6 SUMMARY AND CONCLUSION

Administration of therapeutic agents through intact skin can be employed for topical and systemic effects. It offers several advantages over the conventional dosage forms such as tablets, capsules, and injections. Currently there are few drugs are marketed as gels and patches such as Diclofenac gels, Nimesulide gels, Nitroglycerin (angina pectoris), Clonidin (hypertension), Scopolamine patches (motion sickness), Nicotine patches (smoking cessation), Fentanyl patches (pain) and Estradiol patches (estrogen deficiency). Administration of drugs in conventional manner is not free from toxicity and G.I. tract related problems. Also hepatic metabolism reduces bioavailability of drug and to initiate and sustained therapeutic action higher doses of drug is needed. Only I/V dreeps can solve the above problem, however continuous presence of needle in the circulatory portal is inconvenient to patient. Ultimately patient compliance is reduced, and management of disease suffers. Drug delivery through skin is an answer and also a safe and convenient alternative to I/V dreeps. It obviates hepatic metabolism also, the problems concerned with G.I. tract route are absent. As skin is excellent barrier for drug transport, only potent drug with appropriate physicochemical properties are suitable for transdermal delivery. Meloxicam is an appropriate candidate for transdermal delivery.

6.1 Selection of drug and its characterization

The important drug properties that affect its diffusion from device as well as across the skin includes its moderate oil and water solubility, low melting point (<300°C) and low molecular weight (<500da), potent with daily dose of few mg (<10 mg/day), non-irritating and non-allergic to skin. Meloxicam fits in these criteria and also hydrophobic drug penetrate more as compared to ionic and hydrophilic drug and with this information Meloxicam was selected for research work. It is practically insoluble or moderately soluble in water.
Meloxicam drug was procured and was characterized prior to incorporate into drug delivery device. The drug was subjected to various preformulation parameters namely melting point, partition coefficient, solubility, FT-IR studies, DSC studies also to check the purity of drug. The calibration of standard curve of Meloxicam obeys the Beer-Lambert’s law within the working range with correlation coefficient \( r^2 \) value of 0.999. From solubility studies it was found that phosphate buffer pH 7.4 was suitable to maintain sink condition so, it was selected as a diffusion medium. As the drug shown moderate solubility in phosphate buffer pH 7.4, to enhance solubility of drug, its inclusion complex with β-cyclodextrin was prepared.

FT-IR studies of Meloxicam, β-Cyclodextrin and its complex at different ratio were conducted. Studies of complex showed physical interaction and not the chemical interaction. UV/VIS Spectroscopic data are useful for the preparation of standard curve and estimation of Meloxicam from various formulations.

**6.2 Preparation and characterization of inclusion complex of drug with β-cyclodextrin**

The solid inclusion complex of Meloxicam and β-cyclodextrin was prepared by kneading method. Inclusion complex formation in solid state was assessed by FT-IR and DSC. Solubility enhancement was studied and it was observed that the solubility of Meloxicam was enhanced by 4 folds with the inclusion formation. The solubility of Meloxicam was increased linearly as a function of the concentration of β-cyclodextrin. After confirmation of solubility, ratio of Meloxicam and β-cyclodextrin was determined at which solubility was enhanced. As it was observed that the solubility was enhanced at the ratio 1:2:: drug: β-CD ratio; hence for further studies, same ratio was used.
6.3 Preparation and Characterization of Gels

Gels of Meloxicam and its complex were prepared by using HPMC E 6 and carbopol 940 and solvent blend of Chloroform: Dichloromethane. pH of gel was adjusted with triethanolamine when carbopol gels were prepared. Permeation enhancers like sodium lauryl sulphate and dimethyl sulfoxide were incorporated in different concentrations. Prepared gels were characterized for clarity, color, homogeneity visually, rheological properties including spreadability, extrudability and viscosity. Formulations were translucent opaque and light yellow in appearance with HPMC-E-6 and carbopol 940. pH checked at room temperature was more than 6.1 ± 0.16. Spreadability of the formulations was determined and it was observed that spreadability of HPMC gel was in the range 20.65-26.71 g.cm/sec, Carbopol gel in the range 18.37-20.42 g.cm/sec, and gel containing combination of these two was found to be 22.33 g.cm/sec. Indicated spreadability of Carbopol gel was good as compared to HPMC gel. Extrudability of Carbopol gel formulations was found to be good requiring less pressure to extrude as compared to the gels containing HPMC and combination. The viscosity of all formulated gels was found in range of 8258 to 9664 centipoises. Drug content of all prepared gel formulations was found in the range 98.18-99.84%. As the percentage drug content of formulations was found satisfactory, the methods adopted for gels formulations were found suitable. Formulation DCG 2 showed pH 6.5 ± 0.11, % drug content 99.84%, viscosity 8634 cps, spreadability 19.57 g.cm/sec and extrudability 210 ± 1.41 gm./cm², proving the formulation better than remaining formulations.

6.3.1 In-vitro drug diffusion Studies: Results of in-vitro drug release across the cellophane membrane using Franz diffusion cell revealed that incorporation of DMSO in Carbopol and HPMC gel shown significant rise in diffusion rate. The rate was increased with the addition of Meloxicam-β-CD complex when Meloxicam was replaced with complex. Formulation DCG-2 containing complex, carbopol and DMSO showed best in-vitro release of 97.48%. Similarly, formulation DCG-2 showed in-vitro release of 88.66 % when the diffusion studies were done across goat abdominal skin. The difference in release rate across cellophane membrane and goat skin might be
because of barrier properties of skin.
After studying all the physicochemical parameters, \textit{in-vitro} drug release it was concluded that the formulation DCG-2 should be optimized. Hence the formulation DCG-2 was subjected for kinetic studies and further animal studies including skin irritation and anti-inflammatory studies.

\textbf{6.4 Preparation and Characterization of Patches/Films}

The patches/films of Meloxicam and Meloxicam-\(\beta\)-cyclodextrin complex were prepared by solvent evaporation method using both hydrophilic and hydrophobic polymers HPMC- E-6 and ethyl cellulose at different concentrations respectively. Films of each polymer were prepared with different concentration of polymer 200, 250, 300, 350 and 400. Formulation with 200 mg of polymer could not form a proper film with both HPMC and EC. Formulation with 400 mg of polymer forms a thick film for both HPMC and EC. Therefore 300 and 350 mg quantity of polymers was used. Formulations with 300 and 350 mg polymer have produced uniform, smooth, transparent and reproducible films. Prepared films were characterized for the physicochemical parameters including physical appearance, weight uniformity, thickness uniformity, folding endurance, tensile strength, drug content and water vapour transmitted. All the prepared films were satisfactory when visually inspected for colour, clarity, flexibility and smoothness. The average weight of all the films ranged between 384 - 393 mg, showing relatively similar weights. Thickness was found in between 0.336 to 0.344 mm in all the batches. Folding endurance of the films was determined by repeatedly folding a strip at the same place till it break. Formulations DCP 2 and DCP 3 showed good folding endurance as compared to other formulations. The tensile strength was relatively good. All the films have shown reasonable tensile strength and no sign of cracking in the films observed. However, it was observed that films containing \(\beta\)-cyclodextrin complex have good tensile strength.
Drug content determination of the films was carried out to ascertain that the loading of drug is uniform in the formulation. All the formulations were found to contain 94.21-98.11\% of drug indicating uniformity of drug content. Formulation DCP 3 has shown maximum drug content of 98.11\%. The water vapor transmission influences permeation...
of drug from the barriers. The films containing HPMC showed water vapor transmission in the range $5.41 \times 10^{-2}$ to $9.7 \times 10^{-2}$, whereas films containing EC showed in the range $1.3 \times 10^{-2}$ to $8.7 \times 10^{-2}$, which might be due to the hydrophilic and hydrophobic natures of polymers respectively. Effect of Eugenol on release of Meloxicam across goat abdominal skin in PBS pH 7.4 was observed and it was found that cumulative % drug release was increased when penetration enhancer eugenol was used in concentration of 5%.

6.4.1 In-vitro drug diffusion Studies: Results of in-vitro drug release across the cellophane membrane using Franz diffusion cell revealed that incorporation of eugenol in the formulations shown significant rise in diffusion rate. The rate was found to be increased with the addition of Meloxicam-β-CD complex when Meloxicam was replaced with complex. Formulation DCP-3 containing complex, HPMC and eugenol showed best in-vitro release of 98.321%. Similarly, formulation DCP-3 showed in-vitro release of 98.354% when the diffusion studies were done across goat abdominal skin. The difference in release rate across cellophane membrane and goat skin might be because of barrier properties of skin.

After studying all the physicochemical parameters, in-vitro drug release it was concluded that the formulation DCP-3 should be optimized. Hence the formulation DCP-3 was subjected for kinetic studies and further animal studies including skin irritation and anti-inflammatory studies.

The flux observed with the gels prepared using Meloxicam was less as compared with the gels containing drug-β-CD complex. The gels containing drug has shown flux in the range 7-9 μg/cm²/h whereas the flux observed with gels containing drug-β-CD complex was in the range 10-12 μg/cm²/h. The permeability coefficients observed with gels containing Meloxicam were in the range $1.18 \times 10^{-2}$ to $2.50 \times 10^{-2}$ cm/h and with gels containing complex were $1.15 \times 10^{-2}$ to $3.01 \times 10^{-2}$ cm/h.

Similarly, the flux observed with the patches prepared using Meloxicam was less as compared with the patches containing drug-β-CD complex. The patches containing drug has shown flux in the range 6-9 μg/cm²/h whereas the flux observed with patches
containing drug-β-CD complex was in the range 9-12 μg/cm²/h. the permeability coefficients observed with patches containing Meloxicam were in the range 1.67 x 10⁻² to 2.20x 10⁻² cm/h and the patches containing complex has shown about 1.89 x 10⁻² to 2.40 x 10⁻² cm/h.

This may be due to the enhancement in solubility and thereby bioavailability of drug.

6.5 Skin Irritation and Anti-Inflammatory studies of Gels

6.5.1: Skin Irritation Studies: Skin irritation is important adverse effect of topical formulations in humans which may depend on many factors, including the concentration, duration and frequency of exposure, exposed skin site, rate of penetration and intrinsic toxic potential of the substance. Thus the assessment of skin irritation of topical pharmaceutical compounds is a significant step in the evaluation of their biocompatibility in human being. So the protocol was devised for evaluation of skin irritation in such a manner that the signs at the site of application would be assessed. The score for skin irritation in terms of erythema and edema in all rabbits was found to be in the category of negligible irritant. Erythemas were not observed in any animal after topical application of test formulation. No any animal indicates edema formation after 24 hrs of topical application of formulation. From those findings it was concluded that the primary irritation score of tested formulation was under the category of negligible irritant.

6.5.2 Anti-inflammatory activity studies: Edema was induced on the right hind paw by subplantar injection of carrageenan. Before carrageenan injection the volume of contralateral paw was measured and was considered the initial (control) reading. Topical application of drug, drug-β-CD complex, gel containing drug, gel containing drug-β-CD complex and standard drug treatments demonstrated to decreased the paw edema volume significantly (P<0.001) compared to vehicle treated animals (control group). The results after 7 hrs of topical application indicated to decrease the edema volume by 25.74% in complex drug, drug by 5.13%, complex drug gel by 13.89% and drug gel by 2.39 %. The maximum percentage of edema volume was decreased by standard gel (28.84%). The
overall results indicated complex drug and its gel decreased the maximum paw edema compared to drug and its formulation which was comparable to standard gel Diclofenac.

6.6 Skin irritation and anti-inflammatory studies of patches/ films:

6.6.1 Skin Irritation Studies: Skin irritation is important adverse effect of topical formulations in humans which may depend on many factors, including the concentration, duration and frequency of exposure, exposed skin site, rate of penetration and intrinsic toxic potential of the substance. Thus the assessment of skin irritation of topical pharmaceutical compounds is a significant step in the evaluation of their biocompatibility in human being. So the protocol was devised for evaluation of skin irritation in such a manner that the signs at the site of application would be assessed. The score for skin irritation in terms of erythema and edema in all rabbits was found to be in the category of negligible irritant. Erythemas were not observed in any animal after application of test formulation. No any animal indicates edema formation after 24 hrs of application of formulation. From those findings it was concluded that the primary irritation score of tested formulation was under the category of negligible irritant.

6.6.2 Anti-inflammatory activity studies: Edema was induced on the right hind paw by subplantar injection of carrageenan. Before carrageenan injection the volume of contralateral paw was measured and was considered the initial (control) reading. The results of present study indicated to reduce the carrageenan paw edema volume. The results of present study demonstrated the treatment of test samples indicated to reduce the carrageenan paw edema volume. Results of the study indicated to increase in the paw edema formation in all animals after 1 hrs of carrageenan injection. Test samples decreases the edema formation. Application of drug, drug-β-CD complex, patch containing drug, patch containing drug-β-CD complex demonstrated to decreased the paw edema volume significantly (P<0.001) compared to vehicle treated animals (control group). The results after 7 hrs of topical application indicated to decreases the edema volume by 25.74% in complex drug, drug by 5.13%, complex drug patch by 13.10% and drug patch by 7.43%. The maximum percentage of edema volume was decreased by
standard gel (28.84%). The overall results indicated complex drug and its patch decreased the maximum paw edema compared to drug and its formulation.

6.7 Comparison of Anti-inflammatory studies between optimized gel, patch and marketed gel

It was observed that the optimized formulation of gel and patch showed greater in vitro drug release and permeation respectively. In addition, the permeability coefficients were found to be higher for these formulations.

The anti-inflammatory activities of optimized formulations of optimized gel and patch were evaluated using the carrageenan induced hind paw edema method. Mean percent edema of all groups was calculated. Percent inhibition of edema was determined with respect to control group in those groups in which formulations were applied. Inhibition of edema was found to be highest in the groups in which gel was applied. Results after 7 hrs of topical application indicated to decrease the edema volume by 25.74% in complex drug, gel containing drug-β-CD complex by 13.89% and patch containing drug-β-CD complex by 13.10%. The maximum percentage of edema volume was decreased by standard gel (28.84%).

The results indicated that the complex drug and its gel decreased the maximum paw edema compared to patch formulation which was comparable to standard gel Diclofenac.

6.8 Kinetics of drug release

To find out the kinetics and mechanism of drug release, the data obtained from the cumulative release from optimized formulation of gel (DCG 2), was treated according to Zero order, First order, Higuchi square root law and Korsmeyer Peppas kinetic. The results indicated that the release of Meloxicam from optimized formulation exhibits diffusion characteristics, closely following zero order equation and is highly correlated with Korsmeyer’s and Peppas release kinetics.

The release profile of optimized patch formulation (DCP 3) was fitted in the various kinetic models. The results showed that the coefficient of determination ($r^2$) was found to be much closer to 1 for Korsmeyer-Peppas equation. The mechanism of release was
determined by n value of Korsmeyer Peppas model. It indicates that the optimized formulation follow non-fickian diffusion with n values (0.5<\text{n}<1.0) of 0.996. However, it was also observed that although the coefficient of determination \((r^2)\) with Higuchi equation was closer to 1; the n value was greater than 1.0 with Higuchi equation. This means that the formulation is following more than one type of release profiles possibly owing to chain disentanglement and swelling of hydrophilic polymers.

6.9 Accelerated Stability studies.

Stability testing was carried out to determine the quality of formulation under the influence of temperature and humidity over time as per ICH guidelines. Optimized formulation of gel (DCG 2) was subjected to accelerated stability testing in stability chamber. It was stored at different temperature and humidity conditions for a period of three months in a stability chamber and appearance, pH, viscosity, spreadability and drug content were determined. It was found that the formulation remained stable and no significant changes were observed.

Similarly the optimized formulation of patch (DCP 3) was subjected to accelerated stability testing in stability chamber for the period of three months in a stability chamber and the parameters appearance, weight, folding endurance, tensile strength and \% drug content were determined. It was found that the formulation remained stable and no significant changes were observed.