Chapter 8
Summary & Conclusion

SUMMARY AND CONCLUSION

In the present investigation, we propose bioadhesive delivery systems for FU and MTX using polymer admixtures for administration through buccal route.

It is envisaged that the proposed delivery systems will overcome first pass metabolism and wastage of drug through gastrointestinal tract which will result in increased bioavailability. It is expected to provide sustained release of drugs which will reduce the frequency of administration of conventional dosage form and will improve patient compliance as there is no need of swallowing. Bioadhesive patch, tablet and gel formulations were designed and optimized. They were characterized for their physicochemical properties.

The *in vitro* permeation properties across buccal mucosal membrane were investigated for both the drugs i.e. FU and MTX. Both the drugs were found to be poorly permeable (52.3 ±8.1 % FU was found to permeate through the buccal mucosa in 8 hours with permeability coefficient of 0.799 (±0.31) x 10^{-4} cm/s; 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). Permeation studies were performed in the presence of penetration/absorption enhancers.

For FU permeation, STGC was found to be best enhancer as highly significant increase *($p<0.001$)* in permeation was observed, with 90.13 ± 7.2 % FU permeated through the mucosal membrane in 8 hours with Kp [5.80 (±0.62) x 10^{-4} cm/s] and EF (1.679).

GMS (0.1M) enhanced MTX permeation significantly as evident from Kp and EF values which were 1.67(±0.75) x 10^{-4} and 2.41 respectively as compared to control for which (Kp) is 4.69(±0.15) x 10^{-5} cm/s and EF is 1. When Oleic acid(3%)+Propylene glycol (5%)+ethanol (10%) were used, further improvement in MTX permeation was observed as evident from Kp and EF values (1.83 (±0.64) x 10^{-4} and 2.73, respectively.

Therefore, optimized formulations viz. buccal bioadhesive patches, bioadhesive tablets and bioadhesive gels were prepared for both the drugs (FU and MTX) using permeation enhancers and these were then subjected to *in vitro* release studies, *in vivo* studies, stability studies and histopathological studies to finally determine their safety and efficacy.

5-Fluorouracil

The **patches** were prepared by solvent casting technique and consisted of two layers, medicated layer and backing layer. Medicated layer contained HPMC K4M and Gantrez MS 955 as polymers while backing layer was constructed of Ethyl cellulose. Glycerin was used as plasticizer. During patch formulation, the incorporation of FU into the bioadhesive layer presented problems, mainly due to its limited aqueous solubility. The problem of crystallization from aqueous gels and fissuring from the volatile solvent gels was overcome when an ethanol/water blend (1:3) was used. This was found to produce films with small crystal size, and a visually even crystal distribution.

Patch formulations were evaluated for assay, bioadhesion, swelling, surface pH, tensile strength, folding endurance and drug release studies.

The assay values were within pharmacopoeial limits (95 % - 105%). The bioadhesion appeared to increase with a corresponding increase in the Gantrez content. Maximum mucoadhesive force (14.99±2.50 x10^3 dynes/cm^2) was exhibited by FP11 containing HPMC and Gantrez in 1:2.5 proportions. The maximum swelling was attained in FP11containing highest proportion of Gantrez (Swelling index=2.4±0.09), while the batch containing highest proportion of HPMC (FP1) displayed lesser swelling index (2.24 ±0.08). Surface pH ranged from 5.5-6.2 depicting the suitability of formulations when applied to buccal mucosa. The *in vitro* release was increased when the polymers were incorporated at low concentrations, but was decreased when incorporated at higher concentrations. Considerable erosion was noticed from the patches in the later phase of dissolution. The goodness of fit for various models investigated for FU patch formulations ranked in the order of Higuchi ≈ Peppas > zero order > first-order. All the kinetic models, other than the first order, fitted well. The values of diffusional exponent

'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.51 and 0.76 indicating non-fickian behaviour.i.e drug release is the combination of erosion and diffusion. Formulation FP6 (containing HPMC and Gantrez in 1:1 proportions), was found to permeate poorly through buccal mucosa, hence STGC (0.05M) was incorporated during the formulation stage. No significant difference *($p>0.05$)* in adhesive bond strength, tensile strength and moisture content was observed between STGC containing and STGC free patches. Addition of STGC to patch formulations resulted in an increase in release rate.

Compatibility studies (DSC, XRD and FTIR) indicated absence of any kind of interaction between drug and polymers. Three month's storage under 30 ± 2°C/ 65±5%RH and 40 ±2°C/ 75 ±5%RH did not change the release behavior and assay although tensile strength decreased.

FU Bilayer bioadhesive 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. FU bioadhesive tablets were prepared as per 3^2 Factorial Design.

HPMC K4M (4mg), HPMC K15M (4-16 mg), Gantrez MS 955(10-30 mg) were used as polymers. Bioadhesion [Y_1], release until 8 hours (rel_{8hrs}) [Y_2], time taken to release 50% of drug ($t_{50\%}$) [Y_3] and diffusion coefficient (n) [Y_4] were taken as the response variables. Physical parameters and drug assay for these formulations were within the acceptable limits. On analyzing the data of all the 9 formulations prepared as per 3^2 Factorial design using Design Expert® software, various polynomial equations, response surface and contour plots were generated. Gantrez was found to have more pronounced effect on bioadhesion than that of HPMC K15M as an increase in bioadhesion was observed with rise in levels of Gantrez. High level of HPMC K15M gave low value of percentage FU release at 8 h at all the levels of Gantrez 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 Gantrez increase in FU Rel_{8hr} was observed at all corresponding

levels of HPMC K15M. As Gantrez MS 955 is a water soluble polymer, the above outcome can be explained by the phenomenon that when a soluble polymer is used, the void formed in the matrix by dissolution initially contain a rather viscous solution, characteristic of dissolved polymers. The high viscosity in the pores serves to retard the diffusion of drug at the early stages of release. At the later stages, the polymer solution becomes dissipated and resistance to diffusion is decreased. Linear relationship was obtained between the fraction of HPMC K 15M and $t_{50\%}$, and it was observed from response surface and contour plots that as the fraction of HPMC K15M(X_2) increased, the value of $t_{50\%}$ increased, at all the 3 levels of Gantrez. On increasing the amount of Gantrez(X_1), nonlinear trend in the response curve was observed i.e. "region of maximum" for $t_{50\%}$ at intermediate levels of Gantrez (X_1) at all the 3 levels of X_2. Diffusion exponent ranged from 0.32 to 0.57. Formulation FT5 was considered optimum on the basis of *in vitro* release and other characterization parameters.

The permeation of FU from tablet formulation (FT5) through buccal mucosa was found very low (Kp =1.750 (±0.67) $x10^{-5}$ cm/sec) when compared with that of FU solution (0.799 (±0.31) x 10^{-4} cm/sec). The formulation (FT5) was prepared further using STGC as a permeation enhancer (0.05M) for *in vivo* studies. The bioadhesive strength of these tablets was 9.92±0.95 dynes/cm^2 while hardness was 4.6 ± 0.2 kg/cm^2. No significant difference in adhesive bond strength and hardness was observed between STGC containing and STGC free tablets. Addition of STGC to tablet formulations resulted in an increase in release rate. DSC and FTIR studies indicated no interaction between FU and other tablet components.

The γ- scintigraphic images of the rabbits taken after administration of placebo buccoadhesive tablets (containing ^{99m}Tc), showed the presence of tablets in the same place in buccal cavity indicating that the tablets prepared using optimized combination of HPMC and Gantrez remained adhered to the buccal cavity at least for 4 hours. Drug content, hardness, friability and release values of the formulations carried out at 3 months showed no significant differences ($p>0.05$) indicating a stable formulation.

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

An increasing trend in the bioadhesive strength with increase in the amount of HPMC content (B2, B3, B4 and B5) was observed, but the increase was not statistically significant ($p>0.05$). However, detachment force increased significantly ($p< 0.001$) when Gantrez S-97 was added to the gels (B6-B11). Maximum bioadhesive force ($18.33\pm2.6 \times 10^3$ dyne/cm^2) was found in the formulation B 11, containing 4% Gantrez. The significant enhancement ($p < 0.05$) in bioadhesion due to Gantrez can be attributed to its carboxylic groups that gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus membrane.

All the formulations were found to exhibit assay values in the range 95-105 % depicting good content uniformity. The pH was measured in each gel and found to be within the satisfactory limit of 5.5 – 7.5 which implies that the gel would not cause irritation in the buccal cavity. The apparent viscosity of the Formulation B7 at 40 RPM (at 25±1°C) was found to be 544.49 ± 5.9 cps.

Increasing the concentration of Poloxamer- 407 significantly decreased the cumulative percent drug released. 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.

As the content of HPMC K 15M and Gantrez was increased, decrease in release was discerned. Because, hydrophilic polymer would decrease the amount of free water in the water channels, and affect molecular orientation of the gel matrix. Thus both HPMC and Gantrez decreased the rate of FU release from the gel.

The goodness of fit for various models investigated for gel formulations ranked in the order of Higuchi ≈ Peppas > zero order > first-order. All the kinetic models,

other than the first order, fitted well. The values of diffusional exponent 'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.4486 and 0.7244. Only three formulations B2, B3 and B4 tended to exhibit Fickian diffusional characteristics, as the corresponding values of 'n' were lower than 0.5. Rest of the formulations B5 - B11 has 'n' value in the range 0.5 - 1.0 indicating anomalous (non-Fickian diffusion). The non-Fickian behaviour (n=0.5-1.0) of the formulations may be attributed due to the gelling property of Poloxamer 407 (as it is present in higher proportions in B5-B11 than in B2-B4). An increase in temperature increased the release rate of drug despite an increase in bulk viscosity; this small increase in release rate is likely due to increase in drug diffusion at higher temperature. The effect of pH on FU release was studied using B7 gel and it was observed that there was no significant difference ($p>0.05$) due to pH in the release profiles of FU from the gels between pH 5.5, 6.8 and 7.4.

The permeability coefficients (Kp) of gel across cellulose membrane and buccal mucosal membrane were 1.06×10^{-4} cm/sec and 3.94×10^{-5} cm/sec respectively. The penetration of FU from gel formulation (B7) through buccal mucosa was found very low when compared with cellulose membrane which may be due to the fact that the complexity of the composition of the mucosa offered more resistance to the penetrating drug molecules during the diffusion process. The final formulation (B 7) selected on the basis of *in vitro* release study and other characterization parameters was prepared further using STGC (0.05M) as a permeation enhancer and no significant difference in adhesive bond strength was observed between STGC containing and STGC free gels. DSC, XRD and FTIR studies indicated absence of any kind of interaction between FU and polymers. FU bioadhesive gels exhibited good stability over three month storage at 25 ± 2°C.

In histology studies, no significant loss of superficial cell layers was observed, but there was formation of vacuoles especially in the prickle cell layers. In addition, an increase in intercellular space and swelling, especially inside the cells was observed. This can be collaborated by the reports that bile salts (like STGC and SDC) accumulate in the tissue after penetration into the tissue without causing a loss of superficial cell layers, and then interact with the intercellular or membrane lipids thus increasing the permeability of the permeant through the epithelium.

The γ- scintigraphic images of the rabbits taken after 4 h post administration of placebo buccoadhesive gels, showed the presence of gels in the buccal cavity.

Plasma concentration – time profile of FU containing bioadhesive dosage forms (patch, tablet and gel) displayed an increase in AUC and hence absolute bioavailability when compared with oral conventional tablets (prepared in lab) and intravenous administration of marketed preparation. The absolute bioavailability for bioadhesive patch, tablet and gel were 42.27%, 72.4% and 62.01% respectively which indicated improvement in bioavailability of FU when given by buccal route. Highest Cmax (2058.25±214 ng/ml) amongst all tested formulations was observed with oral conventional tablet and amongst buccoadhesive formulations it was found with tablet (1468.25 ± 203 ng/ml) followed by gel (1189.75±362 ng/ml) and patch (1042.4±284 ng/ml). The sustained-release characteristics of the bioadhesive formulations were also reflected in the MRT and MAT of FU in the body. Both these parameters were significantly increased following buccal administration of the bioadhesive formulations as compared to I.V. administration. The average MRT after buccal administration of patch, tablet and gel were 8.32 ± 2.11, 6.43 ± 1.02 and 8.08± 1.85h, respectively, as compared to 1.07 ± 0.68h after I.V. administration.

Methotrexate:

MTX 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)]. PEG (5%w/w) was used as plasticizer. Patch formulations were evaluated for Assay, bioadhesion, Swelling, surface pH, Tensile strength, Folding endurance and Drug release studies. The assay values were within pharmacopoeial limits (95 % - 105%).

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). The bioadhesiveness appeared to increase with a corresponding increase in the Gantrez content. Maximum bioadhesive strength was seen with the highest levels of Gantrez polymer.

The effect of altering Gantrez AN 139 and HPMC concentrations in the patch on drug release was studied. 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. 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 0.477 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).

The permeation of MTX from patch formulation (MP5) through buccal mucosa 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). The formulation (MP5) was prepared further using GMS as a permeation enhancer (0.1M). No significant difference in adhesive bond strength, tensile strength and moisture content was observed between GMS containing and GMS free patches. XRD and FTIR of physical mixture and patch formulation indicated no modification or interaction between the drug and polymers, even after processing into a patch. No prominent physical changes were observed during the stability study. Insignificant difference (p>0.05) in *in vitro* release was observed at all conditions. Tensile strength reduced on storage at 40 ± 2°C/ 75% ± 5% RH.

MTX bioadhesive bilayer tablets were prepared as per 3^2 Factorial Design. HPMC K15M (10-20 mg), Gantrez MS 955 (5-25 mg) were used as polymers. Bioadhesion [Y_1], release until 8 hours (rel_{8hrs}) [Y_2], time taken to release 50% of drug ($t_{50\%}$) [Y_3] and diffusion coefficient (n) [Y_4] were taken as the response variables. Physical parameters and drug assay for these formulations were with in the acceptable limits. On analyzing the data of all the 9 formulations prepared as per 3^2 Factorial design using Design Expert® software, various polynomial

equations, response surface and contour plots were generated. Gantrez was found to have more pronounced effect on bioadhesion than HPMC K15M as an increase in bioadhesion was observed with rise in levels of Gantrez. High level of HPMC K15M gave low value of percentage MTX release at 8 h at all the levels of Gantrez which might be due to increased thickness of the gel layer formed upon contact with dissolution medium, thus retarding the drug diffusion. At higher levels of Gantrez, increase in MTX Rel_{8hr} was observed at all corresponding levels of HPMC K15M. As Gantrez MS 955 is a water soluble polymer, the above outcome can be explained by the phenomenon that when a soluble polymer is used, the void formed in the matrix by dissolution initially contain a rather viscous solution, characteristic of dissolved polymers. The high viscosity in the pores serves to retard the diffusion of drug at the early stages of release. At the later stages, the polymer solution becomes dissipated and resistance to diffusion is decreased. Linear relationship was obtained between the fraction of HPMC K 15M and $t_{50\%}$, and it was observed from response surface and contour plots that as the fraction of HPMC K15M(X_2) increased, the value of $t_{50\%}$ increased, at all the 3 levels of Gantrez. For $t_{50\%}$ response surface and corresponding contour plots 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. Diffusion exponent ranged from 0.4013 to 0.6761. Formulation M5 was considered optimum on the basis of *in vitro* release and other characterization parameters. The penetration of MTX from tablet formulation (M5) through buccal mucosa 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). The formulation (M5) was prepared further using GMS as a permeation enhancer. FTIR and XRD studies indicated no interaction between MTX and other tablet components. The γ- scintigraphic images of the rabbits taken after 4 h post administration of buccoadhesive tablets(containing ^{99m}Tc), showed the presence of tablets in the same place in buccal cavity. Drug content, hardness, friability and release values of the formulations carried out after 3 months showed no significant differences ($p>0.05$) indicating a stable formulation.

Bioadhesive gels containing MTX were formulated using Poloxamer-407 and Gantrez in various combinations. An increasing trend in the bioadhesive strength

with increase in the amount of Gantrez was observed. All the formulations were found to exhibit assay values in the range 95-105 % depicting good content uniformity. The pH was measured in each gel and found to be within the satisfactory limit of 5.5 – 7.5 which implies that the gel would not cause irritation in the buccal cavity. The apparent viscosity of the Formulation B7 at 40 RPM (at 25±1°C) was found to be 550.52 ± 3.5cps. Increasing the concentration of Poloxamer- 407 significantly decreased the cumulative percent drug released. As the content of Gantrez was increased, decrease in release was discerned.

The goodness of fit for various models investigated for gel formulations ranked in the order of Higuchi ≈ Peppas > zero order > first-order. All the kinetic models, other than the first order, fitted well. The values of diffusional exponent 'n', obtained from the slopes of the fitted Korsemeyer–Peppas model, ranged between 0.5794 and 0.8874. The non-Fickian behaviour (n=0.5-1.0) of the formulations may be attributed due to the gelling property of Poloxamer 407.

The permeability coefficients (Kp) of MTX from gel across cellulose membrane and buccal mucosal membrane were 1.8275x 10^{-4}cm/sec and 3.75x10^{-5} cm/sec respectively. The penetration of MTX from gel formulation (MG 7) through buccal mucosa was found very low when compared with cellulose membrane. Thus, 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

XRD and FTIR studies indicated absence of any kind of interaction between MTX and polymers. MTX bioadhesive gels exhibited good stability over three month storage at 25 ± 2°C.

Histology studies revealed no significant loss of superficial cell layers, 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. The γ- scintigraphic images of the rabbits taken after 4 h post administration of placebo buccoadhesive gels, showed the presence of gels in the buccal cavity

Plasma concentration – time profile of MTX containing bioadhesive dosage forms (patch, tablet and gel) displayed an increase in AUC and hence absolute bioavailability when compared with oral conventional tablets (prepared in lab) and intravenous administration of marketed preparation. The absolute bioavailability for bioadhesive patch, tablet and gel were 48.44%, 40.72% and 44.59% respectively which indicate improvement in bioavailability of MTX when given by buccal route. 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 significantly increased following buccal administration of the bioadhesive formulations as compared to I.V. administration.

Conclusion:

The permeation properties of the two drugs (FU and MTX) were studied and found to be poorly permeable across buccal mucosa and their permeation properties were improved with enhancers. Mucoadhesive formulations (patches, tablets and gels) were prepared for these actives. Greater mucoadhesion was observed for formulations containing more amount of Gantrez. The formulations did not show any significant difference in their *in vitro* release profiles when prepared with penetration enhancers. Histology studies revealed insignificant differences in the buccal mucosal tissues after application of the dosages forms indicating the suitability of the formulations.

In the *in vivo* study, the absolute bioavailability of perorally administered FU tablets was only 32.16%, significantly lower than the absolute bioavailability of the FU buccoadhesive tablet and gel formulations. The absolute bioavailability for FU buccal bioadhesive patch, tablet and gel were 42.27%, 72.4% and 62.01% respectively, which indicate improvement in bioavailability of FU when given by buccal route. Both MRT and MAT were considerably increased following buccal administration. The increased bioavailability of FU from the buccal bioadhesive formulations was due to the increased penetration enhancement of FU and the potential avoidance of first-pass hepatic metabolism.

In the *in vivo* study, the absolute bioavailability of perorally administered MTX tablets was only 19.84%, significantly lower than the absolute bioavailability of the

MTX buccoadhesive tablet, patch and gel formulations. The absolute bioavailability for MTX buccal bioadhesive patch, tablet and gel were 48.44%, 40.72% and 44.59% respectively which indicate improvement in bioavailability of MTX when given by buccal route. Both MRT and MAT were considerably increased following buccal administration. The increased bioavailability of MTX from the buccal bioadhesive formulations was due to the increased solubility and permeation of MTX.

Hence the developed buccal dosage forms of FU and MTX can be potentially useful clinically in treatment of oropharyngeal cancer because side effects associated with the usual higher therapeutic dose of the drug could be greatly reduced. Thus, the results of the present investigations conclusively indicate enhancement in bioavailability of the drugs, 5-Fluorouracil and Methotrexate when administered via buccal route. Moreover, they provide sustained release and also avoid need of swallowing.

Hence, the developed buccal dosage forms of FU and MTX hold promise as better alternative to the conventional formulations. However, further investigations in human beings and under clinical conditions are necessary before they can be fully exploited.