Chapter 6
Experimental
(Methotrexate)

6.1 *IN VITRO* BUCCAL PERMEATION STUDIES OF METHOTREXATE:

The buccal mucosa was isolated as per the procedure described in section 4.2. The isolated buccal mucosa was mounted between donor and receiver chambers of the Franz diffusion cells with a diffusion area of 3.7 cm^2 and a compartment volume of 20 ml. After an equilibrium period of 30 minutes with phosphate buffer on receptor side, 2 ml of MTX solution (0.5%w/v) in phosphate buffer was filled on the donor side. The diffusion studies were carried out at 37 ±1°C. Samples of 1 ml were taken from the acceptor side at 1 hour intervals for 8 hours, replaced with the same amount of fresh buffer solution, and subsequently assayed using UV spectrophotometer at 303 nm.

The cumulative amount of permeated drug (from mucosal side to serosal side) was plotted versus time, and the flux was calculated from the steady state part of the curve. The permeability coefficient (Kp) was calculated using equation 4.1 described in section 4.2.

The efficacy of the different enhancers was determined by comparing the permeation rate of drug in the presence and absence of enhancers. It was defined as the enhancement factor (EF) which was calculated using the equation (6.1) (Shin et al., 2000):

Enhancement Factor,

$$EF = \frac{\text{MTX permeation rate at steady state in the presence of enhancer}}{\text{MTX permeation rate at steady state in the absence of enhancer}} \quad \text{-- (6.1)}$$

6.2 FORMULATION OF BIOADHESIVE PATCHES OF METHOTREXATE (MTX):

The patches were prepared by casting procedure using viscous solutions of drug and polymers, and subsequent drying. Aqueous polymer solutions were prepared using the required weight of Gantrez AN 139 [a copolymer of methyl vinyl ether and maleic anhydride (PMVE/MA)], added to ice-cooled water and stirred vigorously. The mixture was heated and maintained between 95 and 100 °C until a clear solution was formed.

MTX (25 mg) was added to 15 ml Ethanol: Water (2:1) with stirring and PEG – 200 was added to it. The mixture was heated to 55 – 60 °C until the drug is solubilized. HPMC was added to the drug containing solution with stirring until a clear solution is formed. Both the solutions were mixed for 1 hour and final weight adjusted to 25g with water and ethanol mixture so that proportion of Ethanol: Water remains 2:1. Air bubbles were removed by refrigeration at 4°C.

The gel (20 g) was casted into the petri plates and allowed to dry under vacuum at room temperature for 3 days. At this point residual water was determined at 8.0 ±3.5 % (w/w), as found using Karl Fischer titration (McCarron et al, 2005). Table 6.1 and 6.2 give the compostions of bioadhesive patches and backing layer respectively.

The mechanical properties of the bioadhesive matrices were evaluated using the film formulation without any non-adhesive backing film (McCarron et al, 2005).For the formulation of backing layer, ethyl cellulose (20 cps) was dissolved in acetone and casted on the dried 1st layer and allowed to dry for 24 hrs. After drying, the patch was removed from the casting plate (Reinhold and Merkle, 1989). The fully assembled bi-laminar patch was used for bioadhesive performance and other evaluation purposes (McCarron et al, 2005).

Table 6.1. Compositions for mucoadhesive layer

Quantity/patch	MP1	MP2	MP3	MP4	MP5	MP6	MP7	MP8	MP9
MTX (mg)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
HPMC K4M (mg)	87.5	75	62.5	50	50	50	50	50	50
Gantrez AN 139 (mg)	50	50	50	50	40	62.5	75	87.5	100
PEG (%w/w)	5	5	5	5	5	5	5	5	5

Table 6.2. Compositions for protective layer

Excipients	Quantity
Ethyl cellulose (mg)	22
Propylene glycol (%)	4
Acetone (ml)	10

6.2.1 *Evaluation of bioadhesive patches:*

6.2.1.1 Assay:

MTX content was determined by dissolving an accurately weighed quantity of formulation (equivalent to 2.5 mg) in 50 ml of pH 6.8 phosphate buffer, filtering through 0.45μ filter. Absorbance of the resulting solution was taken at 303 nm after dilution with phosphate buffer.

6.2.1.2 Bioadhesion:

The bioadhesion measurements were performed as per the procedure mentioned in section 4.3.1.2.

6.2.1.3 Swelling rate:

The swelling rate of formulations was evaluated using a 1% w/v agar gel plate as per the method described in section 4.3.1.3.

6.2.1.4 Surface pH:

The micro environmental pH of the formulation was measured so as to determine its effect on buccal mucosa. The formulation was first allowed to swell by adding an adequate amount of distilled water to formulation surface. The surface pH was then recorded after bringing glass electrode of pH meter (Elico, Mumbai) near the surface of the formulation and allowing it to equilibrate for 1 min (Bottenberg, P. et al, 1991).

6.2.1.5 Tensile strength:

The strength required to break the patch was recorded by using INSTRON tensile strength tester with microprocessor force gauge (Instron Universal Testing Instrument, Model 1121, Instron Ltd., Japan) using same procedure as given in 4.3.1.5 (Patel et al., 2007)..

6.2.1.6 Folding endurance: Same procedure as given in 4.3.1.6 was followed for determination of folding endurance.

6.2.1.7 Drug release studies:

The drug release study for patches was carried out by using USP dissolution apparatus V at 37± 0.1°C (Wong, C. F., 1999) as described in section 4.3.1.7. Volume of dissolution medium i.e phosphate buffer (pH 6.8) was 500 ml and the speed of the paddle was 50 rpm. The aliquots (5 ml) were withdrawn at 60, 120, 180, 240, 360, 420 and 480 minutes. The removed volume was replaced by the same volume of phosphate buffer. Analysis of the withdrawn samples was carried out by UV spectroscopy at 303 nm.

6.2.1.8 Mathematical modeling of release kinetics

Data obtained from *in vitro* release studies were fitted to various kinetic equations to understand the mechanism of drug release from formulated extended release patches. The kinetic models used were zero order, first order, Higuchi and Peppas equation (Tas et al, 2004; Jones et al, 2004; Costa et al, 2001).

The following plots were plotted: Q_t *vs.* t (zero order kinetic model); log (Q_0-Q_t) *vs.* t (first order kinetic model,) and Q_t *vs.* square root of t (Higuchi model) and log M_t/M_∞ = nlog t + k (Peppas equation). Where Q_t is the amount of drug released at time t and Q_0 is the initial amount of drug present in formulation (Korsmeyer et al, 1983). M_t/M_∞ is the fraction of drug released after time t in respect to amount of drug released at infinite time, k is the rate constant and n is the diffusional exponent which characterizes the transport mechanism (Peppas, 1985). Statistical comparisons were made using one way ANOVA by using the software PRISM (Graphpad, San Diego, CA). The level of significance was considered at $p < 0.05$.

6.2.2 *Histology Studies:*

The histological and morphological changes in the buccal mucosa due to treatment with formulations were studied by light microscopy (using Olympus microscope).The guinea pig buccal tissue was stored in Kreb's buffer and was used within 2 hours upon removal. Most of the underlying tissues were removed from the mucosa with surgical scissors, and then the epithelium was separated from most of the connective tissue making sure that basal membrane was intact. The tissue was mounted on Franz diffusion cell and incubated with the formulation on one side and pH 6.8 phosphate buffer (20 ml) on the other side. For control, pH 6.8 phosphate buffer was kept on the both sides of cell. Stirring in the acceptor side was carried out using magnetic bead.

At the end of the exposure period (8 hour), small portions of tissue were fixed in 10% buffered formalin solution and dehydrated. All specimens were then embedded in paraffin wax. Sections were cut with a microtome in direction perpendicular to the epithelial surface (at Baroda Pathology Lab, Baroda). They were then stained with hematoxylin eosin (HE) and examined under light microscope (Olympus, Japan) (Aioi et.al, 1993; Shin et.al.,2000).

6.2.3 *Compatibility studies:*

To investigate the interaction between MTX and polymers, Fourier Transform Infrared (FTIR) spectrometry and X-ray diffraction (XRD) studies were carried out.

FTIR spectra were recorded using a FTIR Shimadzu -8300 spectrophotometer (Tokyo, Japan). Samples were prepared in KBr discs by means of a hydraulic press. The scanning range was 500 to 4000 cm^{-1} and the resolution was 4 cm^{-1}.

X- Ray Diffractograms were recorded on a X-ray diffractometer (Philips PW 1710) under the following conditions: Ni-filtered Cu-Ka radiation; 40 KV voltage: 30 mA current, scan speed 2°/min in terms of 2θ angle.

6.2.4 *Stability study of MTX bioadhesive patch formulations:*

Patches were packed in glass Petri dishes lined with aluminum foil and kept in a stability chamber maintained at 40 ± 2 °C and 75 ± 5% RH (Accelerated) and 30 ±2°C /60±5%RH (Intermediate term) for 3 months. Changes in the appearance, release behavior and drug content of the stored bioadhesive patches were investigated after 3 months as per the procedures described previously (Nafee et al, 2003; ICH Guidlines).

6.3 FORMULATION OF BUCCAL BIOADHESIVE TABLETS OF METHOTREXATE (MTX):

Bilayer tablets (consisting of a backing layer and adhesive drug reservoir layer) were prepared by covering one side of the single-layer tablet with an inert ethylcellulose layer. Ethylcellulose was selected as a backing material because this hydrophobic polymer has very low water permeability thus providing an impermeable backing layer that prevents drug loss in oral cavity (Miyazaki et al, 2000).

Bilayered bioadhesive tablets were formulated using HPMC K15 M and Gantrez MS 955 as polymers as per the compositions given in Table 6.3 and 6.4. Drug containing layer of tablets was prepared by direct compression (On 8 station Rotary machine, Jaguar, D-Tooling: **General Machines Ltd., Mumbai**) of drug blended with HPMC, Gantrez MS, and other excipients. Drug, polymers, Tablettose, and lubricant were mixed and then compressed using 9.5 mm flat faced punches at a lower hardness (2-3 kg/cm^2) and then backing layer made of EC (20 cps) was compressed on the drug containing layer to obtain bilayered tablets with final hardness of tablets at 4-5 kg/cm^2.

6.3.1 *Preliminary studies*

During preliminary studies, various combinations of HPMC K15M and Gantrez in different ratios were formulated to choose the best possible combination for optimization using factorial design. The formulations employed for pre-liminary investigations are enlisted in Table 6.3.

Table 6.3. Preliminary batches for MTX tablets

Composition (mg)	Formulation				
	PMT1	PMT2	PMT3	PMT4	PMT5
MTX	2.5	2.5	2.5	2.5	2.5
Tablettose 80	100	100	110	116	111
Gantrez MS 955	20	10	10	5	20
HPMC K15	20	30	20	20	10
Magnesium stearate	2	2	2	2	2
Ethyl cellulose,20 cps	70	70	70	70	70

6.3.2 *Optimization by factorial design*

Section 2.8.2 gives description about optimization methodologies and factorial designs. A 3^2 factorial design was employed for optimization of tablet formulation in which the amount of two carriers (factors) was varied at three levels as required by the design (Ahuja and Singh, 2002). The amounts of Gantrez (X_1) and HPMC K 15M (X_2) were selected as factors, studied at 3 levels each. Table 6.4 summarizes the 9 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study. Time taken to release 50% of drug ($t_{50\%}$), release until 8 hours (rel_{8hrs}), diffusion coefficient (n) and bioadhesion were taken as the response variables.

6.3.3 *Optimization Data Analysis*

Various RSM (Response Surface Methodology) computations for the current optimization study were performed employing Design Expert® software (version7.1.2, Stat-Ease Inc, Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple regression analysis (MLRA) approach as described in section 4.4.3.8.

Table 6.4. Factor combinations as per 3^2 factorial design

Trial No.	Coded factor levels	
	Factor 1 (X_1)	Factor 2 (X_2)
1	-1	-1
2	-1	0
3	-1	1
4	0	-1
5	0	0
6	0	1
7	1	-1
8	1	0
9	1	1

Translation of coded levels in actual units

Coded level	-1	0	+1
X_1: Gantrez MS 955 (mg)	5	15	25
X_2:HPMC K15M(mg)	10	15	20

All batches contained 2.5 mg MTX, HPMC K15M (10-20 mg), Gantrez MS 955 (5-25 mg), Magnesium stearate (2mg), and quantity sufficient of Tablettose 100.
Drug containing layer's weight was 145 mg; Backing layer contained Ethyl cellulose-20cps (70 mg).

6.3.4 *Characterization of Tablets:*

6.3.4.1 Diameter and thickness:

The diameter and thickness of the formulated tablets were measured using Vernier Caliper (Mitutoyo, Japan).

6.3.4.2 Hardness:

Hardness of the tablets was tested using a Monsanto hardness tester (Campbell Electronics, Mumbai). Five tablets from each batch were used, and average values were calculated.

6.3.4.3 Weight variation:

To study weight variation, 20 tablets were weighed using an electronic balance (Afcoset-USA), and the test was performed according to the method described in Indian Pharmacopoeia (Indian Pharmacopoeia, 1996).

6.3.4.4 Friability:

It was determined by using Roche Friabilator (Campbell Electronics, Mumbai). The tablets were carefully dedusted prior to testing. Accurately weighed 20 tablets were placed in the friabilator which was rotated for 100 revolutions, and tablets were removed. Any loose dust from the tablets was removed and tablets were accurately weighed. A maximum weight loss of not more than 1% of the weight of the tablets being tested was considered acceptable. (USP XXV, 2002)

6.3.4.5 Surface pH: Procedure same as described in section 4.3.1.4 was employed.

6.3.4.6 Assay: 10 tablets were crushed in mortar and powder equivalent 2.5 mg MTX was dissolved in 50 ml of pH 6.8 phosphate buffer. After filtering through 0.45μ filter, absorbance of the resulting solution was taken at 303 nm after dilution with phosphate buffer.

6.3.4.7 *In vitro* release study:

Tablets were stuck from backing membrane side onto the bottom of the dissolution vessel with a drop of cyanoacrylate adhesive so that, only one side of tablet was in contact with the dissolution medium [pH 6.8 phosphate buffer] (Akbari et. al, 2004). Then the prewarmed (37±0.5 °C) dissolution medium (500ml) was gently poured into the vessel and dissolution conducted as per United States Pharmacopoeia (USP) method II with a paddle speed of 50 rpm. Samples (5.0 ml) were withdrawn at regular intervals and replaced with an equal volume of medium. Samples were filtered and subsequently analyzed using UV spectrophotometer at 303 nm.

Various kinetic models like Zero order, First order, Higuchi and Peppas equation were applied to predict the mechanism of drug release as per that for the patches (section 4.3.1.8).

6.3.5 *Histology studies:*

Histology studies were performed as per the same procedure used for patches as described in section 4.3.2.

6.3.6 *In vivo bioadhesion study:*

The bilayered placebo tablets (without drug) were prepared using the optimized formula for tablet formulation. The backing layer was pulled off and a small hole was drilled through the matrix layer (An electric drill (Shiv Shakti Enterprises, Faridabad, India) was used to drill holes of 1.4mm diameter to a depth of 1.0-1.2 mm through the centre of the tablets) for introducing radioactive solution. 1 ml of ^{99m}Tc (2 mCi/ml) was mixed with 0.1 ml of stannous chloride solution (1mg/ml) and the pH was adjusted to 7 using sodium bicarbonate solution. About 10µl of above aqueous solution of ^{99m}Tc was instilled into the drilled holes, avoiding contact with the surface of the tablets. The solution was allowed to dry for 1 hour in a fume cupboard.

A mixture of HPMC K15M and Gantrez MS-955 in 1:1 proportion was prepared and used to seal the drilled holes so that it covered an area slightly greater than that

of the hole. Then the backing layer of Ethyl cellulose was stuck over it using cyanoacrylate adhesive (Araldite®). These tablets were left to dry overnight before use.

Animals (albino rabbits) were lightly anesthetized by an i.m. injection of a 1:5 mixture of Diazepam (1.9 mg/kg) and ketamine (9.3 mg/kg). Following induction of anesthesia, bioadhesive tablets were first moistened with a drop of water and inserted in the mouth and positioned in such a way that tablet surfaces were in contact with the cheek.

Gamma scintigraphy

For determination of the tablet position and the residence time in the buccal cavity, the rabbits were fixed on a board and imaging was performed using a Single Photon Emission Computerized Tomography gamma camera (SPECT, LC 75-005, Diacam, Siemens, USA).

6.3.7 *Compatibility study*:

To investigate the compatibility of matrix materials with MTX, Fourier Transform Infrared (FTIR) spectrometry and X- Ray Diffraction (XRD) studies were carried out.

FTIR spectra were recorded using a FTIR Shimadzu -8300 spectrophotometer (Tokyo, Japan). Samples were prepared in KBr discs by means of a hydraulic press. The scanning range was 500 to 4000 cm^{-1} and the resolution was 4 cm^{-1}.

X- Ray Diffractograms were recorded on a X-ray diffractometer (Philips PW 1710) under the following conditions: Ni-filtered Cu-Ka radiation; 40 KV voltage: 30 mA current, scan speed 2°/min in terms of 2θ angle.

6.3.8 *Stability study of bioadhesive tablet formulations containing MTX*:

The stability study of formulations was carried out as per ICH guidelines. Tablet formulations were wrapped in aluminium foils and subjected to stability study at 40 ±2°C /75±5 %RH (Accelerated) and 30 ±2°C /60±5 %RH (Intermediate) for 3

months and then samples were observed for physical appearance and analyzed for drug content.

6.4 FORMULATION OF BUCCAL BIOADHESIVE GELS CONTAINING MTX:

Poloxamer 407 gels were prepared using the method described by Schmolka. (1972). Table 6.5 gives composition of various gel formulations. Required quantity of Poloxamer-407 was dissolved in 10 ml water with gentle stirring. The solution was left in a refrigerator overnight to ensure complete polymer dissolution. MTX was dissolved in 10 ml water and ethanol mixture (1:1) and 2.5 ml PEG-200 (Polyethylene glycol) was added and heated at 50 °C temperature. Once the drug is solubilized, Gantrez S- 97 was added to this solution and mixed by strirring.

Then both the solutions (Poloxamer and drug solution with Gantrez)were mixed and stirred at 40-45 °C for 2 hours till a gel was formed (Shin et al, 2004). The prepared gel formulations were filled in amber colored glass containers (Well stoppered) and stored at room temperature.

Table 6.5. Compositions of the MTX bioadhesive gel formulations (%w/w).

Batch No.	MTX	Poloxamer - 407	Gantrez S-97
MG1	0.1	16	3
MG2	0.1	16	4
MG3	0.1	16	5
MG4	0.1	16	6
MG5	0.1	17	3
MG6	0.1	17	4
MG7	**0.1**	**17**	**5**
MG8	0.1	17	6
MG9	0.1	18	3
MG10	0.1	18	4
MG11	0.1	18	5

6.4.1 *Characterization:*

6.4.1.1 Measurement of bioadhesive force

The method is same as described in section 4.3.1.2 except few changes. Here, two circular pieces (diameter 3.6 cm) of guinea pig buccal mucosa were cut and separated from connective tissue and glued with cyanoacrylate adhesive on the ground surface of two tissue holders made of plexiglass. Tissues were immediately used after separation. The gel (0.5 g) was applied to the mucosal tissue on one of the tissue holders. Thickness of gel layer was 1.0±0.2 mm. Thereafter, both the tissue holders with mucosa and gel formulation were allowed to come in contact with each other with uniform and constant pressure for 5 min (preload time) to facilitate adhesion bonding. Rest of the method is same as in section 4.3.1.2.

6.4.1.2 *In Vitro* diffusion studies

Prevalidated Franz diffusion cells with a receiver compartment volume of 20 ml and effective diffusion area of 3.14 cm^2 were used in this study. The synthetic cellulose membrane (Molecular cut off value: 12,000) was mounted on top of receptor compartment of diffusion cells. The receptor phase (pH 6.8 phosphate buffer) was continuously stirred by magnetic bead and kept at a temperature of 37±0.5°C during the experiments. Amount of the prepared gels equivalent to 2.5 mg of MTX was spread on the cellulose membrane at the donor compartment; the donor cap was covered with parafilm and clamped (Tas et. al., 2004).

At appropriate times upto 8 hours, 500 µl sample was withdrawn from the receiver compartment and the same amount of fresh buffer solution was added to keep the volume constant. This dilution of the receiver content was taken into account when evaluating the data. The samples were analyzed by UV spectrophotometer at 303 nm and the concentration of MTX in each sample was determined from a previously calibrated standard curve. Each data point represented the average of three determinations.

6.4.1.3 Assay

Method for estimation of drug content of the gel formulations is same as that discussed in section 6.3.4.6.

6.4.1.4 pH measurements

The pH was measured in each gel, using a pH meter (Elico, Mumbai), which was calibrated before each use with buffered solutions at pH 4, 7, and 10.

6.4.1.5 Viscosity measurement

Viscosity of gel was measured using Brookfield programmable rheometer (Rheocalc V 2.5, Model DV-III Ultra) using CP 40 spindle at 40 RPM (25±1°C). Effect of shear rate on shear stress and viscosity were studied.

6.4.2 *Histology Studies:*

The histological and morphological changes in the buccal mucosa due to treatment with gel formulations were studied as per the method described under section 4.3.2.

6.4.3 *In vivo bioadhesion of gels:*

Radiolabelling of the gels and their application

1 ml of technetium-99m [^{99m}Tc] (2 mCi/ml) was mixed with 0.1 ml of stannous chloride solution (1mg/ml) and the pH was adjusted to 7 using sodium bicarbonate solution. 10µl of above aqueous solution of ^{99m}Tc was added to approximately 2 g of gel formulation (placebo) to be tested and thoroughly mixed. The average final activity per dose and per subject, at the time of administrations ranged from 3995 to 4210 kcpm (kilocounts per minute). Rabbits were anaesthetized as described previously and 200 mg of the gel formulation was applied with a small Teflon spatula on an area of approximately 1 cm^2 of the oral mucosa. Each animal was used only once throughout these trials.

Gamma scintigraphy

For the determination of residence time of gel formulations in the buccal cavity, the rabbits were fixed on a board and imaging was performed using a Single Photon

Emission Computerized Tomography gamma camera (SPECT, LC 75-005, Diacam, Siemens, USA).

6.4.4 *Stability study of bioadhesive gel formulations containing MTX:*

Gels were stored in amber colored glass containers (well stoppered) for three months in the dark at room temperature (25±2°C). They were checked for physical appearance, pH, assay and release after preparation and regularly throughout this period. Physical evaluation of stability of the samples was carried out by visual inspection (Tas et al., 2004).

6.5 *IN VIVO* STUDIES OF MTX CONTAINING BIOADHESIVE PATCHES, TABLETS AND GELS:

6.5.1 *Animal treatment*

Albino rabbits (either sex) weighing 2.0–2.6 kg were housed individually over 2 weeks in a temperature-controlled environment (20–25°C). The relative humidity varied between 50 and 60%. The animals were fasted overnight prior to the experiment but had free access to water.

Rabbits were lightly anesthetized by an i.m. injection of a 1:5 mixture of Diazepam (1.9 mg/kg) and ketamine (9.3 mg/kg). Following induction of anesthesia, a catheter was placed in the marginal ear vein for blood sample collection. Bioadhesive tablet/patch containing MTX (2.5 mg) were first moistened with a drop of water and inserted in the mouth and positioned in such a way that tablet/ patch surfaces are in contact with the cheek. The gel formulations were applied directly on the cheek of rabbits. 0.5 ml blood samples were collected before and 1, 2, 3, 4, 6, 8 and 24 h after buccal administration of the mucoadhesive buccal patch/tablet/gels and 15 min and 1, 2, 4 h after intravenous administration. The blood samples were collected into a heparinized microcentrifuge tubes.

The light plane of anesthesia was maintained by an i.m. injection of one-third of the initial dose of Diazepam and Ketamine mixture as needed. Then the samples were subjected to centrifugation on a laboratory centrifuge (Sigma, 3K30) at 10 000 rpm for 10 minutes at 0°C, and supernatant plasma was collected into another

microcentrifuge tube and kept at –20°C until analysis (Alur et al., 1999).The plasma samples were analyzed by HPLC as per method described in section 3.4.

6.5.2 *Pharmacokinetic data analysis*

The noncompartmental pharmacokinetic analysis was performed (Shin et al, 2000). Trapezoidal method was employed to calculate the AUC of plasma concentration (Cp) as a function of time (t). Mean residence time (MRT) was calculated as area under the first moment curve (AUMC) divided by area under the curve (AUC). AUMC was determined using a plot of plasma concentration multiplied by time (C x t) versus time and calculation of its area under the curve by the Trapezoidal method. All the pharmacokinetic parameters were calculated in MS-Excel software. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (t_{max}) were determined by visual inspection of the experimental data as well as the plasma concentration curve using MS –Excel software. The elimination rate constant (K_{el}) was calculated by the regression analysis from the slope of the line and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability of MTX after buccal administration per the I.V. administration was calculated using equation 4.6 (Alur et al., 1999).

The statistical significance of the differences between the formulations was tested by the Student's paired t-test. All the values were reported as mean ± standard error of mean (S.E.M.) of six determinations.

REFERENCES

Ahuja, N and Singh, B., Development of Controlled-Release Buccoadhesive Hydrophillic Matrices of Diltiazem Hydrochloride: Optimization of Bioadhesion, Dissolution and Diffusion Parameters. *Drug Dev. Ind. Pharm.*, 28 (2002) 433-444.

Aioi, A., Kuriyama, K., Shimizu, T., Yoshioka, M., Uenoyama, S., Effects of vitamin E and squalene on skin irritation of a transdermal absorption enhancer laurylsarcosine. *Int. J. Pharm.*, 93 (1993)1–6.

Akbari, J., Nokhodchi, A., Farid, D., Adrangui, M., Siahi-Shadbad, M. R., Saeedi, M., Development and evaluation of buccoadhesive propranolol hydrochloride tablet formulations: effect of fillers. *Farmaco.*, 59 (2004)155-61.

Alur, H. H., Pather, S. I., Mitra, A. K., Johnston, T. P., Transmucosal sustained-delivery of chlorpheniramine maleate in rabbits using a novel, natural mucoadhesive gum as an excipient in buccal tablets. *Int. J. Pharm.*, 188(1999)1-10.

Bottenberg, P., Cleymaet, R., Muynek, C.D., Remon, J.P., Coomans, D., Slop, D., Development and testing of bioadhesive, fluoride-containing slow-release tablets for oral use. *J Pharm Pharmacol.*, 43(1991) 457-464.

Costa, P. and Sousa Lobo, J.M., Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.*, 13 (2001) 123-33.

ICH Guidelines, Guidance for Industry, Q1C Stability testing for new dosage forms, USFDA –CDER, Nov 1996.

Indian Pharmacopoeia, Ministry of Health and Family Welfare, Govt. of India, 1996.

Jones, D. S., Lawlor, M. S., Woolfson, A. D. Formulation and characterisation of tetracycline-containing bioadhesive polymer networks designed for the treatment of periodontal disease. *Current Drug Del.*, 1 (2004)17-25.

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.

McCarron, P. A., Donnelly, R. F., Zawislak, A., Woolfson, A. D., Price, J. H., McClelland, R., Evaluation of a water-soluble bioadhesive patch for photodynamic therapy of vulval lesions. *Int J Pharm.*, 293(2005)11-23.

Miyazaki, S., Kawasaki, N., Nakamura, T., Iwatsu, M., Hayashi, T., Hou, M., Attwood, D., Oral mucosal bioadhesive tablets of pectin and HPMC: in vitro and in vivo evaluation. *Int. J. Pharm.*, 204(2000) 127–132.

Nafee, N.A., Boraie, M.A., Ismail, F.A., Mortada, L.M., Design and characterization of mucoadhesive buccal patches containing cetylpyridinium chloride. *Acta Pharm.*, 53(2003)199-212.

Peppas, N.A., Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta. Helv.*, 60 (1985) 110-11.

Reinhold, A.and Merkle, H.P., Evaluation of laminated mucoadhesive patches for buccal drug delivery. *Int. J. Pharm.*, 49 (1989) 231-240.

Schmolka, I.R., Artificial skin I. Preparation and properties of pluronic F-127 gels for treatment of burns. *J. Biomed. Mater. Res.*, 6 (1972) 571–582.

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(2000)37–43.

Shin, S.C., Cho, C. W., Yang, K.H., Development of lidocaine gels for enhanced local anesthetic action. *Int. J. Pharm.*, 287 (2004) 73–78.

Tas, C., Ozkan,Y., Savaser,A., and 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.

USP 25/NF 20 (United States Pharmacopoeial Convention, Inc., Rockville, MD, 2002).

Wong, C. F., Yuen, K. H., Peh, K. K., Formulation and evaluation of controlled release Eudragit buccal patches. *Int. J. Pharm.*, 178 (1999)11-22.