-5} while the EF values were 1.04 and 1.05 respectively. This means that the capacity of the drug to diffuse through the buccal mucosa is not modifiable in the presence of cyclodextrins or EDTA. So other enhancers were then tested.

In the presence of SDC (0.1M), MTX permeation was observed to improve with Kp value 9.70 (±0.51) x 10^{-5} and EF as 1.78. When STGC (0.1M) was used no significant deference to SDC was noticed as Kp was 9.96 (±0.44) x 10^{-5} and EF was 2.01. GMS (0.1M) enhanced permeation significantly as evident from Kp and EF values which were 1.67(±0.75) x 10^{-4} and 2.41 respectively.

When PEG-200(5%) +EtOH (10%) were used, Kp and EF values were 7.73(±0.35) x 10^{-5} cm/s and 1.85 while for PG-200(5%) + EtOH (10%) these values were 7.99(±0.48) x 10^{-5} cm/s and 2.03, respectively.

On using oleic acid (3%) + Polyethylene glycol-200 (5%) + ethanol (10%), permeability coefficient and EF values were 1.58(±0.34) x 10^{-4} and 2.27 respectively which differed significantly from control ($p<0.05$). But when Oleic

acid(3%) + Propylene glycol (5%) + ethanol (10%) were used, further improvement in permeation was observed as evident from Kp and EF values (1.83 (±0.64) x 10^{-4} and 2.73, respectively).

Thus, permeability of MTX increased most significantly ($p<0.05$) in the presence of Oleic acid (3%) + Propylene glycol (5%) + ethanol (10%) and GMS (0.1M). The value of Kp augmented 6.14 fold in presence of Oleic acid (3%)+Propylene glycol (5%) + ethanol (10%) and 5.60 fold in presence of GMS as compared to MTX alone. Hence Oleic acid (3%) + Propylene glycol (5%) + ethanol (10%) and GMS (0.1M) were selected for further studies. **Oleic acid** acts by reducing the lipid packing order in buccal epithelial cell membranes (Turunen et al, 1994). Extraction of buccal intercellular lipids would seem an appropriate mechanism of action for ethanol, since ethanol is a lipid solvent (Nicolazzo et al, 2005).

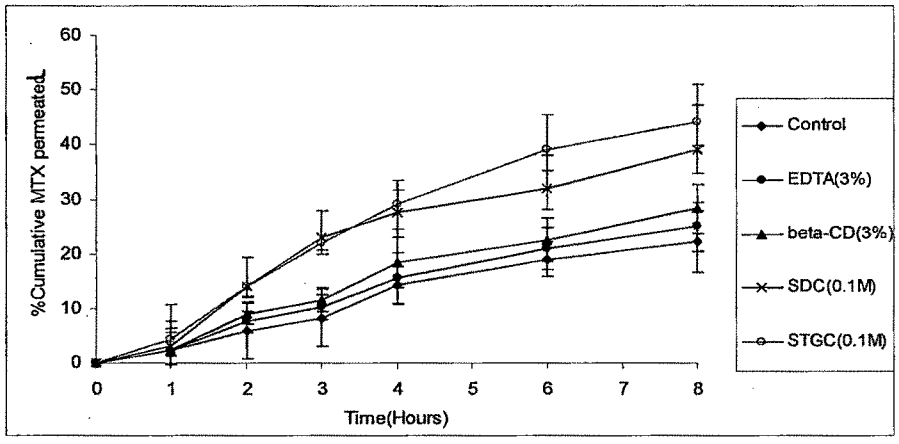


Fig 7.1a. Permeation of MTX in presence of enhancers across buccal mucosa.

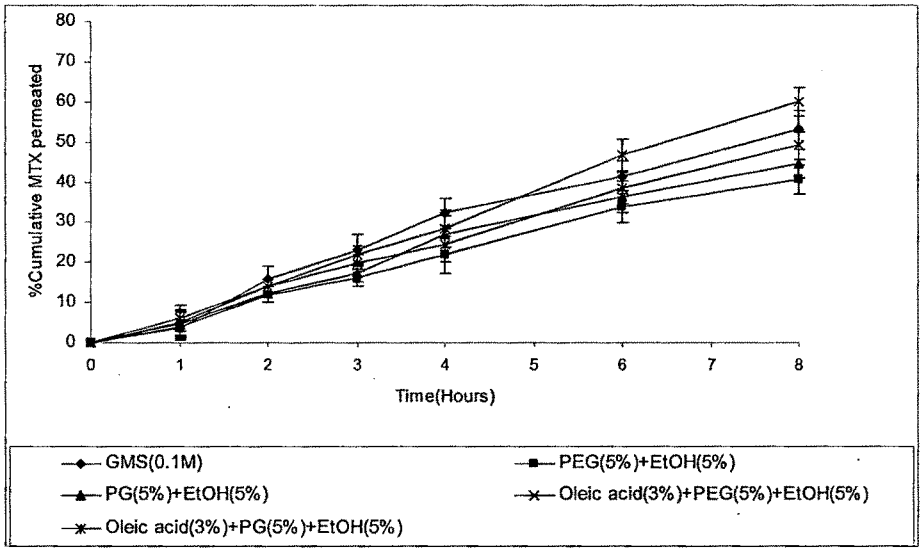


Fig 7.1b. Permeation of MTX in presence of enhancers across buccal mucosa.

Table 7.1. Permeability of MTX across buccal mucosa in presence of penetration enhancers

Batch	Permeability Coefficient, Kp (cm/s)	Enhancement factor (EF)
Control	$4.69(\pm0.15) \times 10^{-5}$	1
EDTA (3%)	$5.49 (\pm0.60) \times 10^{-5}$	1.04
β-CD (3%)	$6.17 (\pm0.52) \times 10^{-5}$	1.05
SDC(0.1M)	$9.70 (\pm0.51) \times 10^{-5}$	1.78
STGC(0.1M)	$9.96 (\pm0.44) \times 10^{-5}$	2.01
GMS(0.1M)	$1.67(\pm0.75) \times 10^{-4}$	2.41
PEG-200(5%)+EtOH(10%)	$7.73(\pm0.35) \times 10^{-5}$	1.85
PG (5%) + EtOH (10%)	$7.99(\pm0.48) \times 10^{-5}$	2.03
Oleicacid(3%)+PEG200 (5%)+ETOH(10%)	$1.58(\pm0.34) \times 10^{-4}$	2.27
Oleic acid (3%) +PG (5%) + ETOH (10%)	$1.83 (\pm0.64) \times 10^{-4}$	2.73

GMS:Glyceryl monostearate; SDC: Sodium deoxyxholate; STGC: Sodium tauroglychocholate; EtOH: Ethyl alcohol; PEG:Polyethylene glycol; EDTA: Ethylene diamine tetraacetic acid; β-CD: β-Cyclodextrin.

7.2 FORMULATION OF MTX BUCCAL BIOADHESIVE PATCHES

The patches consisted of two layers, medicated layer and backing layer. The polymers employed were HPMC K4M and Gantrez AN 139 [a copolymer of methyl vinyl ether and maleic anhydride (PMVE/MA)].

Incorporation of air bubbles into the hydrogel solutions was a regular problem associated with mechanical mixing. Centrifugation of the water-based systems was not possible, as suspended solid material would settle out. Therefore, refrigeration at 4°C was the preferred method of clearing gels of entrapped air. Air bubbles also occasionally became introduced into the gel during the casting process. Although this was rare and mostly associated with highly viscous materials, it was nonetheless problematic, both in terms of film appearance and subsequent characterization. The problem was solved by placing the cast film under a slowly drawn vacuum, forcing air bubbles to the surface of the material.

Optimization of formulation composition was carried out by varying the ratios of HPMC K4M and Gantrez AN 139 to get the desired characteristics (viz. release, bioadhesion and other physicochemical properties). The plasticizer composition was finalized by studying the mechanical strength using tensile strength tester. Physicochemical characterization parameters such as thickness, average weight, assay, pH, bioadhesion, tensile strength values are given in Table 7.2.

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

7.2.1 *Morphology of Patch*

The prepared patches containing MTX were yellow colored and clear. The scanning electron photomicrograph of the film at 1000 X magnification showed smooth surface without any scratches or transverse striations (Fig. 7.2).

Table 7.2. Physicochemical parameters of MTX mucoadhesive patches

Parameters							
Batch	**Assay**	**pH**	**Tensile strength(g)**	**Folding Endurance**	**Mucoadhesion ($x10^3$ dynes/cm^2)**	**Patch Thickness (mm)**	**Average weight (mg)**
MP1	102.01 ±1.82	5.89±0.12	724±17	228±18	5.58±2.17	1.21±0.12	148±5.2
MP2	98.01 ±2.13	5.81±0.18	691±24	235±14	6.45±1.40	1.25±0.10	136±4.8
MP3	98.32 ±3.10	5.79±0.11	734±18	250±24	8.57±1.54	1.24±0.09	129±7.6
MP4	99.87 ±3.14	5.98±0.24	846±13	229±17	9.63±1.96	1.19±0.09	121±7.7
MP5	101.32 ±2.48	6.04±0.08	1120±29	248±26	12.32±2.07	1.17±0.14	115±4.5
MP6	99.38 ±1.54	6.12±0.21	1345±24	265±11	14.93±1.62	1.21±0.12	132±8.2
MP7	98.71 ±1.84	5.86±0.13	1549±38	274±12	17.24±2.24	1.22±0.14	134±3.9
MP8	98.34 ±2.42	6.27±0.10	1599±35	239±16	18.2±2.54	1.23±0.11	156±4.5
MP9	98.69 ±2.09	6.07±0.18	1662±54	254±12	21.28±3.14	1.25±0.07	167±2.1

(Mean ± SEM; n=3)

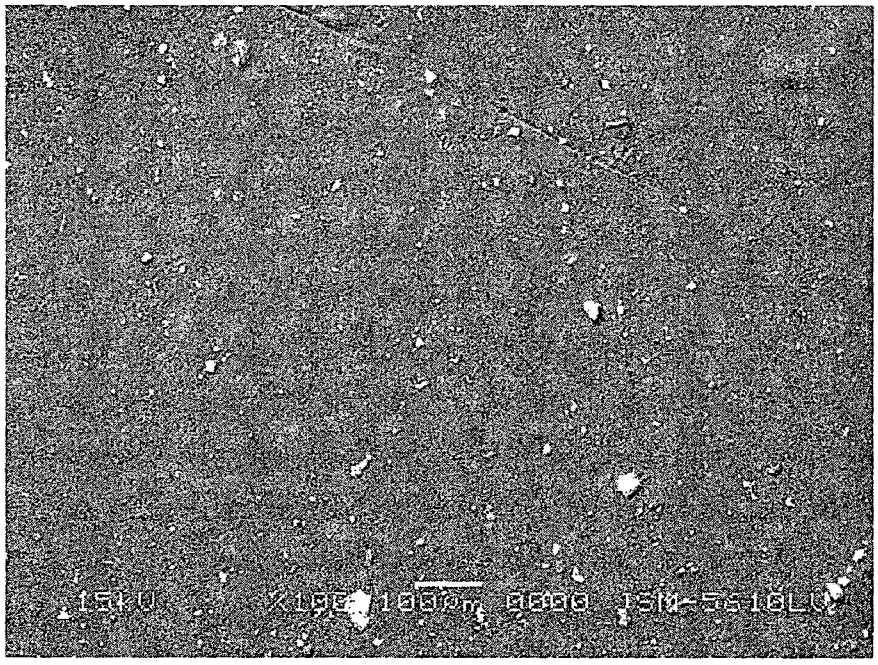


Fig.7.2. Scanning Electron Photomicrograph of MTX patch at 1000 X magnification.

7.2.2 *Swelling studies:*

Swelling profile is helpful in interpreting the mechanism of bioadhesion and understanding release from formulations (Tur et al., 1998). The swelling profiles of MTX bioadhesive patches are shown in Fig 7.3a and 7.3b. The swelling index of the prepared buccoadhesive patches decreased in the order MP9> MP8> MP7> MP1> MP2> MP6> > MP3> MP4> MP5.

It can be seen that at the initial stage, the swelling occurs very 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 in the patches, and similar trend was observed with Gantrez (Fig 7.3a and 7.3b). The maximum swelling was attained in MP9 containing highest proportion of Gantrez (Swelling index=2.54±0.84), while the batch containing highest proportion of HPMC (MP1) displayed lesser swelling index (2.1±0.09). Differences in swelling of the tested

polymers could be explained by the difference in resistance of the matrix network structure (hydrogen bond) to the movement of water molecules.

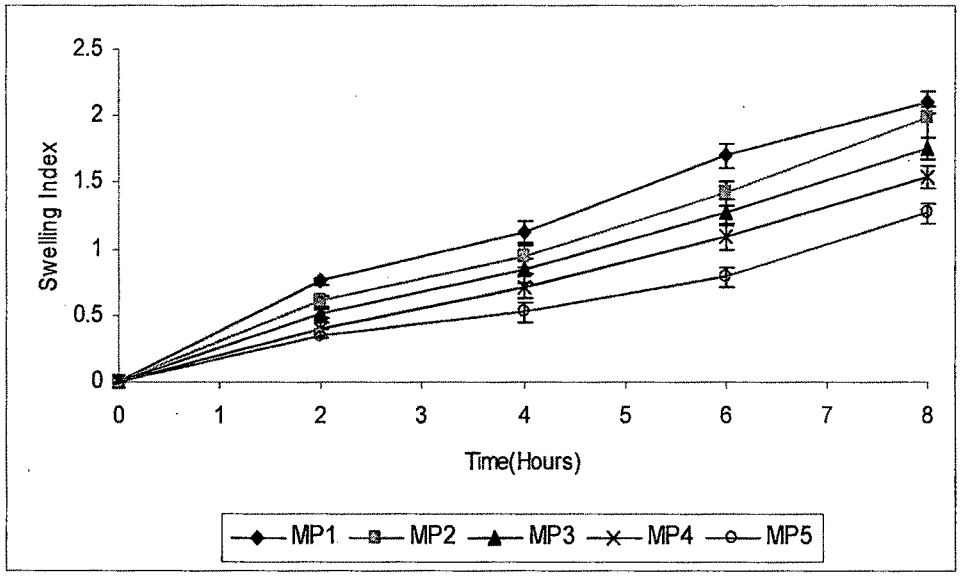


Fig 7.3a. Swelling index of MTX patch formulations (MP1 – MP5)

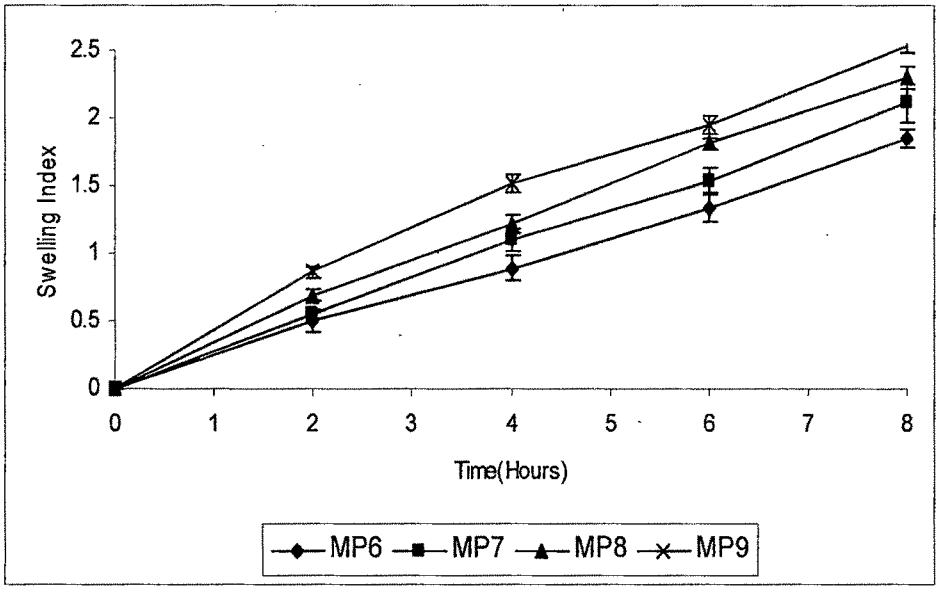


Fig 7.3b. Swelling index of MTX patch formulations (MP6 – MP9)

7.2.3 *Mucoadhesion:*

The data of bioadhesive force is shown in Table 7.2. An increasing trend in the bioadhesive strength with increase in the amount of polymer(s) was observed. Maximum bioadhesive strength was seen with the highest levels of Gantrez polymer.Fig.7.4 construes an increasing trend in the bioadhesive strength with the decrease in the amount of HPMC content (MP1-MP4), keeping the Gantrez content constant. This may be due to availability of more number of free carboxylic acid groups (present in Gantrez) for bioadhesion. Maximum bioadhesive force was exhibited by MP 9 containing HPMC and Gantrez in 1:2 proportion. Increasing the concentration of Gantrez AN 139 increased bioadhesion significantly *($p< 0.05$)* upto batch MP7 and thereafter no noticeable increase was discerned.

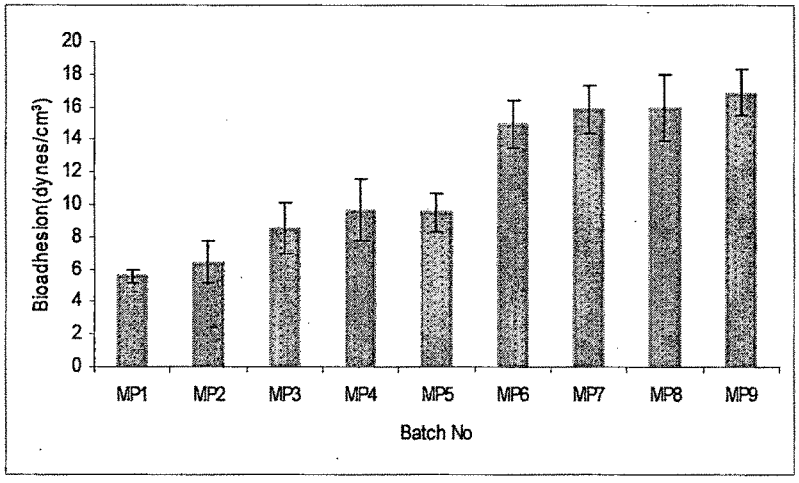


Fig. 7.4. Bioadhesive force of MTX patch formulations (n=3)

7.2.4 *In vitro release:*

In vitro release profile of the designed formulations is shown in Fig.7.5a and 7.5b. In formulations MP1, containing polymers in 1.75:1 proportion, 74±3.5% MTX was released in 8 hours. As the HPMC proportion is reduced, there is increase in release as evident in release profile of formulations MP1- MP4. 100.64 ±4.5 % MTX was released in 8 hours from MP5 containing HPMC and Gantrez in 1:0.8 proportion.

Batches MP6 - MP9 contained increasing amount of Gantrez and fix amount of HPMC K4M.

The effect of altering Gantrez AN 139 and HPMC concentrations in the patch on drug release is shown in Fig.7.5a and 7.5b. The reduction in release is observed as the concentrations of both polymer components increased. Increasing the concentration of Gantrez or HPMC in the casting gel and, thus, in the resultant patch, increased the viscosity of the gel and, consequently, the patch thickness. Such an increase will raise the micro-viscosity of the patch and provide a more concentrated matrix from which the drug will be released more slowly. The results were in accordance with the results reported by Woolfson et al. (1998) who reported that an increase in Gantrez content in the patch system resulted in release retardation.

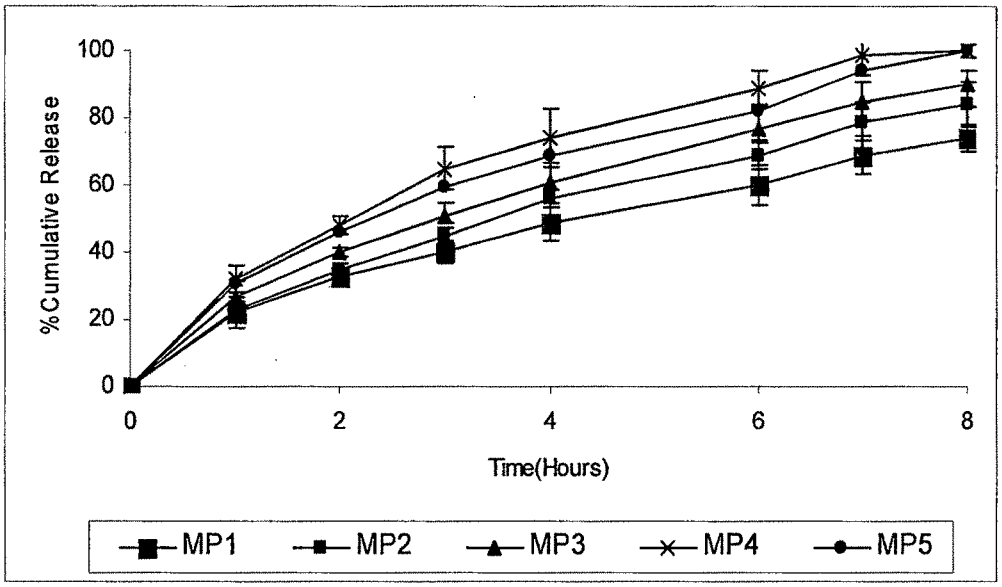


Fig. 7.5a *In vitro* release profiles of MTX from different buccoadhesive patches in phosphate buffer (pH 6.8) (values are expressed as mean ± SEM).

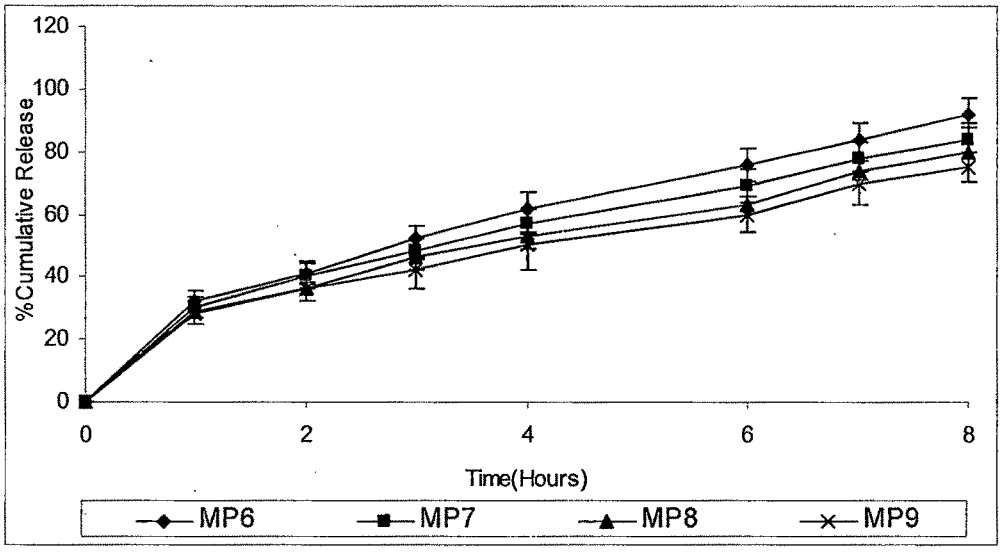


Fig. 7.5b. ***In vitro*** **release profiles of MTX from different buccoadhesive patches in phosphate buffer (pH 6.8) (values are expressed as mean ± SEM).**

To investigate the kinetics of MTX 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 7.3. To use Peppas equation, it is necessary that release occurs in one dimensional manner and that the system width/thickness or length/thickness relation (aspect ratio) be at least 10/1 (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. When aspect ratio is <10, the critical values of n for declaring Fickian diffusion and zero-order release were found to be 0.45 and 0.89, respectively (Peppas and Sahlin, 1989; Singh et al., 2006).

The goodness of fit for various models investigated for MTX 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

Table 7.3. Release kinetics of MTX bioadhesive Patches

Formulation code	Zero order model		First order model		Higuchi model		Peppas model	
	k	R^2	k	R^2	k	R^2	n	R^2
MP1	7.24	0.9913	0.0694	0.9354	28.42	0.9947	0.5805	0.9976
MP2	8.61	0.9886	0.0746	0.9241	33.88	0.9967	0.6282	0.9987
MP3	8.92	0.9886	0.0696	0.9235	35.173	0.999	0.5864	0.9997
MP4	9.60	0.9552	0.0648	0.8756	38.30	0.9903	0.5585	0.9912
MP5	9.51	0.9799	0.0664	0.9092	37.56	0.9959	0.5628	0.997
MP6	8.48	0.9948	0.0628	0.9575	33.19	0.9933	0.5176	0.9908
MP7	7.57	0.9949	0.0604	0.9578	29.64	0.9934	0.4979	0.9934
MP8	7.1849	0.9925	0.0605	0.966	28.003	0.9829	0.494	0.9823
MP9	6.6473	0.9954	0.0587	0.9736	25.87	0.9820	0.477	0.9821

(Mean ± SEM; n=3)

between 0.477 and 0.628. Only three formulations MP7, MP8 and MP9 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 MP1 – MP6 has 'n' value in the range 0.5 - 1.0 indicating anomalous behaviour (non-Fickian diffusion).

7.2.5 *In vitro* diffusion of MTX from patches across buccal mucosa

The penetration of MTX from patch formulation (MP5) through buccal mucosa (Fig. 7.6) was found very low (Kp =2.93(±0.50) x10^{-5} cm/sec) when compared with that of MTX solution (4.69 (±0.15) x 10^{-5} cm/sec) (Fig.7.1a; Table 7.1). This difference 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.

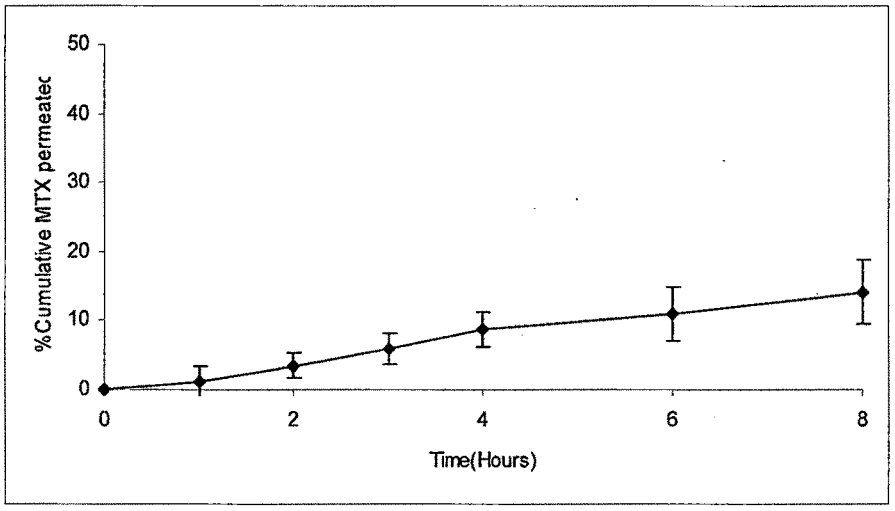


Fig. 7.6. ***In vitro*** **diffusion of MTX from patches across buccal mucosal membrane.**

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

The formulation (MP5) was prepared further using GMS as a permeation enhancer (0.1M) (Section 7.1). The bioadhesive strength of these patches was 11.22±1.29 dynes/cm^2, tensile strength 1060± 22g and moisture content 11.2± 2.4% w/w. No significant difference in adhesive bond strength, tensile strength and moisture content was observed between GMS containing and GMS free patches.

Addition of GMS to patch formulations resulted in an increase in release rate (Fig 7.7). 51.13 ±3.79%, 76.54 ±5.22% and 104.2± 4.4% MTX was released from MTX patch formulations containing GMS as compared to 46.13 ± 4.2%, 68.54 ± 3.1% and 100.12 ± 3.2% from GMS free patches(MP5) after 2, 4 and 8 hours, respectively.

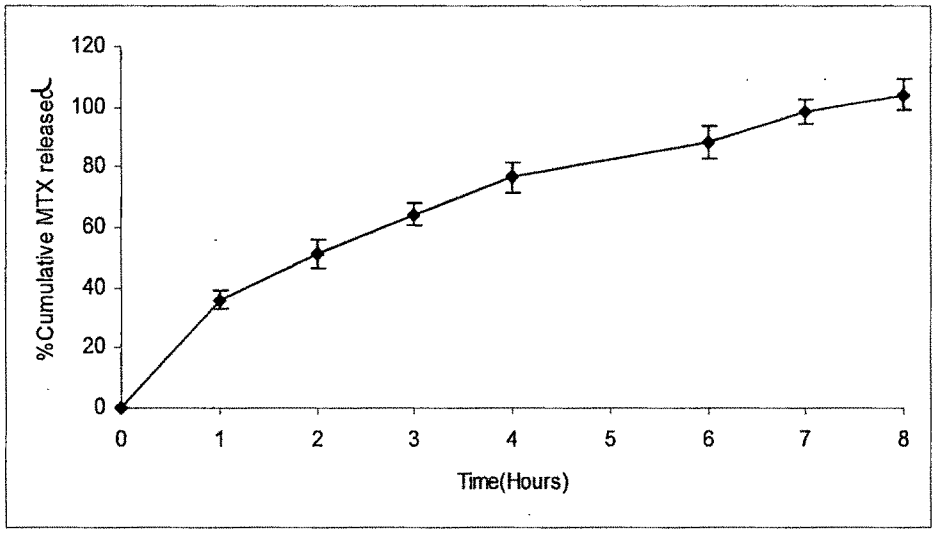


Fig 7.7. *In vitro* release profiles of MTX from buccoadhesive patches containing GMS in phosphate buffer of pH 6.8 (values are expressed as mean ±SEM).

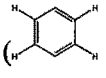
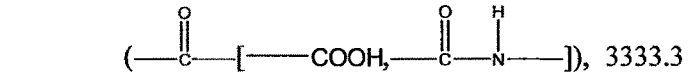
7.2.6 *Compatability studies*:

7.2.6.1 X Ray Diffraction (XRD): The X-ray diffractogram of MTX show sharp peaks at diffraction angles (2θ) 9.32°, 11.44°, 12.84°, 14.34°, 17.66°, 19.30°, 24.27°, 25.49°, 21.78°,26.98°, 28.880°, and 31.15° indicative of crystalline sample. All major characteristic crystalline peaks also appeared in the diffractogram of physical mixture (MTX: Gantrez: HPMC K 4M:: 1:5:5) and patch (Fig 7.8 and Table 7.4). Moreover, the relative intensity and 2θ angle of these peaks remained unchanged, clearly ruling out the possibility of interaction.

Table 7.4. Characteristic XRD peaks of MTX, MTX physical mixture with patch components and patch

S.No.	Formulation	Characteristic peaks at 2θ (degrees)*Intensity in Parentheses
1.	MTX	9.32°(92.1),11.47°(72.7),12.87°(90.7),14.38°(74.8),17.69°(62.6),19.45°(66.2),23.47°(38.6),24.22°(65.6),25.46°(66.0),26.94°(100),28.83°(37.4),31.13°(37.9)
2.	MTX patch physical mix	9.31°(76.2),11.50°(82.9),12.86°(100),14.35°(95.4),17.66°(85),19.53°(87.5),23.4°(59.5),24.27°(64.8),25.46°(64.2),26.96°(73.7),28.82°(50.4),31.10°(47.4)
3.	MTX patch	9.37°(74.5),11.52°(84.3),12.90°(100),14.36°(96.9),17.73°(85.7),19.48°(86.7),23.37°(64.8),24.24°(63.2),25.48°(61.2),27.02°(67.8),28.88°(52.1),31.17°(47.9)

7.2.6.2 Fourier Transform Infrared (FTIR) Spectroscopy:

IR spectra of MTX, its physical mixture with various polymers and solid dispersions with polymers are presented in Fig 7.9. Pure MTX spectra showed sharp characteristic peaks at 840.3 cm^{-1}(), 1538.4 - 1515.1 cm^{-1} (Amide II, Aryl systems), 1612.9 cm^{-1} (Aryl systems), 1694.9-1639.3 cm^{-1} (), 3333.3 -2500.0 cm^{-1} (-COOH), 3448.2-3225.8 cm^{-1} (H_2O, $-NH_2$). All the above characteristic peaks appeared in the spectra of physical mixtures and MTX patch at same wavenumber indicating no modification or interaction between the drug and polymers, even after processing into a patch.

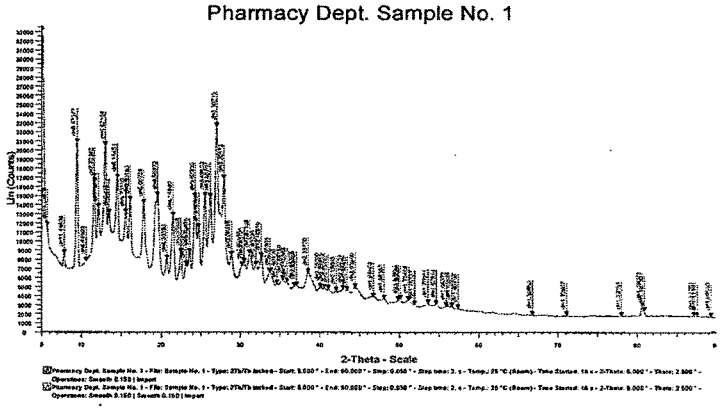


Fig.7.8a XRD spectra of MTX

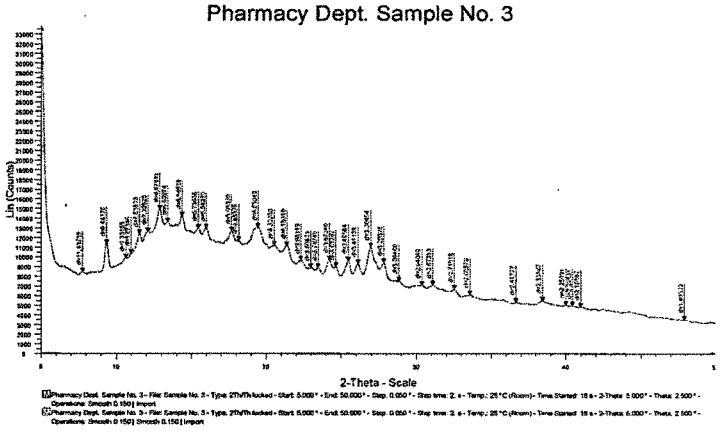


Fig.7.8b XRD spectra of MTX patch physical mixture

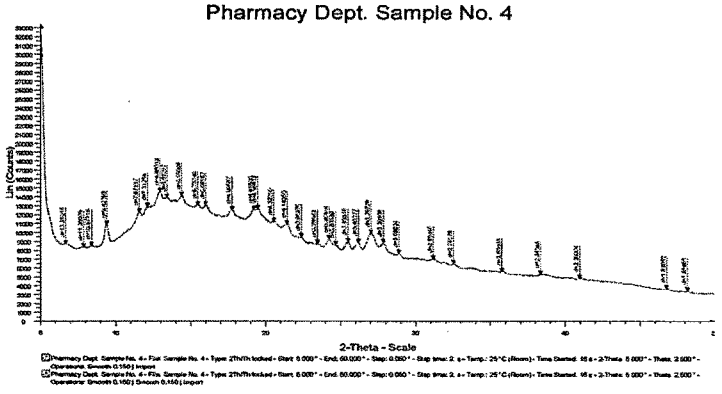


Fig.7.8c XRD spectra of MTX patch formulation

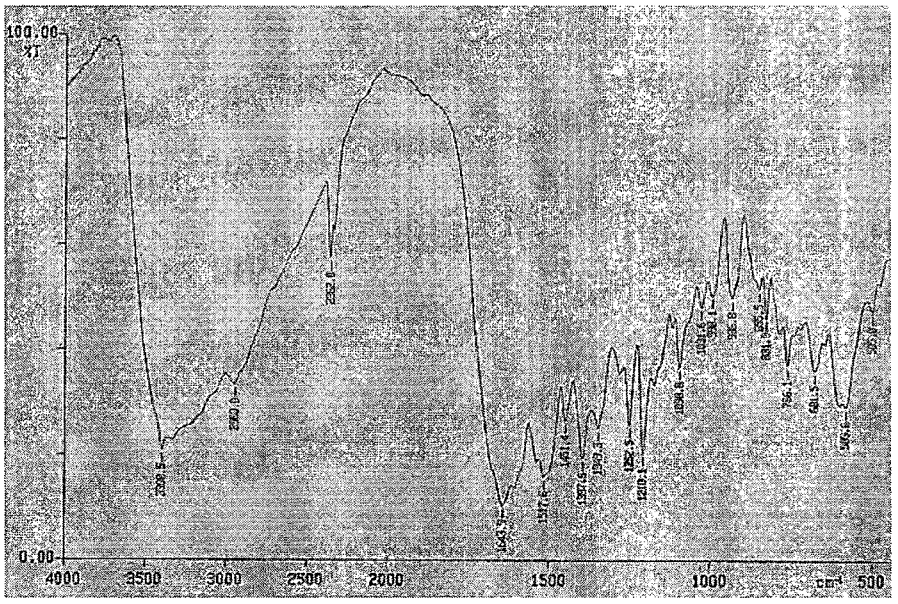


Fig. 7.9 a. FTIR spectra of MTX

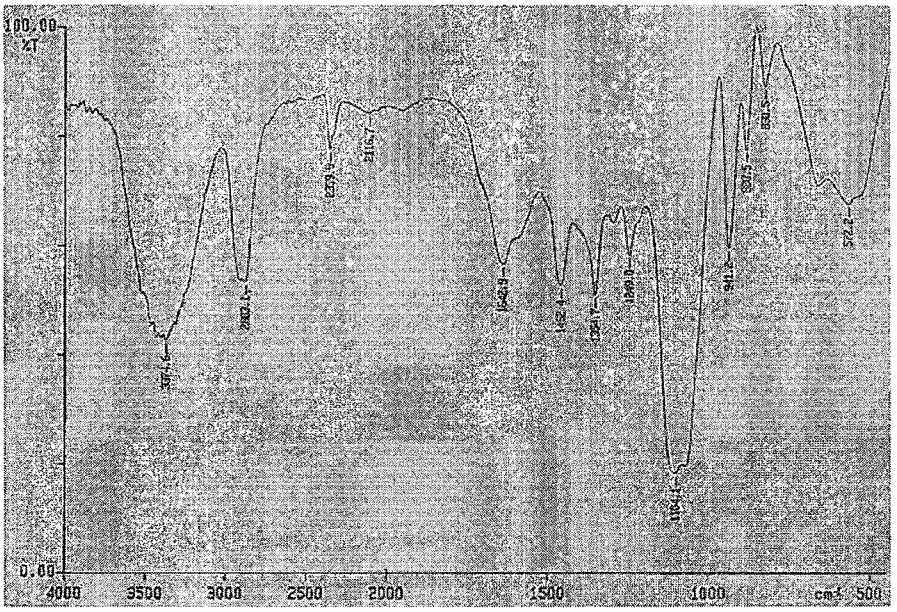


Fig. 7.9 b. FTIR spectra of MTX patch physical mixture.

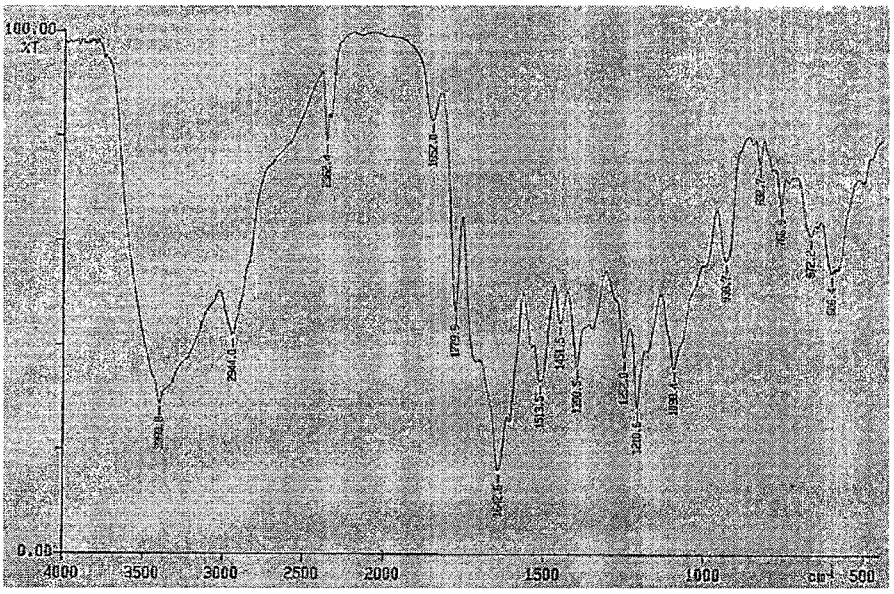


Fig. 7.9 c. FTIR spectra of MTX patch(Formulation)

7.2.7 Stability study:

Table 7.5 enlists different parameters examined and observations during the study. Patches exhibited drug content within acceptable limits of 95-105% over the storage period at different conditions. No prominent physical changes were observed during the study. Insignificant difference ($p>0.05$) in *in vitro* release was observed at all conditions (Table 7.5). Tensile strength reduced on storage at 40 ± 2°C/ 75% ± 5% RH, whereas, moisture content increased significantly ($p<0.05$) on storage at 40 ± 2°C/ 75% ± 5% RH for 3 months indicating the impact of humidity and temperature on mechanical properties of the patch.

Table 7.5. Stability results for MTX bioadhesive patches

Parameter	Initial	3 Months	3 Months
		30 ± 2°C/ 65% ± 5% RH	40 ± 2°C/ 75% ± 5% RH
Tensile strength (kg/cm^2)	1060(±22)	989(± 89)	765(±108)
Moisture content (%w/w)	11.2(±2.4)	14(±2.4)	22(±4.8)
Assay(%w/w)	101.32 (±2.48)	99.34 (± 0.15)	97.25(±0.24)
Release (8 hour)	104.2(±4.4)	98.48(±3.38)	103.04(±2.05)

7.3 Formulation of MTX Buccoadhesive tablets:

7.3.1. Preliminary studies: During preliminary studies, different polymer combinations of Gantrez MS-955 and HPMC K15M were explored and the dissolution results of same are enlisted in (Table 7.6, Fig. 7.10). Physical parameters of all the preoptimization batches were evaluated and the results are shown in Table 7.7.

Based on the criterion of maximum values of bioadhesion and complete release between 6-8 hours, different formulations having varying polymer combinations were studied during preliminary studies. An appropriate range of the factor ingredients (polymers) was selected to obtain the desired response parameters for further optimization studies.

Table 7.6. Mean values of percent cumulative drug released for the MTX bioadhesive tablets prepared during preliminary studies

Time (Hours)	Cumulative percent drug released ± SEM				
	PMT1	PMT2	PMT3	PMT4	PMT5
1	20.57±2.6	21.1±3.5	26.30±5.1	32.11±3.3	30.01±4.3
2	38.95±2.4	36.61±4.0	44.34±3.5	49.21±3.7	47.43±3.6
3	48.04±3.4	45.38±2.9	59.01±2.1	63.24±5.1	62.04±1.9
4	62.19±3.5	58.01±2.2	71.32±6.7	76.22±4.2	78.31±3.6
6	74.23±1.7	72.10±5.3	86.21±2.8	92.03±2.9	98.24±4.1
8	93.23±6.1	82.30±2.8	98.01±3.6	100.40±4.6	103.88±4.2

7.3.2 *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 7.8 displays the values of physical parameters and drug assay for these formulations. The tablet weights ranged from 213 ± 2 to 217 ± 3 mg, diameter 9.48 ± 0.02 to 9.53 ± 0.03 mm, thickness between 2.1 ± 0.1 to 2.2 ± 0.1 mm and hardness between

4.3±0.2 to 4.7±0.1 kg/cm^2. The MTX content varied between 99.24±1.6 to 102.00±1.1 % and friability ranged between 0.19±0.06 to 0.42±0.03%. Thus, all the formulations were found to comply with the compendial requirements. pH of all the formulations was in the range 6.11±0.023 to 6.47±0.041.

Table 7.7. Physical parameters and drug assay for preliminary batches of MTX tablets

Batch	Weight (mg)	Hardness (kg/cm^2)	Diameter (mm)	Thickness (mm)	Friability (%)	Assay (%)	Bioadhesion (x10^{3}dynes/cm^2)
PMT1	216±3	4.8±0.2	9.53±0.04	2.1±0.1	0.25±0.05	100.25±2.8	10.1±1.08
PMT2	217±2	4.7±0.1	9.47±0.01	2.1±0.1	0.32±0.04	101.20±2.1	9.8±1.1
PMT3	213±2	4.6±0.4	9.48±0.02	2.2±0.1	0.17±0.01	102.52±1.4	9.4±1.67
PMT4	215±4	4.7±0.3	9.49±0.04	2.1±0.1	0.26±0.04	99.107±2.5	8.9±1.28
PMT5	216±3	4.8±0.3	9.51±0.02	2.2±0.1	0.18±0.02	99.35±0.92	11.8±1.3

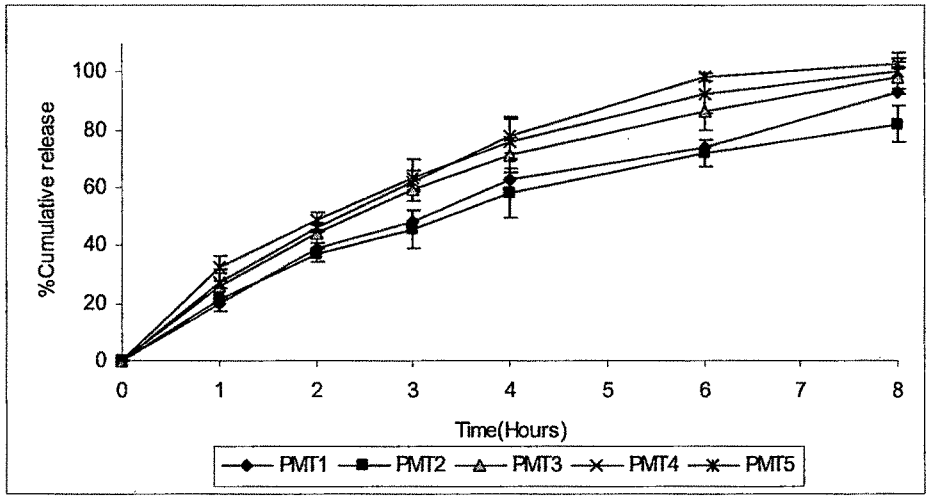


Fig. 7.10: Dissolution profile of preliminary batches of MTX tablets

Table 7.8 Physical characterization and drug assay for MTX tablets

Batch	Weight (mg)	Hardness (kg/cm^2)	Diameter (mm)	Thickness (mm)	Friability (%)	Assay (%)	pH
M1	218±2	4.3±0.2	9.53±0.03	2.1±0.1	0.40±0.02	99.24±1.6	6.32.±0.020
M2	214±2	4.6±0.2	9.49±0.02	2.1±0.1	0.36±0.05	101.08±1.9	6.40±0.024
M3	216±3	4.7±0.1	9.52±0.04	2.1±0.1	0.42±0.03	101.25±2.2	6.21±0.020
M4	215±3	4.5±0.2	9.49±0.03	2.1±0.1	0.29±0.04	102.00±1.1	6.11±0.023
M5	216±3	4.6±0.3	9.48±0.02	2.2±0.1	0.25±0.03	99.89±1.3	6.25±0.019
M6	214±2	4.4±0.2	9.53±0.03	2.1±0.1	0.19±0.06	101.75±1.2	6.24±0.016
M7	213±2	4.5±0.3	9.48±0.02	2.2±0.1	0.24±0.08	99.94±1. 2	6.34±0.014
M8	217±3	4.6±0.2	9.50±0.01	2.1±0.1	0.27±0.06	101.70±1.4	6.26±0.018
M9	215±4	4.6±0.2	9.49±0.03	2.2±0.1	0.31±0.05	100. 4±2.2	6.47±0.041

Table 7.9 enlists the response parameters of all the nine formulations prepared as per 3^2 factorial design, while Fig. 7.11a, b and c portrays their individual drug release profiles. Bioadhesion (Y_1), release until 8 hours [rel_{8hrs} (Y_2)], time taken to release 50% of drug [$t_{50\%}$ (Y_3)], diffusion coefficient [n (Y_4)] were taken as the response variables. The best release performance is exhibited by formulation M 5, i.e., prepared using the intermediate levels of Gantrez MS-955 and HPMC K 15M.

Table 7.9. Response parameters for formulations of MTX bilayered buccal tablets prepared as per 3^2 factorial design

Formulation code	Formulation composition		Release till 8 hrs (rel_{8hr})	$t_{50\%}$ (min)	n	Bioadhesion(B) ($x10^3$dynes x cm^2)
	Gantrez(mg)	HPMCK15M (mg)				
M1 (-1,-1)	5	10	104.6	72	0.4013	8.60
M2 (-1,0)	5	15	103.1	118	0.5277	8.9
M3 (-1, 1)	5	20	99.23	132	0.5778	9.3
M4 (0, -1)	15	10	103.20	108	0.4643	10.62
M5 (0, 0)	15	15	97.98	171	0.6585	11.23
M6 (0, 1)	15	20	88.11	207	0.6761	12.9
M7 (1,-1)	25	10	103.14	116	0.5055	12.83
M8 (1, 0)	25	15	101.02	139	0.5483	13.9
M9 (1, 1)	25	20	99.95	149	0.5850	14.2

Table 7.10. Observed and Predicted values of response parameters for Methotrexate bioadhesive tablets.

Batch	Response parameters											
	Y1			Y2			Y3			Y4		
	Observed	Predicted	%RE	Observed	Predicted	%RE	Observed	Predicted	%RE	Observed	Predicted	%RE
M1	8.60	8.40	2.38	104.60	104.38	0.211	72.00	71.75	0.348	0.40	0.39	2.56
M2	8.90	8.89	0.11	103.10	103.54	0.425	118.00	118.50	0.422	0.53	0.53	0.00
M3	9.30	9.51	2.21	99.23	99.01	0.222	132.00	131.75	0.190	0.58	0.59	1.69
M4	10.62	10.88	2.39	103.20	103.64	0.425	108.00	108.50	0.461	0.46	0.51	9.80
M5	11.23	11.54	2.69	97.98	97.09	0.917	171.00	170.00	0.588	0.66	0.63	4.76
M6	12.90	12.33	4.62	88.11	88.55	0.497	207.00	207.50	0.241	0.68	0.66	3.03
M7	12.83	12.77	0.47	103.14	102.92	0.214	116.00	115.75	0.216	0.51	0.48	6.25
M8	13.90	13.60	2.21	101.02	101.46	0.434	139.00	139.50	0.358	0.55	0.58	5.17
M9	14.20	14.56	2.47	99.95	99.73	0.221	149.00	148.75	0.168	0.58	0.59	1.69

% RE= % Relative Error

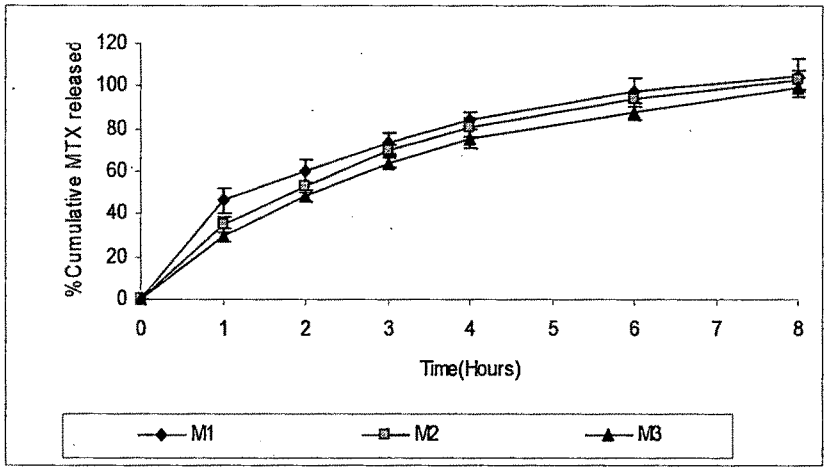


Fig. 7.11a. Dissolution profile of MTX tablets. Mean± SEM.(n=3)

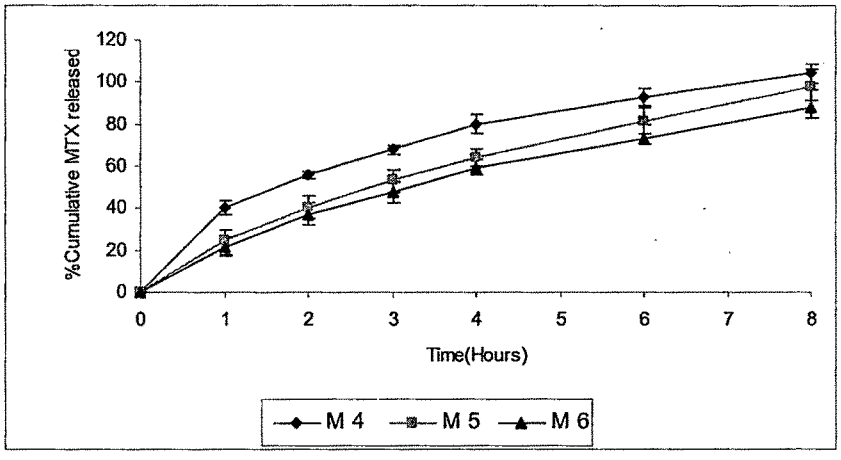


Fig. 7.11b. Dissolution profile of MTX tablets. Mean± SEM.(n=3)

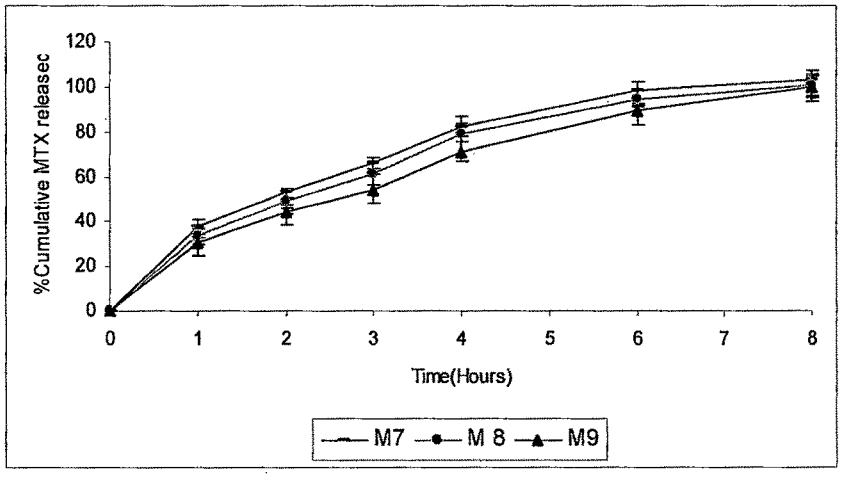


Fig. 7.11c. Dissolution profile of MTX tablets. Mean± SEM.(n=3)

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

On analyzing the data of all the 9 MTX tablet formulations prepared as per 3^2 Factorial design, using Design Expert® software, various polynomial equations, response surface and contour plots were generated (section 4.4.3.8). 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).

7.3.3. (a). Effect of Formulation variables on bioadhesion force (Y_1):

The constant and regression coefficient for Y_1 (bioadhesion force) were found to be significant using **quadratic model** and the equation is as follows:

$$Y_1 \text{ (Bioadhesion)} = +11.54 + 2.36 * X_1 + 0.73 * X_2 + 0.17 * X_1 * X_2 - 0.30 * X_1^2 + 0.065 * X_2^2 \quad \text{---------- (7.1)}$$

R-Squared = 0.9788.

The combined effect of X_1 and X_2 can be elucidated with the help of response surface plot (Fig 7.12a) which reveals that Y_1 varies in somewhat linear fashion with the amount of two polymers. High level of X_1 gave higher values of bioadhesion force at all the 3 levels of X_2 which indicates that X_1 has significantly positive effect on Y_1. Contour plot (Fig 7.12b) reveals that Y_1 varies in somewhat linear fashion with increase in the amount of each polymer. However, the effect of X_1 (Gantrez) seems to be more pronounced as compared with that of X_2 (HPMC K15M). The predicted and observed values of response parameters are shown in Table 7.10. Low values of the relative error showed that there was a reasonable agreement of predicted values and experimental values.

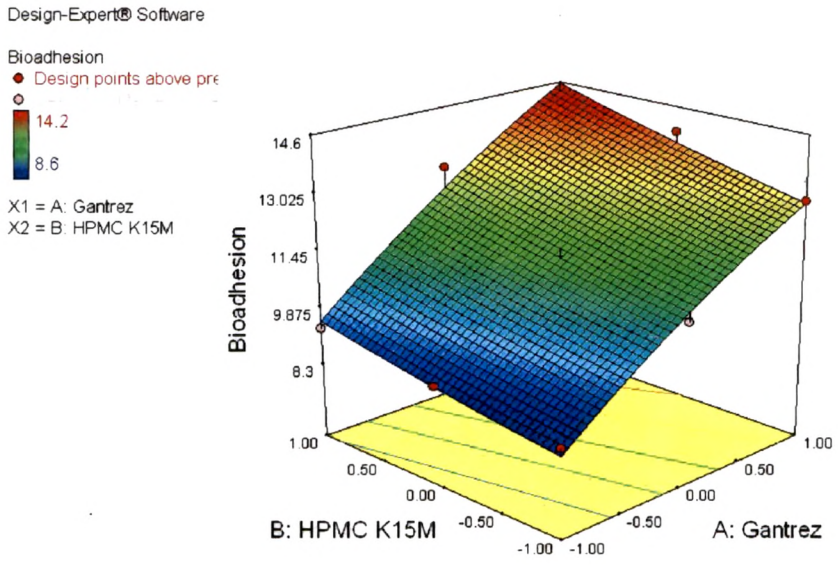


Fig7.12a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on Bioadhesive force.

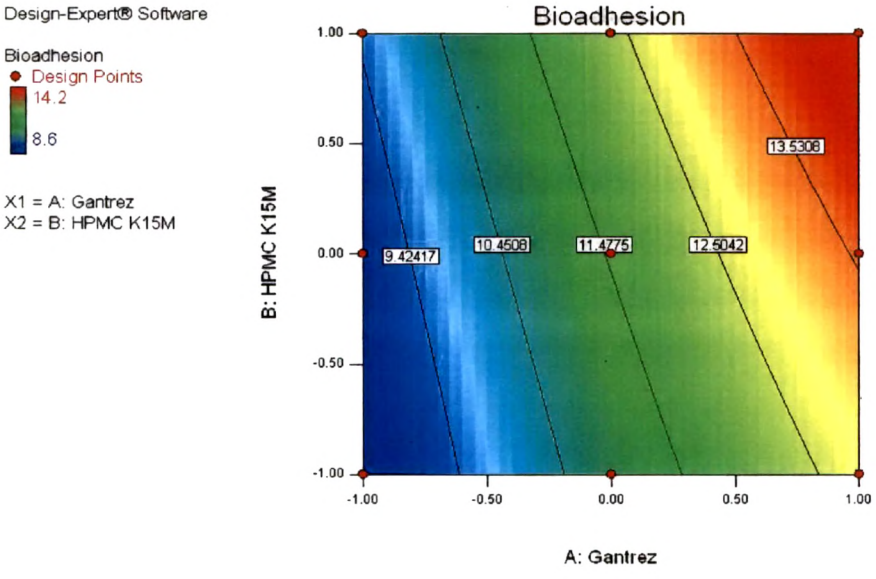


Fig 7.12b: Corresponding contour plot showing the relationship between various levels of both polymers.

7.3.3 (b) Effect of Formulation variables on percentage release at 8 h (Rel_{8hr} or Y_2)

The model terms for Y_2 (Rel_{8hr}) were found to be non significant on using both polynomial and quadratic models.

For polynomial model equation is;

$$Y_2 (Rel_{8hr}) = +97.09 - 1.04* X_1 - 7.54 * X_2 + 0.55 * X_1 * X_2 + 5.41* X_1^2 - 0.99 * X_2^2 + 5.40 X_1^2* X_2 + 0.85* X_1* X_2^2; \quad \text{----------(7.2)}$$

R-squared= 0.9911; PRESS value = 322.38.

However, on reducing the model to a quadratic one, all the model terms were found to be non significant and the R^2 value reduced significantly to 0.7894.

For quadratic model equation is;

$$Y_2 (Rel_{8hr}) = +97.09 - 0.47* X_1 - 3.94 * X_2 + 0.55 * X_1 * X_2 + 5.41* X_1^2 - 0.99 * X_2^2; \quad \text{----------(7.3)}$$

R-squared= 0.7894; PRESS value = 499.92.

As discussed in section 4.4.3.8, we used PRESS (Predicted Residual Sum of Square) values as the criteria for model selection. The PRESS value for the polynomial model was 322.38 whereas, for quadratic model it was found to be 499.92. As low PRESS values indicates adequate fitting of model (Huang et al, 2005). The polynomial model with the lower PRESS value was selected.

Response surface plot of Rel_{8hr} displays a non-linear relationship at all the levels of X_2 (Fig. 7.13a) whereas in case of X_1 a decreasing trend was observed upto intermediate level. At the intermediate position a nadir was observed. Increasing the polymer concentration (X_1) to the higher side leads to enhancement of release till 8 hours.

The contour plot (Fig.7.13b) shows that Gantrez (X_1) has a comparatively greater influence on the response variable than HPMCK15 M. High value of percentage MTX release at 8 h was observed in formulations having low value of either of the

independent variables (Table 7.9), 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 MTX 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 MTX 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 contains 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 MTX release at 8 h. Low values of the relative error (Table 7.10) showed that there was a reasonable agreement of predicted values and experimental values indicating the correctness of the model.

7.3.3. (c). Effect of Formulation variables on $t_{50\%}$ (Y_3):

The polynomial model for (Y_3) was found to be significant. Factors X_1, X_2 along with interaction factor X_1X_2 were found to be significant. Thus, model becomes:

$$Y_3 (t_{50\%}) = +170 +10.5*X_1+49.5 * X_2 -6.75*X_1*X_2 -41*X_1^2 -12* X_2^2 -26.5 X_1^2*X_2 + 4.75 X_1*X_2^2 \quad \text{..... (7.4)}$$

$R^2 = 0.9998$

For $t_{50\%}$ response surface (Fig.7.14a) and corresponding contour plots (Fig.7.14b) show a non linear trend in case of X_1, i.e. increasing trend was observed up to intermediate levels and further increase in concentration showed a declining phase.

The value of $t_{50\%}$ varied from 72 min to 207 min indicating that the variables significantly affected *($p<0.05$)* this parameter (Table 7.9). Linear relationship was

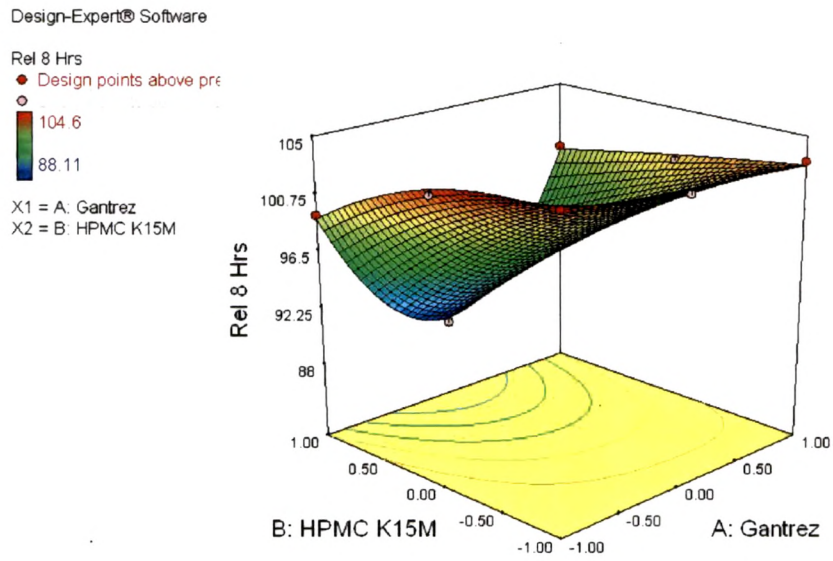


Fig 7.13a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on Rel_{8hrs}.

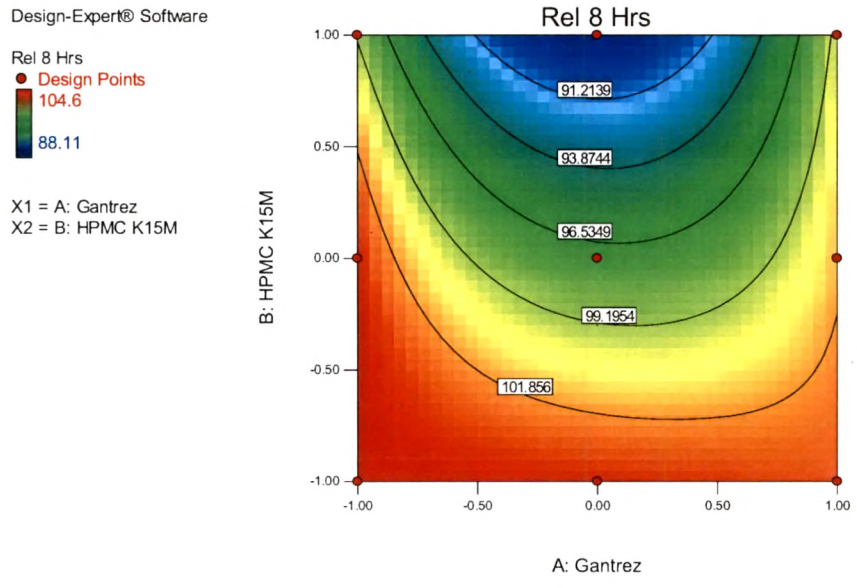


Fig 7.13b: Corresponding contour plot showing the relationship between various levels of both polymers.

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 7.14a and 7.14b).

This 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 exhibited visible erosion and faster release. Low values of the relative error (Table 7.10) showed that there was a reasonable agreement of predicted values and experimental values indicating the correctness of the model.

7.3.3. (d). Effect of Formulation variables on diffusion exponent 'n' (Y_4):

Dissolution profiles were fitted with the power law equation (Korsmeyer et al, 1983; Peppas, 1985) to calculate the values of diffusional exponent (n). To use Peppas equation, it is necessary that release occurs in one dimensional manner and that the system width/thickness or length/thickness relation (aspect ratio) be at least 10/1 (Peppas, 1985). 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. When aspect ratio is <10, the critical values of n for declaring Fickian diffusion and zero-order release were found to be 0.45 and 0.89, respectively (Peppas and Sahlin, 1989; Singh et al., 2006). When n approximates to 0.45, a Fickian/diffusion-controlled release is implied, where $0.45 < n < 0.89$, non-Fickian (anomalous) transport and $n = 0.89$ for zero order (case II transport).

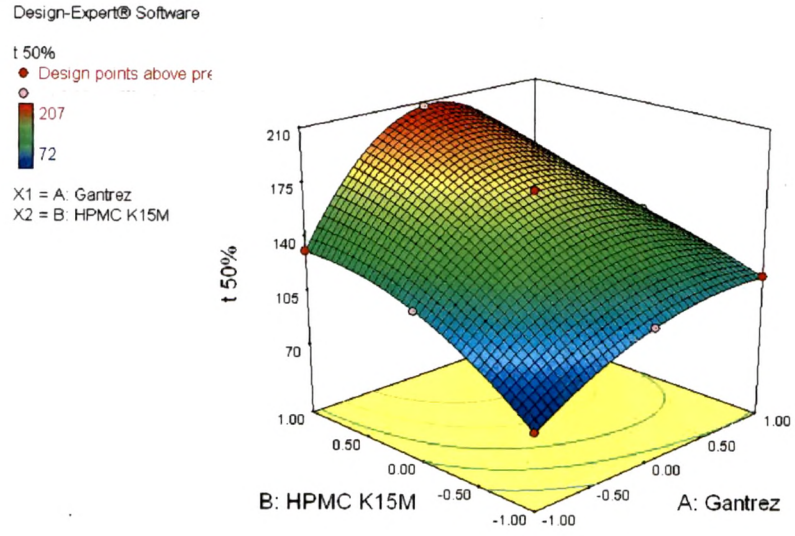


Fig 7.14a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on $t_{50\%}$.

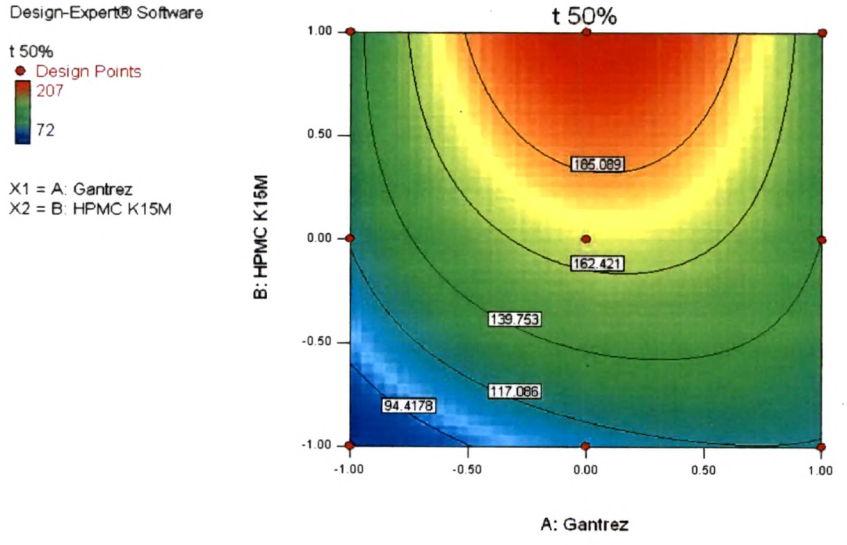


Fig 7.14b: Corresponding contour plot showing the relationship between various levels of both polymers.

Here the release occurred in one dimensional way and the aspect ratio is <10. Using an aspect ratio of 4.4 (calculated from diameter and thickness of tablets), the values of n for declaring Fickian diffusion, non Fickian difusion and zero-order release were taken as 0.45, 0.45 < n < 0.89 and 0.89, respectively (Peppas, 1985). Polynomial and quadratic model for 'n' were found to be non significant, hence PRESS value was used as the criteria for model selection.

Polynomial model equation is:

$$Y4\ (n) = +0.63 + 0.010*X_1 + 0.11*X_2 - 0.024*X_1*X_2 - 0.075*X_1^2 - 0.043*X_2^2 - 0.042\ X_1^2*X_2 + 0.018\ X_1*X_2^2 \quad \ldots\ldots\ldots (7.5)$$

R^2=0.9669; PRESS value = 0.37.

Quadratic equation is:

$$Y4\ (n) = +0.63 + 0.022*X_1 + 0.078*X_2 - 0.024*X_1*X_2 - 0.075*X_1^2 - 0.043*X_2^2 \quad \ldots\ldots\ldots (7.6)$$

R^2=0.9223; PRESS value = 0.049.

As the PRESS value for quadratic model was lower than that for the polynomial one, therefore quadratic model was selected. Results from equation 7.6 shows that both the factors X_1 and X_2 affected the values of release exponent 'n'. The value of correlation coefficient was 0.9223 indicating a good fit.

Response surface and contour plots for 'n' are shown in Fig. 7.15a and Fig. 7.15b. Diffusion exponent ranged from 0.401 to 0.676, 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 7.15a and 7.15b).

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 increase in the response curve for n was observed. All the

formulations except M1 displayed n value > 0.45. Thus, the release mechanism is predominantly non - Fickian diffusion. Low values of the relative error (Table 7.10) 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 7.11). The results of ANOVA in Table 7.12 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.

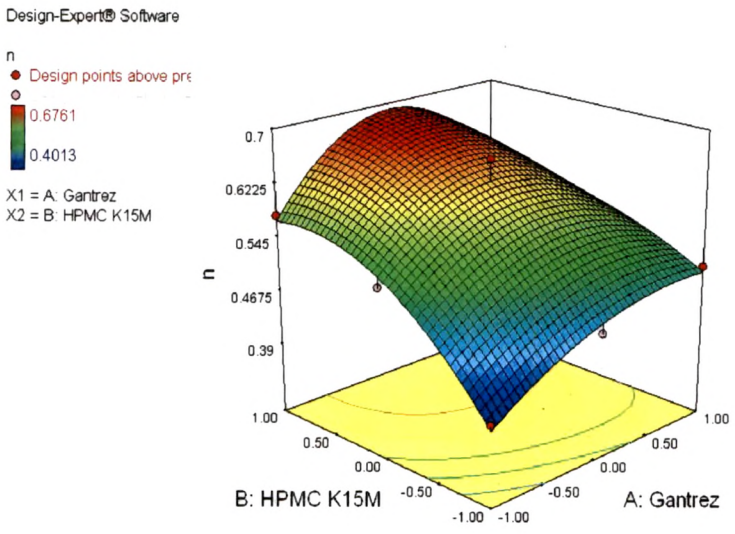


Fig 7.15a: Response surface plot showing the influence of Gantrez and HPMC K15M polymers on diffusion coefficient.

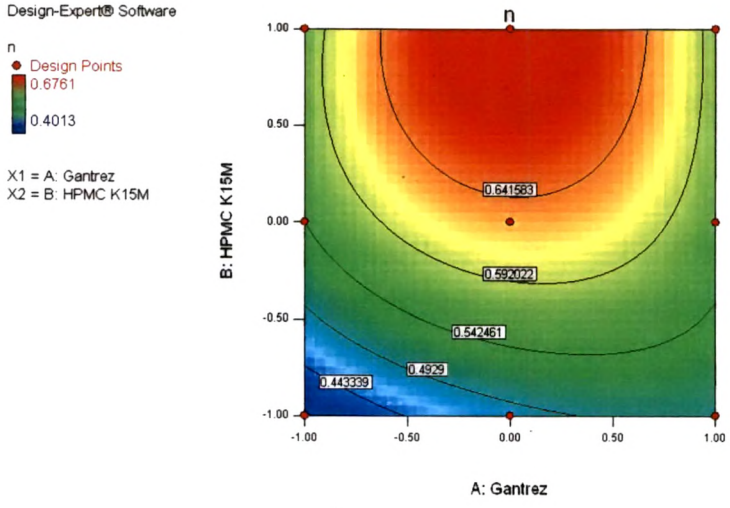


Fig 7.15b: Corresponding contour plot showing the relationship between various levels of both polymers.

Table 7.11. 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	11.54	2.36	0.73	0.17	-0.30	0.065	--	--	0.9788	0.0103
Rel_{8hr}	97.09	-1.04	-7.54	0.55	5.41	-0.99	5.40	0.85	0.9911	0.1912
$t_{50\%}$	170.00	10.50	49.50	-6.75	-41.00	-12.00	-26.25	4.75	0.9998	0.0278
n	0.63	0.022	0.078	-0.024	-0.075	-0.043	--	--	0.9223	0.0685

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

Table 7.12. Results of analysis of variance (ANOVA) for measured response

Parameters	df	SS	MS	F	Significance F
		$t_{50\%}$			
Model	7	12045.75	1720.82	764.81	0.0278
Residual	1	2.25	2.25	--	--
Total	8	12048.00	--	--	--
		Rel_{8hr}			
Model	7	196.18	28.03	15.84	0.1912
Residual	1	1.77	1.77	--	--
Total	8	197.95	--	--	--
		n			
Model	5	0.057	0.011	7.12	0.0685
Residual	3	4.789E-003	1.596E-003	--	--
Total	8	0.062	--	--	--
		Bioadhesion			
Model	5	36.72	7.34	27.68	0.0103
Residual	3	0.80	0.27	--	--
Total	8	37.52	--	--	--

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

7.3.4 Search for optimum formulations

A numerical optimization technique by the desirability approach was used to generate the optimum settings for the formulation (Section 2.8.4). 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 MTX release at 8 h was targeted to >90% with release exponent (n)>0.45 and time required for 50% of drug release ($t_{50\%}$) was kept at a range of 150 to 175 min. Formulation M5 (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 M5) 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 7.13 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 MTX bioadhesive buccal tablets.

Table 7.13. The predicted and observed response variables of the optimal MTX buccal bioadhesive tablets

	Y_1	Y_2	Y_3	Y_4
Predicted	10.80	98.21	162.44	0.63
Observed	10.62±0.91	97.98 ±1.5	171±2.3	0.65 ±0.09
Predicted Error (%)	-1.66	-0.234	5.26	-3.5

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

7.3.5 *In vitro* diffusion of MTX from tablets across buccal mucosa:

The penetration of MTX from tablet formulation (M 5) through buccal mucosa (Fig. 7.16) was found very low (Kp =2.66 (±0. 48) $x10^{-5}$ cm/sec) when compared with that of MTX solution (4.69 (±0.15) x 10^{-5} cm/sec) (Fig.7.1a; Table 7.1).

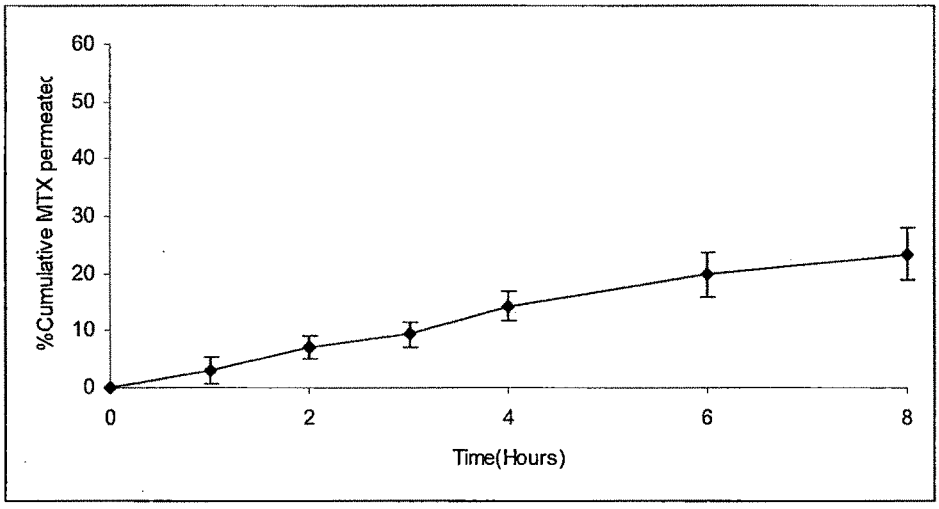


Fig. 7.16. ***In vitro*** **diffusion of MTX from tablets across buccal mucosal membrane.**

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

The formulation (M5) was prepared further using GMS as a permeation enhancer (0.1M) (Section 7.1). The bioadhesive strength of these tablets was 10.12±1.35 dynes/cm^2 while hardness was 4.5 ± 0.2 kg/cm^2. No significant difference in adhesive bond strength and hardness was observed between GMS containing and GMS free tablets. Addition of GMS to tablet formulations resulted in an increase in release rate (Fig 7.17). 45.17 ± 5.5%, 71.84 ± 4.8 % and 103.65± 6.4% MTX was released from MTX tablet formulations containing GMS as compared to 40.61 ± 4.05%, 64.48 ± 3.35% and 97.98 ± 4.6% from GMS free tablets (Fig 7.11) after 2, 4 and 8 hours respectively.

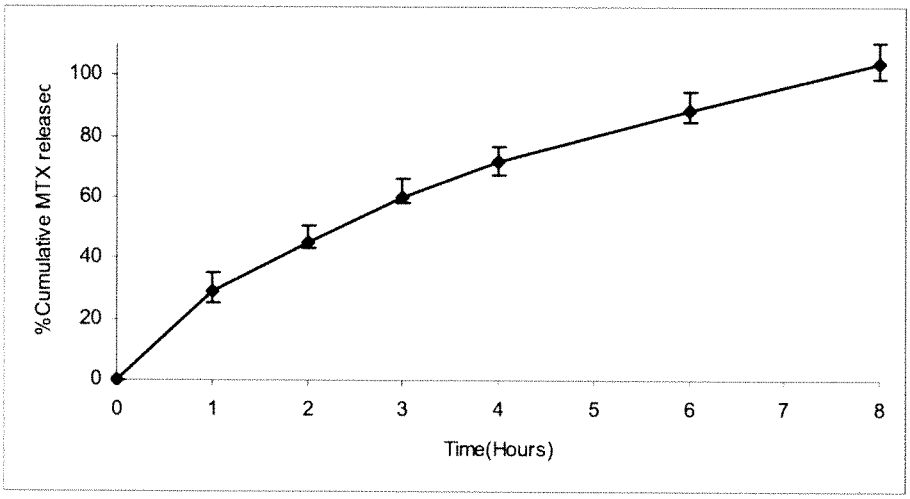


Fig 7.17. ***In vitro*** **release profiles of MTX from buccoadhesive Tablets containing GMS in phosphate buffer of pH 6.8 (values are expressed as mean ±SEM).**

7.3.6 *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.7.18) indicating that the tablets prepared using optimized combination of HPMC and Gantrez remained adhered to the buccal cavity at least for 4 hours.

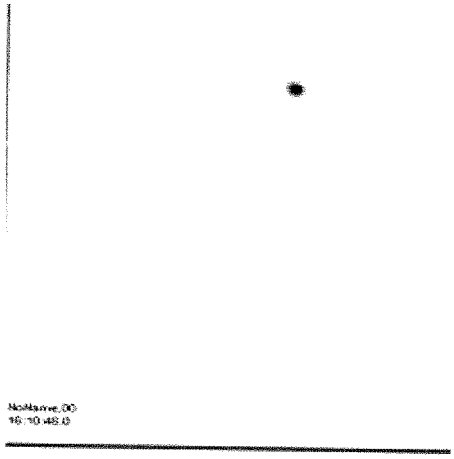
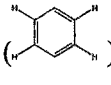


Fig. 7.18 **Scintigraphic Image showing bioadhesive tablet in buccal cavity of rabbit**

7.3.7 Compatibility studies:

IR spectra of MTX, its physical mixture with various polymers is shown in fig. 7.19 a, b. Pure MTX spectra showed sharp characteristic peaks at 840.3cm^{-1} (), 1538.4 - 1515.1 cm^{-1} (Amide II, Aryl systems), 1612.9 cm^{-1} (Aryl systems), 1694.9-1639.3 cm^{-1} (—C(=O)—[——COOH,——C(=O)—N(H)——]), 3333.3 - 2500.0 cm^{-1} (——COOH), and 3448.2 - 3225.8 cm^{-1} (H_2O, -NH_2). All the above characteristic peaks appeared in the spectra of physical mixture of MTX tablet components at same wavenumber indicating no modification or interaction between the drug and polymers.

X-ray diffraction (XRD) spectra of pure compound and binary systems with carriers are presented in Fig 7.20 a, b. The X-ray diffractogram of MTX has sharp peaks at diffraction angles (2θ) 9.32°, 11.44°, 12.84°, 14.34°, 17.66°, 19.30°, 24.27°, 25.49°, 21.78°,26.98°, 28.880°, and 31.15° showing a typical crystalline pattern. The peaks were similar in the MTX and physical mixture, thereby ruling out the drug-carrier interaction (Varma and Pandit, 2005).

7.3.8. Stability study of MTX bioadhesive tablets: MTX 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 (Tables 7.14) indicating a stable formulation at all conditions.

Table 7.14. Stability results for MTX 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)	4.5(±0.20)	4.6 (±0.18)	4.42(±0.14)
Friability (%)	0.25(±0.02)	0.22(±0.06)	0.28(±0.04)
Assay(%w/w)	99. 40 (± 0.214)	98.75 (±0.164)	97.25 (± 0.095)
Release (8 h)	103.65(± 6.4)	92.14 (± 6.15)	95.65 (± 5.64)

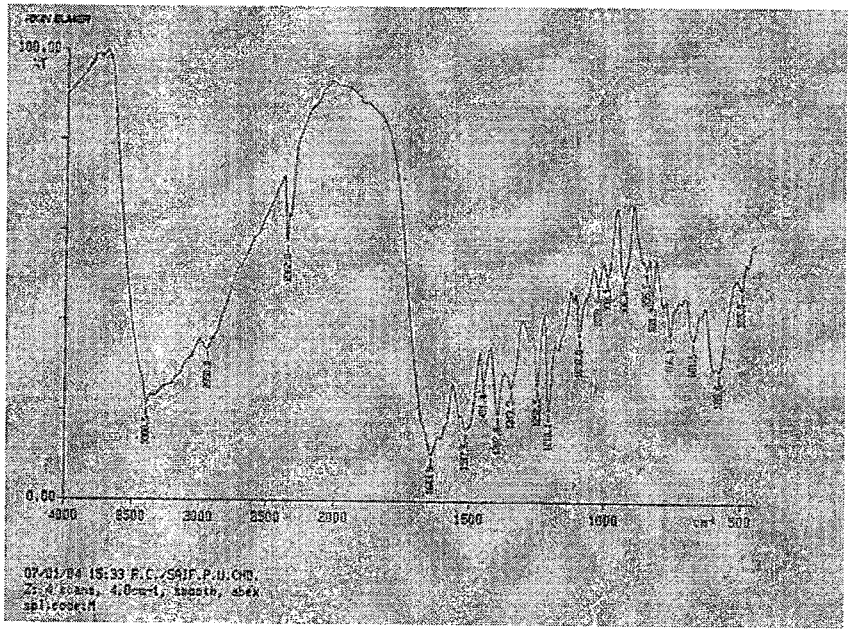


Fig. 7.19 a. FTIR spectra of MTX

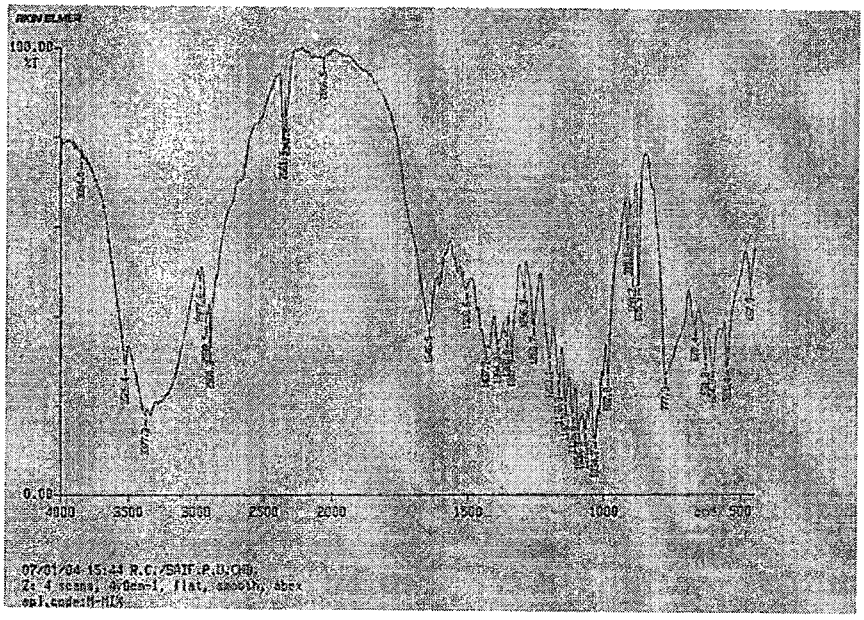


Fig 7.19 b. FTIR spectra of MTX tablet mix

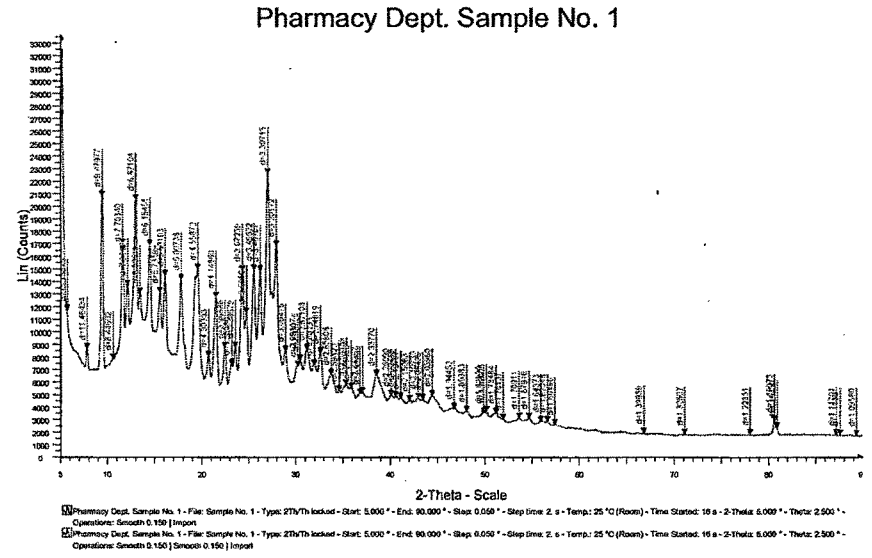


Fig. 7.20 a . XRD spectra of MTX

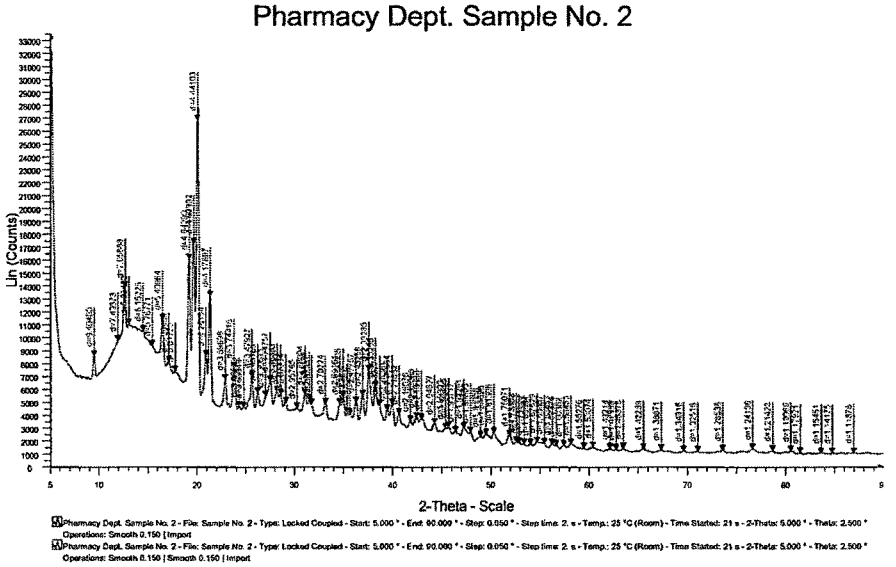


Fig 7.20 b. XRD spectra of MTX tablet mix

7.4. FORMULATION OF MTX GELS

7.4.1. *Preparation of MTX gel formulations:*

Bioadhesive gels containing MTX were formulated using Poloxamer-407 and Gantrez in various combinations. All formulated gels were found to be yellow colored (due to drug), clear, and viscous indicating that drug remained completely dissolved in the gel.

7.4.2. *Evaluation of Gel formulations*

7.4.2.1 Bioadhesive Force

Assessment of the mucoadhesive strength in terms of detachment stress showed that the adhesive properties increased with the addition of Gantrez (Fig 7.21).The detachment force of gels containing 16% Poloxamer -407 and Gantrez S 97 in increasing amounts from 3-6% (MG1-MG4) was less than that of gels containing 17% Poloxamer -407 and increasing proportion of Gantrez S 97 from 3-6% (MG5-MG8).

An increasing trend in the bioadhesive strength with increase in the amount of Gantrez S 97 was observed. Formulation MG11conataining 18% Poloxamer -407 and 5% Gantrez displayed maximum bioadhesive force (19.81±2.02 x 10^3 dyne/cm^2). Minimum adhesion strength was observed in formulation MG1 containing lowest amount of Poloxamer - 407(16%) and lowest Gantrez content (3%). However, statistical analysis showed statistically insignificant difference between the various formulations ($p>0.05$). It seems that increase in overall polymer ratio leads to more adhesion sites (more free functional groups such as carboxyl) and subsequently stronger adhesion is observable (Gurny et.al, 1984).

Gantrez has 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 (Majithiya et al., 2006; Kunisawa et al., 2000).

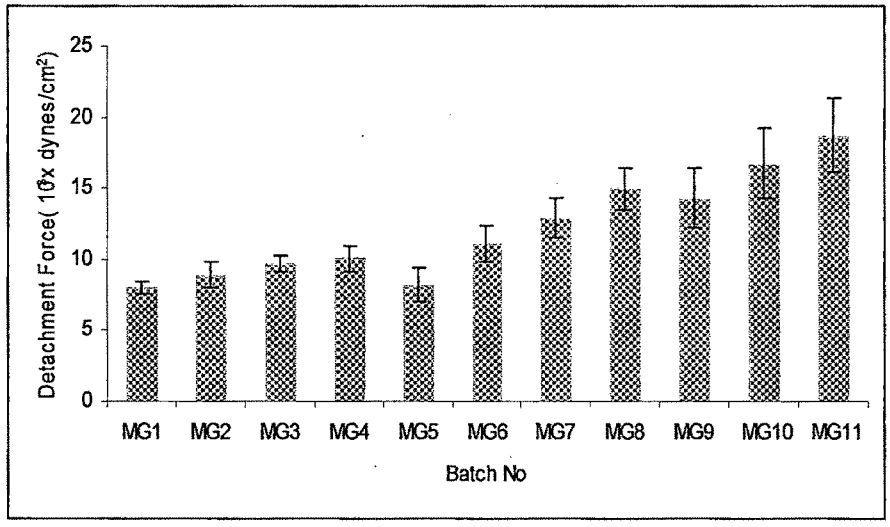


Fig. 7.21 **The bioadhesive strength between the mucosa and MTX gels.** Values are expressed as mean ± S.E.M. (n = 3).

7.4.2.2 Assay: All the formulations were found to exhibit assay values in the range 95-105 % depicting good content uniformity (Table 7.15).

7.4.2.3. pH measurements : Table 7.15 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, indicating that the gel would not cause irritation in the buccal cavity.

7.4.2.4. Viscosity

The apparent viscosity of the Formulation MG7 at 45 RPM (at 25±1°C) was found to be 550.52 ± 3.5cps using CP 40 spindle. However, when it was subjected to variable shearing stress, the behavior indicated plastic flow (Fig.7.22). 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 7.22 insert).

Table 7.15. pH and Assay of MTX bioadhesive gel formulations

Batch No.	pH	Assay (%)
MG1	5.67 (±0.06)	97.24 (±0.151)
MG 2	5.98 (±0.051)	99.02 (±0.094)
MG 3	5.79(±0.042)	99.75 (±0.146)
MG 4	6.29 (±0.064)	99.32 (±0.204)
MG 5	6.38 (±0.016)	98.05 (±0.101)
MG 6	6.44 (±0.048)	99.63 (±0.099)
MG 7	6.21 (±0.035)	99.94 (±0.225)
MG 8	6.61(±0.064)	98.88 (±0.208)
MG 9	6.74 (±0.024)	99.03 (±0.198)
MG 10	6.84 (±0.021)	98.88 (±0.152)
MG 11	6.97 (±0.073)	97.95 (±0.095)

(±SEM): Standard Error Mean, n=3.
Note: Each reading is an average of three determinations.

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 7.22 gave yield value (361.78 dynes/cm^2) which was used to find plastic viscosity. The plastic viscosity of the gel was calculated to be 418.04 cps by using the following formula (Martin, 1999).

U= (F-f)/G; ----------(7.7)

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

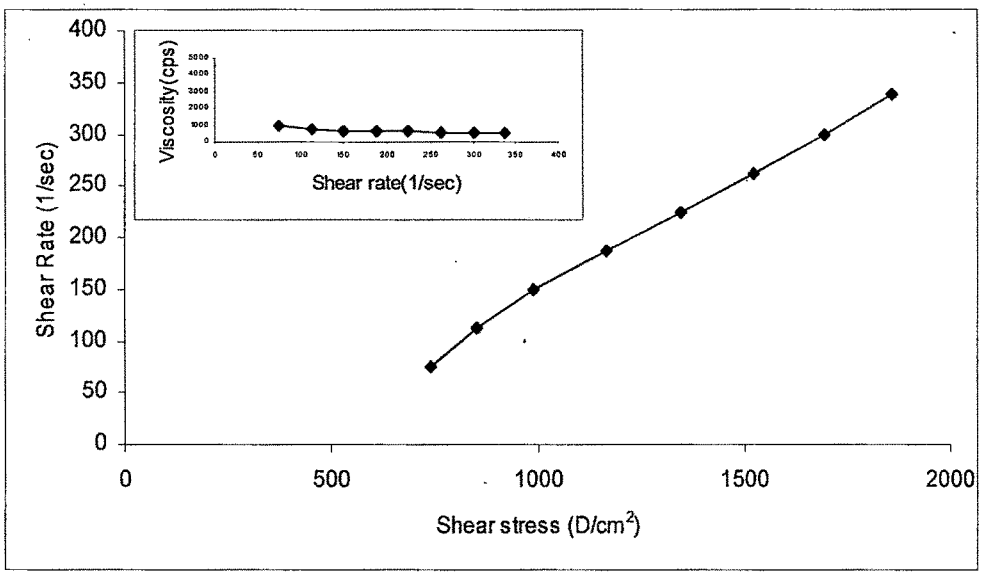


Fig.7.22. Effect of Shear rate on shear stress. The insert represents the effect of shear rate on viscosity of gel.

7.4.3. *In vitro* diffusion study

7.4.3.1 *In vitro* diffusion across cellulosic membrane

The *in vitro* release profiles from various formulations are depicted in Fig 7.23a, 7.23b and 7.23c. Batch MG1, MG2, MG3 and MG4 contained Poloxamer-407 (16% w/w) and varying proportions of Gantrez S 97 (3%, 4%, 5% and 6% respectively).

Formulations MG5-MG8 contained 17% Poloxamer-407 and 3-6% Gantrez while MG9-MG11 contained 18% P-407 and 3-5% of Gantrez. Increase in Poloxamer content displayed decrease in MTX release. As the content of Gantrez was increased, decrease in release was evident from the release profile data.

In the batches (MG1-4), drug release was accompanied by burst release and so the amount of Poloxamer-407 was increased from 16% to 17%. Batches with higher Poloxamer- 407 composition displayed lowering in burst release than those batches with its low content. Increasing the concentration of Poloxamer-407 from 17% to

18% significantly decreased the cumulative percent drug released after 8 h from 96% (MG7) to 81% (MG11).

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**). Another inert, hydrophilic polymer would decrease the amount of free water in the water channels, and affect molecular orientation of the gel matrix. Thus Gantrez® decreased the rate of MTX release from the gel. Similar results have also been obtained in a previous study, where inclusion of PVP, CMC, and HPMC in the P-407 gel decreased the rate of release of ceftiofur (Zhang et al., 2002). Formulation MG 7 was considered for further studies as it displayed nearly complete release of MTX (98.2 ± 4.8%) in 8 hours.

7.4.4. *Drug release kinetics*

Table 7.16 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.

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 0.5794 and 0.8874. 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. All the formulations exhibited 'n' value in the range 0.5 - 1.0 indicating anomalous (non-Fickian diffusion). Also,

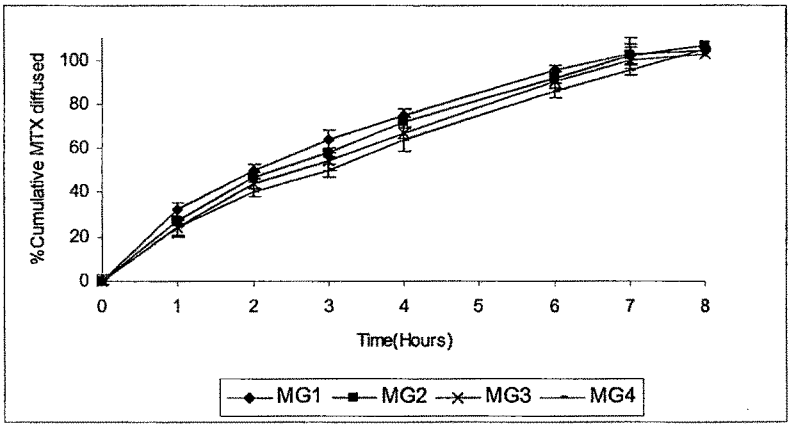


Fig.7.23a. ***In vitro*** **diffusion of MTX from gels across cellulose membrane. Data are expressed as mean ± S.E.M. for** ***n=3.***

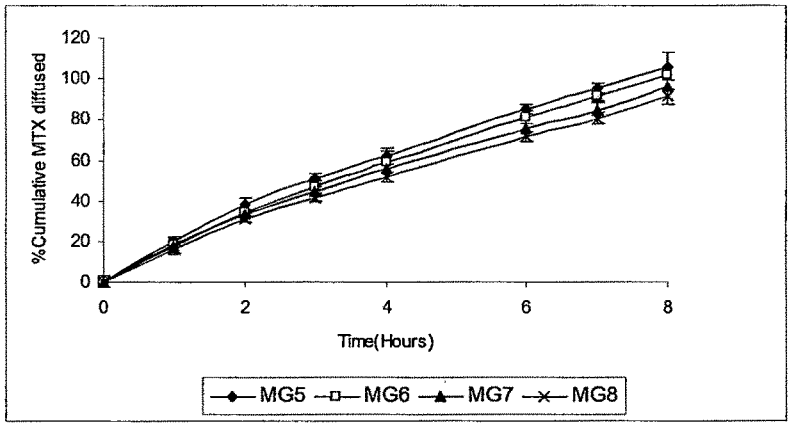


Fig. 7.23b. ***In vitro*** **diffusion of MTX from gels across cellulose membrane. Data are expressed as mean ± S.E.M. for** ***n=3.***

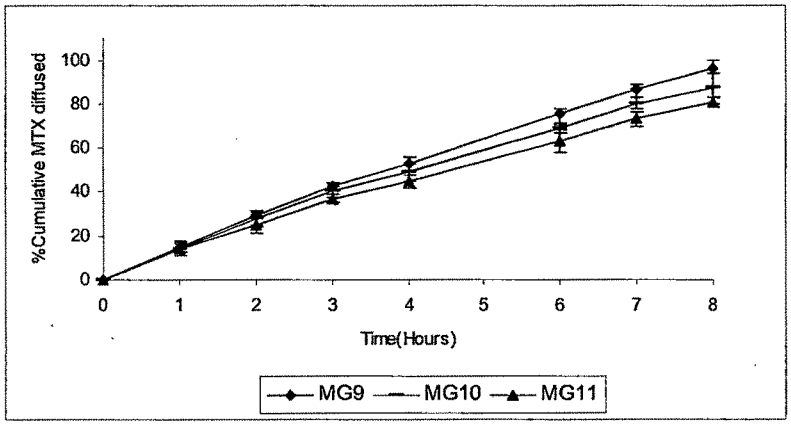


Fig. 7.23c. ***In vitro*** **diffusion of MTX from gels across cellulose membrane. Data are expressed as mean ± S.E.M. for** ***n=3.***

the formulations were observed to yield statistically valid correlations with the Higuchi and Zero-order models too (Table 7.16).

The non-Fickian behaviour (n=0.5-1.0) of the formulations may be attributed due to the gelling property of Poloxamer 407(Ahuja et.al, 2007). For MG 7 (the optimized batch), value of 'n' was observed to be 0.7998 (r^2 = 0.998) and other kinetic parameters were Higuchi (r^2 = 0.9947), Zero order (r^2 = 0.9951), First order (r^2 = 0.9072). The results point out the prevalence of diffusional mechanistic phenomena, in consonance with the results obtained while fitting Korsemeyer–Peppas Model.

Table 7.16. Release Kinetics of MTX containing bioadhesive gels

BatchNo.	Zero order		First order		Higuchi		Peppas	
	r^2	k_0	r^2	k_1	r^2	k_h	r^2	n
MG1	0.9634	10.32	0.8867	0.0675	0.9933	41.075	0.9949	0.5794
MG2	0.9776	11.147	0.8902	0.0767	0.9974	44.097	0.9949	0.657
MG3	0.9799	11.301	0.8918	0.082	0.9951	44.602	0.9937	0.7013
MG4	0.9951	11.41	0.9292	0.0844	0.9943	44.685	0.9986	0.7085
MG5	0.9935	11.894	0.9013	0.0922	0.9966	46.653	0.9971	0.7855
MG6	0.9954	11.719	0.906	0.0968	0.9958	45.906	0.9986	0.8234
MG7	0.9951	10.805	0.9072	0.0941	0.9947	42.309	0.998	0.7998
MG8	0.9963	10.346	0.9102	0.0956	0.9935	40.462	0.998	0.8112
MG9	0.9974	11.465	0.9086	0.1045	0.9932	44.807	0.999	0.8874
MG10	0.995	10.325	0.8988	0.102	0.9947	40.433	0.9972	0.8698
MG11	0.9973	9.4627	0.9195	0.0995	0.9917	36.956	0.9992	0.8397

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

The permeability coefficients (Kp) of MTX from gel across cellulose membrane and buccal mucosal membrane were 1.8275x 10^{-4}cm/sec and 2.97x10^{-5} cm/sec respectively. The penetration of MTX from gel formulation (MG 7) through buccal

mucosa (Fig. 7.24) was found very low when compared with cellulose membrane (Fig. 7.23b) and the difference was statistically significant *($p < 0.05$)*.

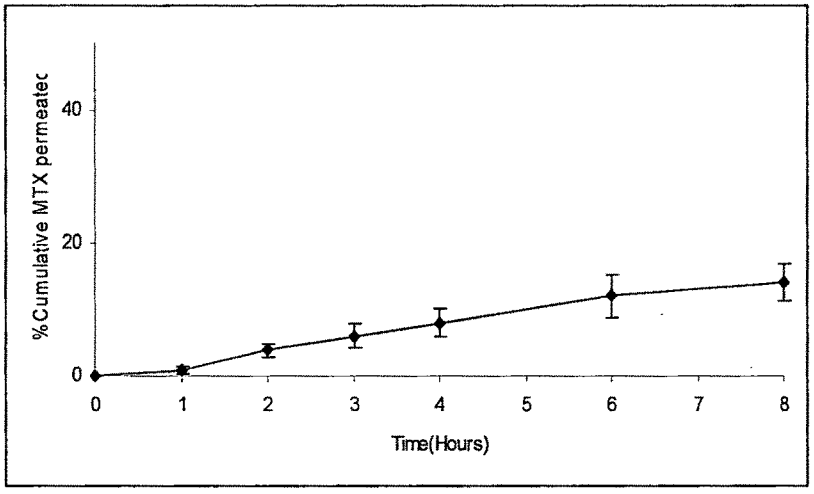


Fig.7.24 Release of MTX from gels across buccal membrane. Data are expressed as mean ± S.E.M. for n=3.

The final formulation (MG 7) selected on the basis of in vitro release study and other characterization parameters was prepared using oleic acid (3%)+PG(5%)+ETOH(10%) as a permeation enhancer (as discussed in section 5.1.1) for *in vivo* studies. The bioadhesive strength of these gels was 10.99 ± 1.41 dynes/cm^2. No significant difference in adhesive bond strength was observed between this formulation and MG 7. Addition of penetration enhancers to gel formulations resulted in an increase in release rate (Fig 7.25). 40.54. ±2.98%, 69.64 ±4.82% and 102.2± 5.2% MTX was released from MTX gel formulations containing enhancer as compared to 33.16 ± 4.2%, 55.64 ± 3.1% and 96.12 ± 3.2% from enhancer free gels (MG7) after 2, 4 and 8 hours, respectively.

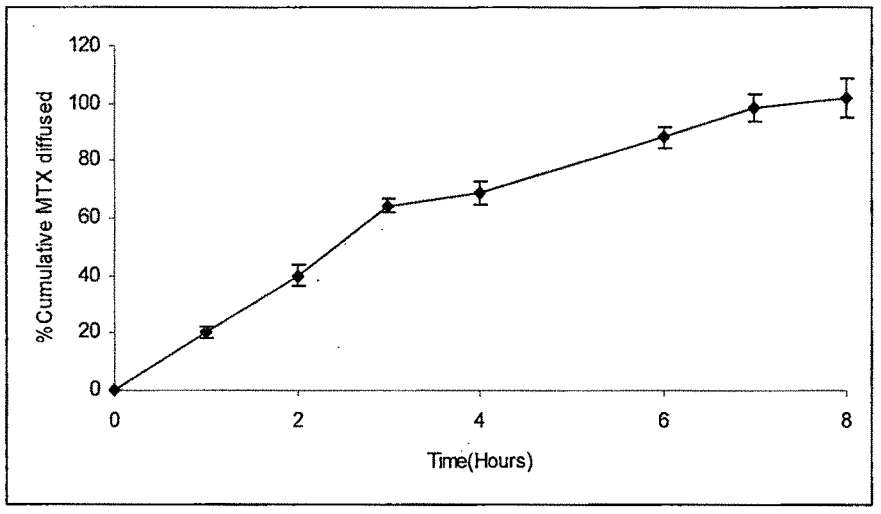
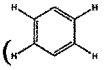
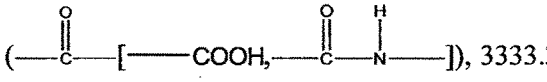


Fig 7.25 ***In vitro*** **diffusion of MTX from buccoadhesive gels containing oleic acid+ propylene glycol and ethanol across diffusion membrane (values are expressed as mean ±SEM; n=3).**

7.4.6 Drug: Polymer compatibility studies:

7.4.6.1 Fourier Transform Infrared (FTIR) Spectroscopy

IR spectra of MTX, its physical mixture with various polymers and solid dispersions with polymers are presented in Fig 7.26. Pure MTX spectra showed sharp characteristic peaks at 840.3 (), 1538.4 - 1515.1 (Amide II, Aryl systems), 1612.9 (Aryl systems), 1694.9-1639.3 (), 3333.3 - 2500.0 (——COOH), and 3448.2 - 3225.8 cm^{-1} (H_2O, $-NH_2$). 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 MTX would be compatible with all these polymers when formulated in a gel (Shin et. al., 2000).

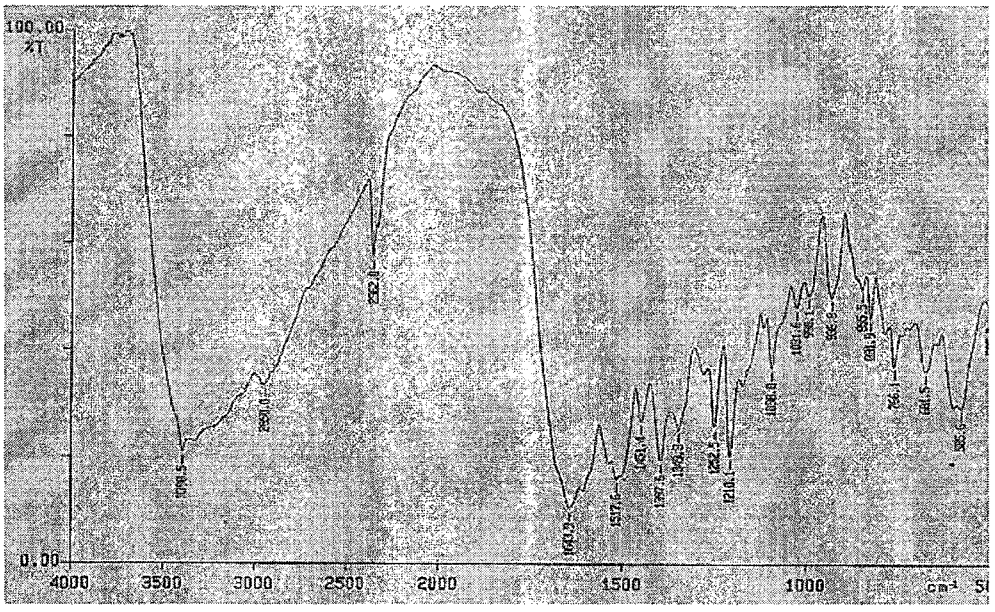


Fig.7.26a. FTIR spectra of MTX

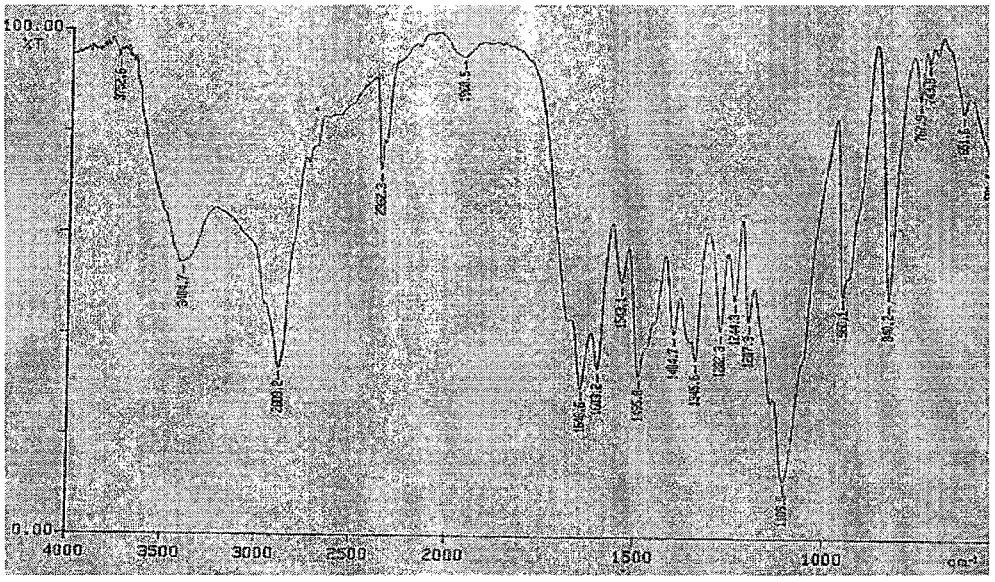


Fig. 7.26b FTIR spectra of MTX + Poloxamer 407 mixture(1:4)

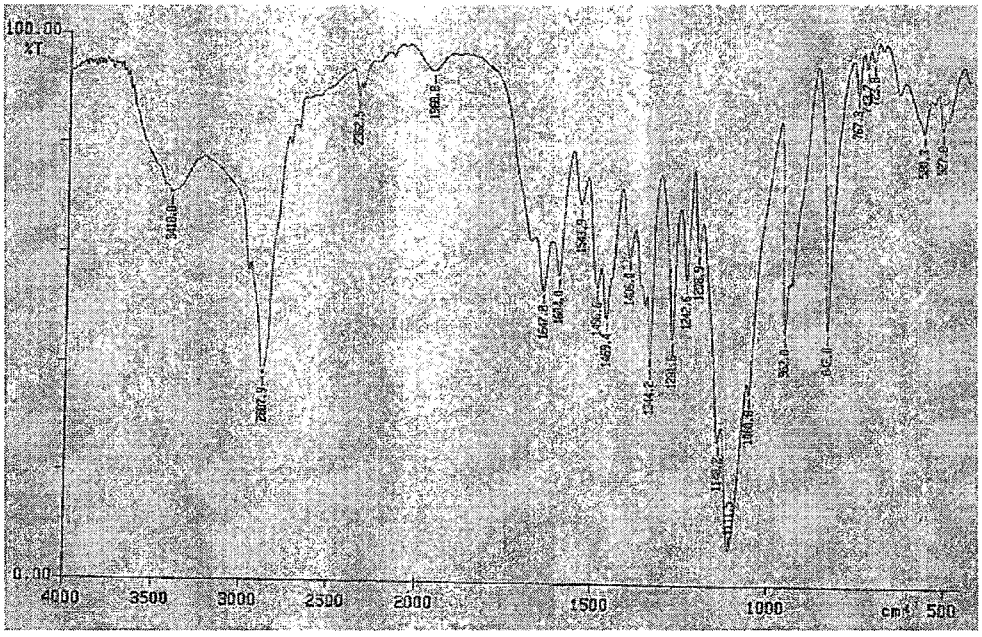


Fig. 7.26c FTIR spectra of MTX+ Poloxamer 407 Solid Dispersion(1:4)

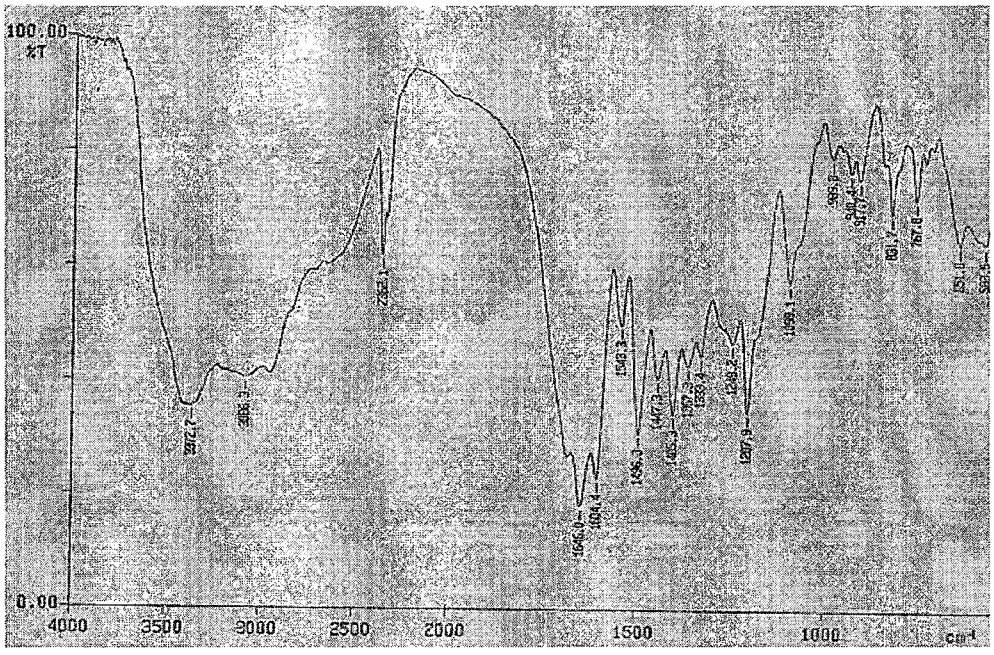


Fig. 7.26d FTIR spectra of MTX+ Gantrez S 97 Mixture(1:4)

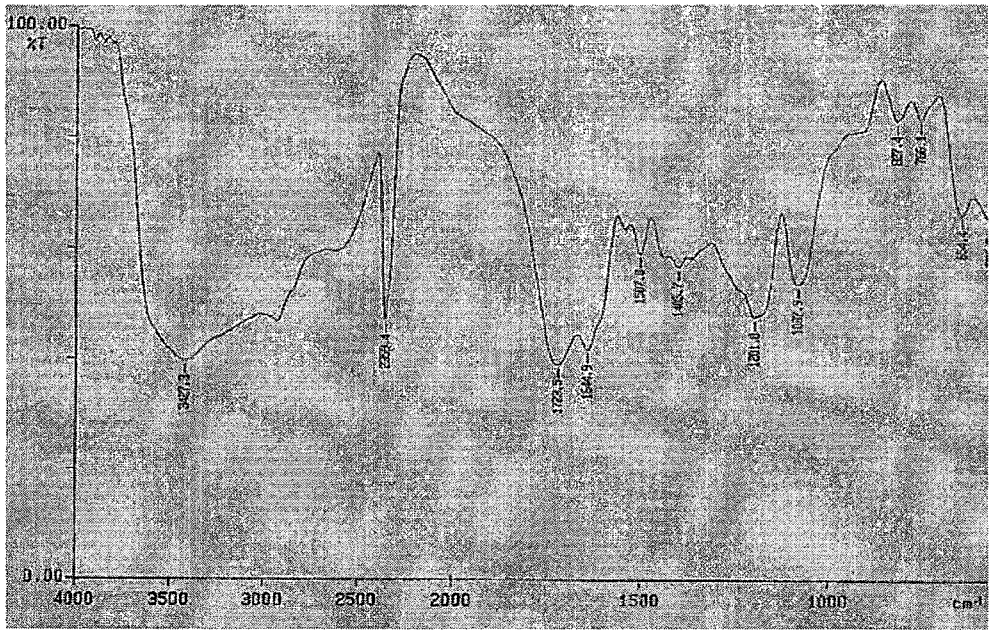


Fig. 7.26e FTIR spectra of MTX + Gantrez S 97Solid Dispersion

7.4.6.2 X ray Diffraction:

X-ray diffraction (XRD) spectra of pure compound and binary systems with carriers are presented in Fig 7.27. The X-ray diffractogram of MTX has sharp peaks at diffraction angles (2θ) 9.32°, 11.44°, 12.84°, 14.34°, 17.66°, 19.30°, 24.27°, 25.49°, 21.78°,26.98°, 28.880°, and 31.15° 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 7.17); clearly suggesting that there is no amorphization of MTX and it is still in its original crystalline form.

Table 7.17. Characteristic XRD peaks of MTX, MTX physical mixture and solid dispersions with polymers

S.No.	Formulation	Characteristic peaks at 2θ (degrees) *Intensity in Parentheses
1	MTX	9.32°(85.3),11.44°(48.3),12.84°(69.1),14.34° (47.9),17.66°(43.0),19.53°(55.0),23.3°(102880), 24.27°(48.8),25.49°(58.8),26.98°(87.5), 28.88°(11.8), 31.15°(14.7)
2	MTX-Gantrez S 97 Physical mixture (1:4)	9.20°(100),11.40°(61.8),12.7°(100),14.3°(82.5), 17.6°(69.2),19.35°(69.2),23.3°(100),24.32° (45.1),25.4°(57.1),26.8°(77.1), 28.80°(11.6), 31.18°(10.8).
3	MTX-Gantrez S 97 solid dispersion (1:4)	9.46°(83.2),12.92°(43.3),14.54°(33.5),17.79° (37.7),19.48°(63.7),23.3°(100),24.32°(49.2),26. 85°(18.3), 28.80°(9.3),30.9°(2.0).

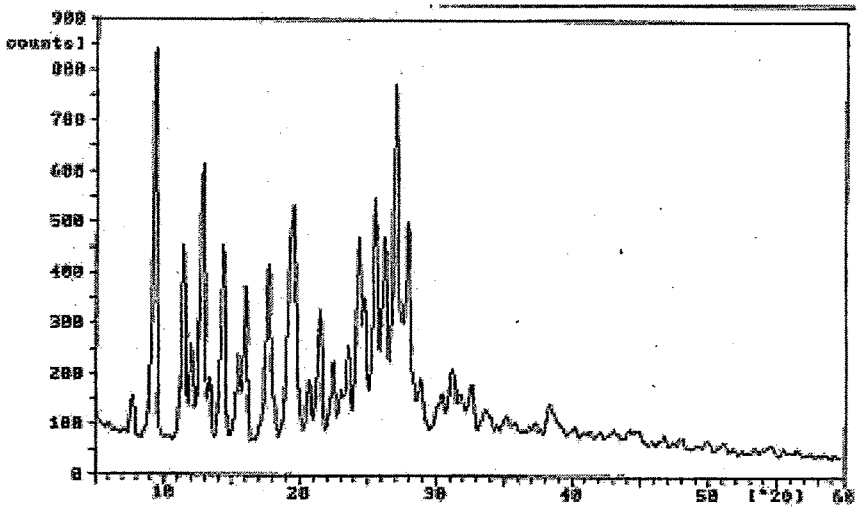


Fig.7.27a XRD spectra of MTX

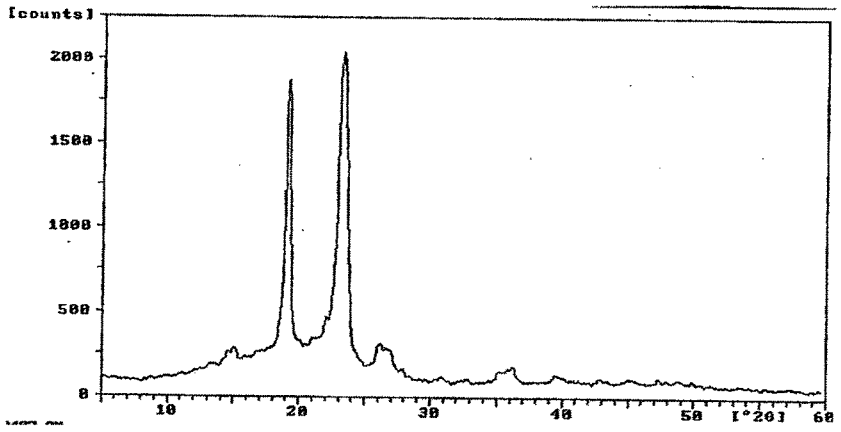


Fig. 7.27b XRD spectra of Poloxamer 407

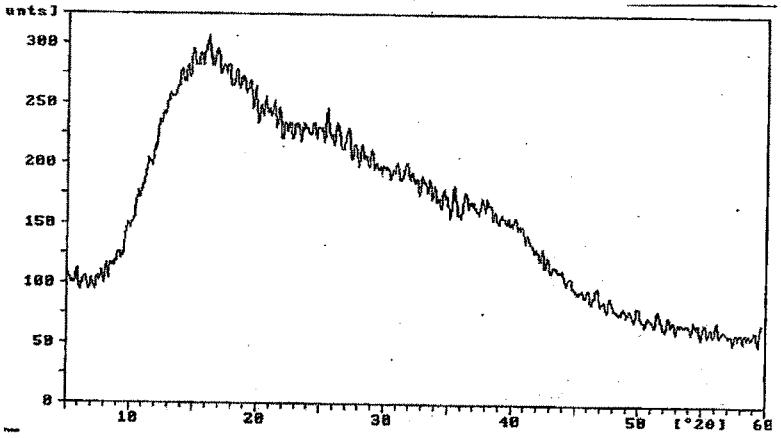


Fig.7.27c XRD spectra of Gantrez S 97

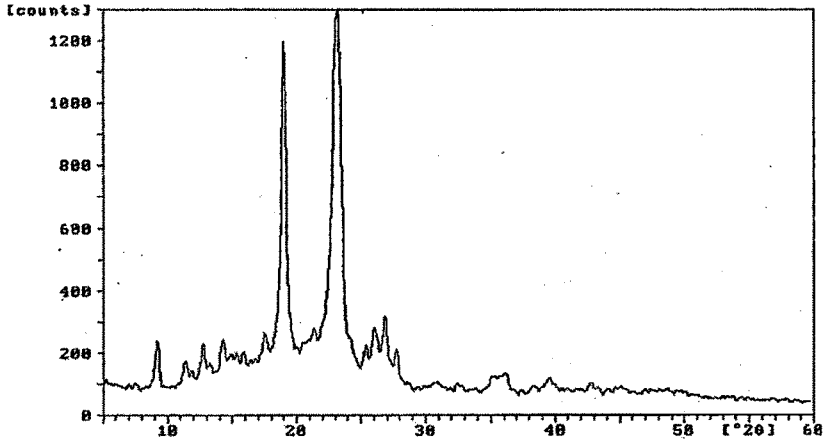


Fig.7.27d XRD spectra of MTX+ Poloxamer 407 MIX(1:4)

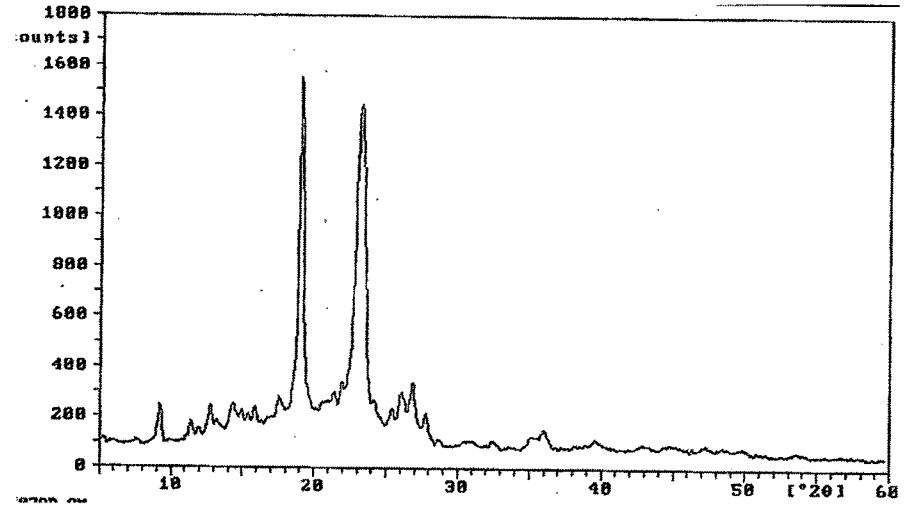


Fig.7.27e XRD spectra of MTX +Poloxamer 407 SD(1:4)

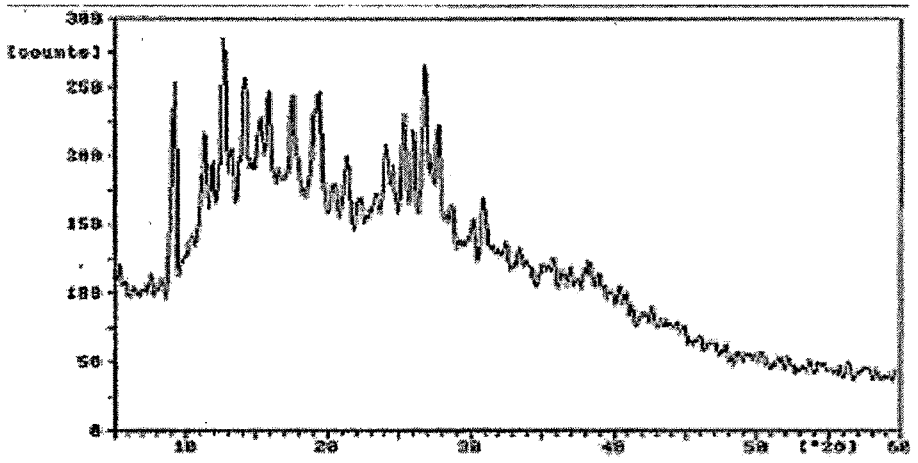


Fig.7.27f XRD spectra of MTX +Gantrez S 97 Mixture(1:4)

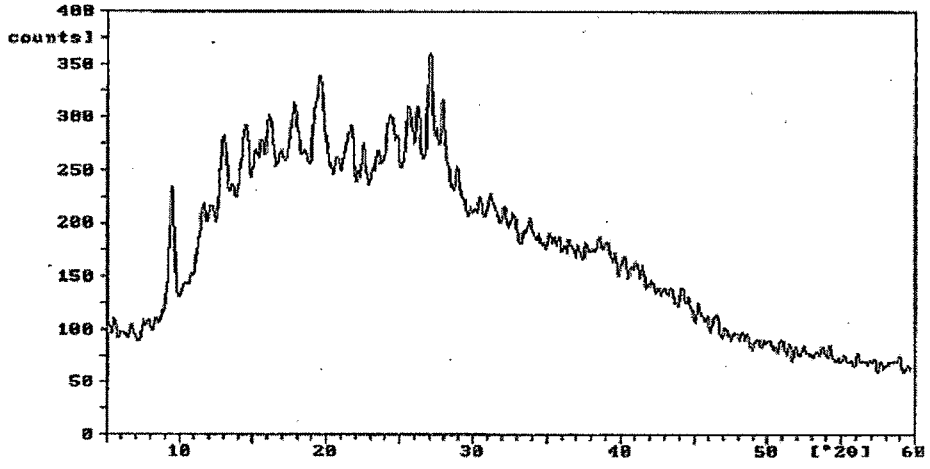


Fig.7.27g XRD spectra of MTX +Gantrez S 97 Solid dispersions (1:4).

7.4.7 *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.7.28). 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.

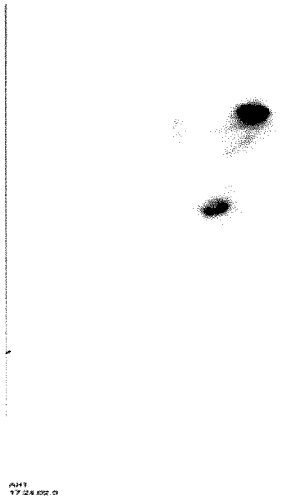


Fig.7.28 Scintigraphic Image showing bioadhesive gel in buccal cavity of rabbit

7.4.8. Stability studies of bioadhesive gels:

Table 7.18 depicts stability results of MTX 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, MTX bioadhesive gels presented good stability.

Table 7.18. Stability results of MTX bioadhesive gels

Parameter	Initial	2 Months	3 Months
		25 ± 2°C	25 ± 2°C
pH	6.51 (±0.035)	6.85(±0.021)	6.98(±0.015)
Assay(%w/w)	99.94 (±0.205)	99.05 (±0.201)	97.05 (± 0.095)
Release (8 hour)	96.80(±6.38)	98.14(± 6.15)	94.65(± 5.64)

7.4.9 Histology studies on buccal mucosa:

Fig 7.29a shows photo micrograph of buccal mucosa. Typical photo micrographs of buccal epithelium after 8 hour treatment with different formulations of MTX (patch, tablet and gel) containing penetration enhancers are shown in Fig 7.29 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. These histological findings explain well the permeation results where the permeation of MTX through the buccal mucosa increased significantly *($p<0.05$)* in presence of enhancers (Section 7.1)

7.5. *IN VIVO* STUDIES ON MTX BIOADHESIVE PATCHES, TABLETS AND GELS:

The plasma concentration- time curve for MTX after the oral conventional tablets (prepared using MTX, lactose and magnesium stearate) and I.V. administration to rabbits of a single 2.5 mg dose {Marketed MTX injection,I.P. (Biotrexate®, Biochem pharmaceutical Industries, Mumbai.} is shown in Fig. 7.30 while Fig 7.31 depicts plasma concentration–time profile of MTX containing bioadhesive dosage forms developed in our lab (patch, tablet and gel). Table 7.19 gives Mean plasma concentration (ng/ml) of MTX from the oral, IV and buccal mucoadhesive administration of MTX formulations. The pharmacokinetic parameters of MTX following administration of oral conventional tablet, I.V. injection, and application of buccal bioadhesive formulations are summarized in Table 7.20.

Fig.7.29.a) Photmicrograph(40X) of Buccal mucosa Control

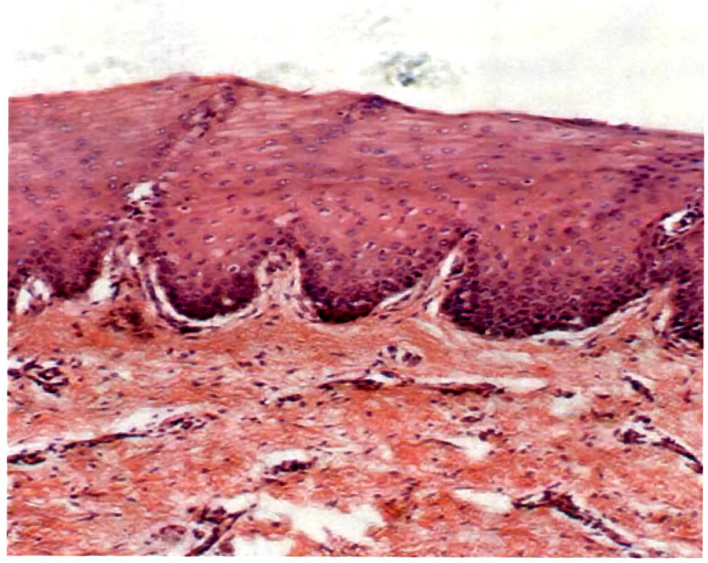


Fig.7.29.b) Photmicrograph(40X) of Buccal mucosa after 8 hour treatment with MTX bioadhesive patches

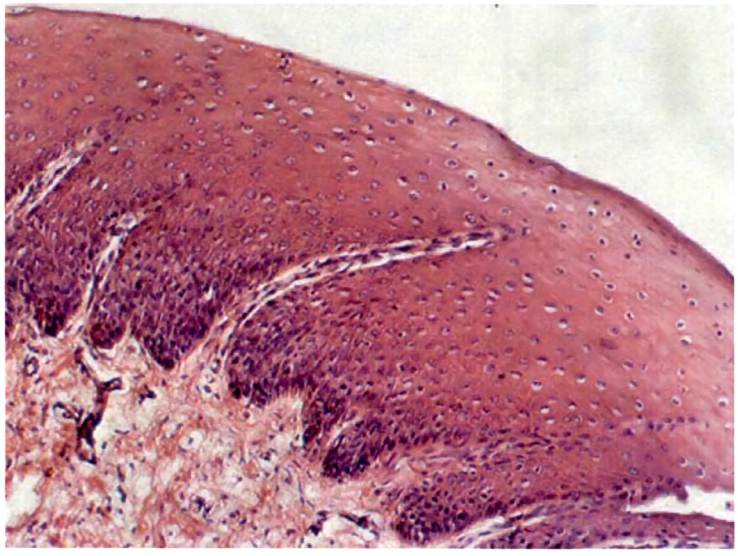


Fig7.29.c) Photmicrograph(40X) of Buccal mucosa after 8 hour treatment with MTX bioadhesive tablets

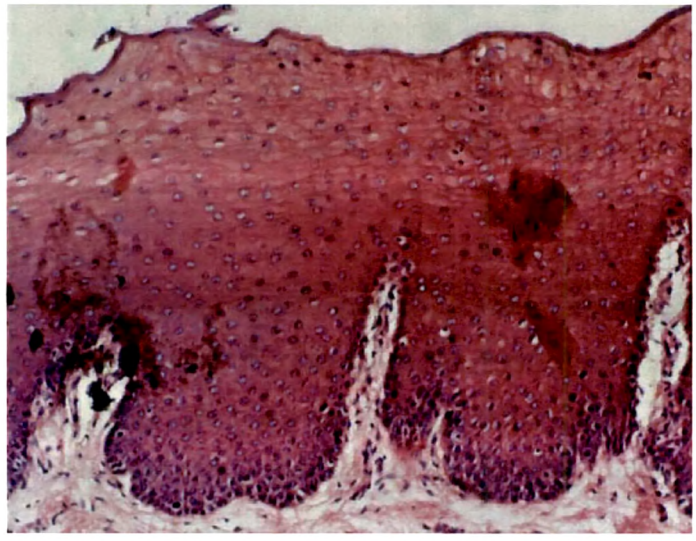


Fig7.29.d) Photmicrograph(40X) of Buccal mucosa after 8 hour treatment with MTX bioadhesive gels

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

The AUC for the intravenous administration and oral conventional tablets were about 4607.83 ± 805 hng/ml and 914.23 ± 144 hng/ml respectively which was significantly different ($p<0.001$; ANOVA followed by Dunnett's multiple comparison test) implying that absolute bioavailability of oral formulation is 19.84% which is as per reports (Collin,1999; Karasulu, et al 2007).

Following buccal administration of bioadhesive patch, tablet and gel formulations to rabbits, the AUC values were 2231.972 ± 163.4, 1876.05 ± 235.02 and 2055.01 ± 175.05 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 48.44%, 48.44% and 44.59% respectively which indicate improvement in bioavailability of MTX when given by buccal route. Highest C_{max} (345±42 ng/ml) amongst all tested formulations was observed with oral conventional tablet and amongst buccoadhesive formulations it was found with gel (309.75±85 ng/ml) followed by and patch (297.4±92ng/ml) and tablet (264.85 ± 70.3 ng/ml).

T_{max} values were 3.0 ± 0.61h, 3.0 ± 1.16h and 3.0 ± 1.02h for buccal administration of patch, tablet and gel. The average $T_{1/2}$ values were 5.98±2.06, 7.086± 1.24 and 5.278± 1.34h, for patch, tablet and gel formulations, respectively, as compared to 0.722±0.048 and 1.10 ± 0.92 h following administration of I.V.injection and oral tablet.

The average K_{el} was 0.9593 ± 0.028 h^{-1} in the intravenous administration group and 0.6292 ± 0.045 h^{-1} in the oral conventional tablets while buccoadhesive formulations depicted lower values 0.1157 ± 0.071, 0.0977 ± 0.005 and 0.1313 ± 0.008 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 MTX 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 8.56 ± 2.05, 9.81 ± 1.58 and 7.339 ± 1.81h, respectively, as compared to 1.179 ± 0.54 after I.V. administration and they

were significantly different ($p<0.001$, ANOVA followed by Dunnett's multiple comparison test).Between the subjects the difference was non significant ($p>0.05$) indicating that there was less intersubject variation.

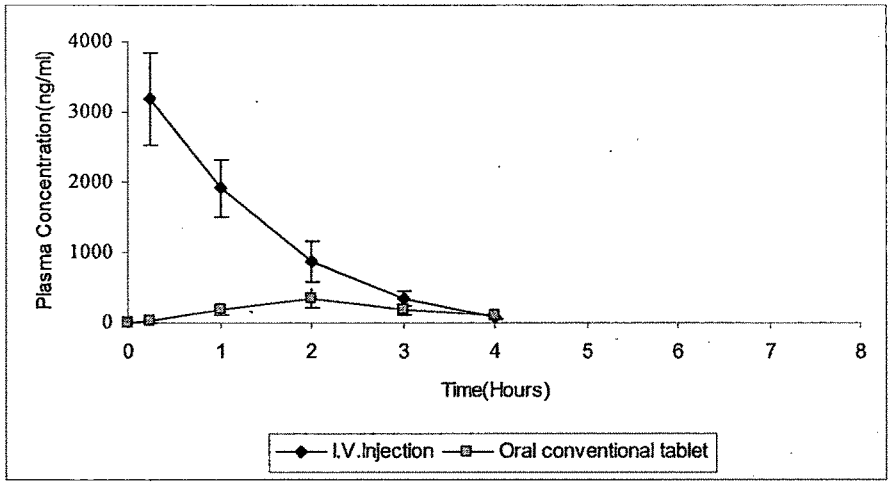


Fig.7.30. Plasma profiles of MTX in rabbits following i.v. administration of injection and oral administration of conventional tablets. (Mean ±.SEM); n=6.

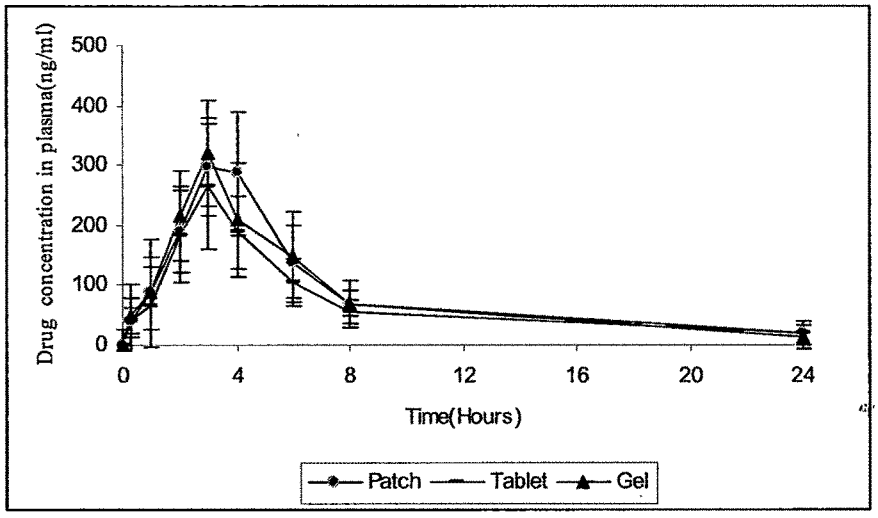


Fig.7.31. Plasma profiles of MTX in rabbits following buccal administration of patches, tablets and gels. (Mean ±.SEM); n=6

Table 7.19. Mean plasma concentration (ng/ml) of MTX from the oral, IV and administration of MTX buccal mucoadhesive formulations.

Time(Hours)	I.V.	Oral Conventional Tablet	Patch	Tablet	Gel
0.25	3189 ±1121	35 ±28.2	38 ±12	42 ±10	48 ±15
1	1910 ±456	184 ±62.3	87 ±16	64 ±21	92 ±32
2	870.56 ±254	345 ±51.2	190 ±41	184 ±74	215 ±35
3	347.75 ±101	186 ±44.7	297 ±32	264 ±84	309 ±11
4	80.25 ±12	98 ±32.1	286 ±58	189 ±65	202 ±40
6	--	--	138 ±26	104 ±24	147 ±33
8	--	--	68 ±12	55 ±16	70 ±14
24	--	--	36 ±10	40 ±18	33 ±22

Table 7.20. Pharmacokinetic Characteristics of MTX 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)	4607.83 ± 805	914.23 ± 144	2231.972 ± 163.4	1876.05 ± 235.02	2055.01 ± 175.05
C_{max} (ng/ml)	-	345±42	297.4±92	264.85 ± 70.3	309.75±85
T_{max} (h)	-	2.0 ± 0. 57	3.0 ± 0.61	3.0 ± 1.16	3.0 ± 1.02
MRT(h)	1.179 ± 0.54	2.71 ± 1.24	8.56 ± 2.05	9.81 ± 1.58	7.339 ± 1.81
MAT(h)	-	1.53 ± 0.70	7.38 ± 1.82	8.63 ± 1.64	6.16± 1.08
K_{el} (h^{-1})	0.9593±0.028	0.6292 ± 0.045	0.1157 ± 0.071	0.0977 ± 0.005	0.1313 ± 0.008
$T_{1/2}$(h)	0.722±0.048	1.10 ± 0.92	5.98±2.06	7.086± 1.24	5.278± 1.34
A. B. (%)	100	19.84	48.44	40.72	44.59

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

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