Chapter 11 Result and Discussion
Transdermal route of administration has been widely recognized as a better route of administration because it has the advantages of the intravenous therapy but overcomes the disadvantages associated with intravenous and oral routes. It also bypasses the first pass metabolism.

By the year 2050, the proportion of those over 60 years is predicted to be one person in three and the median age is expected to rise from 23.4 in 2050 to 31.1. Therefore, aim of the present investigation was directed at the elderly population. Currently over 50% of oral NSAIDs are prescribed for Osteo-Arthritis, which is the most common Rheumatic disease. It is the principal source of pain and disability in the elderly and the overall disease prevalence increases with age. 10-20% of the people over 65 years have the disease for the knee & hips. For several years biomedical research has been involved in the development of anti-inflammatory and antirheumatic compound to be used in these chronic conditions. These drugs should posses, rapid and long-lasting analgesic effect, sustained anti-inflammatory and antiarthritic activity, good tolerability even after prolonged treatment in the elderly patients and easy dose schedule and if possible one single daily administration. In this background Diclofenac and Ketorolac were selected as the drug candidates for the development of transdermal systems for the NSAIDs. Diclofenac has low molecular weight, considerable first pass hepatic metabolism and short plasma half-life. The dosage form was aimed at the elderly for providing relief from pain in the arthritic patients, who are generally suffering from gastrointestinal and renal problems. Avoidance of GI tract should mitigate the common direct toxicities of acute mucosal lesions, nausea, vomiting, and dyspepsia, diarrhea acute mucosal lesions, which occur secondary to high local concentrations of NSAID in the alimentary tract. The formulation also provides for termination of medication if required at any time, which is particularly relevant for elderly patients who should be kept under strict medical control, which is otherwise impossible to perform due to frequently consumed diuretics and co-medication, therefore, the dose calculation for them is also not possible.
Diclofenac and Ketorolac have low inherent permeabilities, therefore, in an attempt to increase the permeation of *Diclofenac diethylammonium* and *Ketorolac tromethamine* naturally occurring terpenes which have been given the status of generally recognized as safe (GRAS) by the US FDA were evaluated as penetration enhancers. Amongst them were Mentha oil, Lemon oil, Eucalyptus oil, Thymol, Geraniol, and Chamomile oil. *Rat abdominal skin* was used for diffusion studies. There is no one species of animal whose cutaneous diffusion barrier to a range of permeants has been shown identical to that of man. From a permeability point of view, of more interest are stratum corneum measurements, the thickness of which is similar in rats and humans. The *rat epidermis* and skin are two thirds as thick as human skin; the relatively thick stratum corneum is due to the composition of the epidermis, which is over 50% stratum corneum by thickness. Though rat skin is more permeable than human skin, the ease and convenience of handling and the lower cost made the rat a good model for the present study.

Chamomile oil, which is an essential oil, used in traditional medicine as anti-inflammatory, analgesic, antispasmodic. Chamomile oil contains the optically active terpene (-) α-Bisabolol. It is obtained from flowers of Chamomile (*Matricaria recutita* L). In the present study it was found to be the best enhancer with an enhancement ratio of 19.78 for diclofenac diethylammonium and 14.48 for Ketorolac tromethamine. Chamomile oil showed good enhancement (19.78) for diclofenac diethylammonium, which is more than that of geraniol, reported to be the best terpene enhancer for diclofenac sodium (Enhancement ratio reported- 18.97, found- 17.17). The enhancement ratio achieved from various enhancers is shown in table 6-31 and table 6-32. It has been suggested that the mechanism of action of terpenes involves the disruption of intercellular lipids of the stratum corneum. It appears, that for hydrophilic drugs, the primary effect of terpene enhancer treatment is to increase drug diffusivity, but also increase the drug partitioning into the stratum corneum. (Cornwell *et al.* in 1996) The cumulative amount of diclofenac diethylammonium and ketorolac tromethamine through rat abdominal skin as a function of time with different drug loading was studied. In all cases a steady state
was reached within three hours and maintained for 24 hours. The lag time was determined by extrapolating the linear portion of the cumulative amount of drug versus time plot to the abscissa. The time to reach the steady state was not influenced by the drug loading. The skin flux increased from 0.108 mg/cm²/h to 0.851 mg/cm² h⁻¹ as the drug loading was increased from 10 mg/3.14 cm² to 100 mg/3.14 cm² for diclofenac diethylammonium using chamomile oil as enhancer and 0.117 mg/cm²/h to 0.888 mg/cm² h⁻¹ using geraniol as enhancer but there was no corresponding increase in the permeability coefficient. For Ketorolac tromethamine flux increased from 0.0439 mg/cm² h⁻¹ to 0.300 mg/cm² h⁻¹ with chamomile oil as enhancer as the drug concentration increased from 5 mg to 50 mg/3.14 cm² and 0.03754 mg/cm² h⁻¹ to 0.267892 mg/cm² h⁻¹ with geraniol as enhancer. This can be attributed to the fact that with increased donor concentration the amount of drug permeated across skin increased (therefore, increased flux) but the increase was not proportional to the increased amount of drug in the donor phase (i.e. decreased permeability coefficient.

For the preparation of an optimized TDDS of Diclofenac diethylammonium and Ketorolac tromethamine various adhesive polymers and plasticizers were tried. Placebo films using pressure sensitive adhesives were prepared. And evaluated with respect to pressure sensitive adhesive nature, ease of pealibility from the system, ease of removal from the skin after use and compatibility with system and skin. The adhesive polymers were selected to formulate a matrix type of adhesive transdermal patches. Selection was based on the adhesive properties of the polymer and compatibility with the drug. The polymer selected for the study were acrylate, silicone and polyisobutylene type of pressure sensitive adhesives. Propylene glycol (5%) and polyethylene glycol (20 %), 0.5% chamomile oil and geraniol were used separately as penetration enhancer. Propylene glycol and polyethylene glycol was selected as plasticizer since propylene glycol is known to act synergistically with terpene enhancers, the synergistic action probably occurs through enhanced lipid disruption at normal skin temperatures. (Cornwell et al., 1996), Polyethylene glycol can also have dramatic effect on NSAID
release rates. (Heyneman et al., 2000). The medicated films were prepared by casting on the backing layer (Alupoly film). The films were dried at room temperature. Low adhesion polyester film was used as covering liner. The formulations were evaluated for physicochemical characteristics viz. aesthetic appearance, adhesion evaluation, cold flow, weight variation, thickness, folding endurance, assay, and identity of the drug.

*In vitro* diffusion studies were conducted on the TDDS using rat skin as the model membrane. The formulations FD-1 and FD-4 were promising for diclofenac diethylammonium showing a cumulative release of 68% and 67% respectively. Formulations FK-1 and FK-4 showed promising results for ketorolac tromethamine exhibiting a release of 41% and 53% which also shows that the formulation prepared using the acrylate adhesive were promising. The plot of cumulative percentage drug release versus square root of time were linear which indicate that the drug diffusion takes place by the mechanism of diffusion and follow a zero order kinetics. The selected formulations were packed in self sealing polythene bags and kept on stability in three conditions – Refrigerator (2°C-8°C), controlled room Temperature (CRT) (25°C and 60% RH) and at 40°C and 75% Relative humidity for a period up to six months. There was no change in physical appearance, assay, dissolution, diffusion and adhesion properties at CRT as well as accelerated conditions of stability. The transdermal patches of diclofenac diethylammonium were stable at 40°C ± 5°C and 75±% RH. The degradation constant was also small, \(-3.30881\times 10^{-5}/\text{day}\). Stability studies for transdermal patches showed no significant change in drug content, so a tentative shelf life of 24 months was proposed for the Diclofenac diethylammonium TDDS. Ketorolac tromethamine patches failed in the stability studies and therefore, studies on ketorolac tromethaine TDDS were not carried out any further.

Pharmacodynamic studies were carried out on the selected TDDS. The preliminary skin irritation studies were carried out. The TDDS did not show any erythema or necrosis and therefore passed the skin irritation test. The anti-inflammatory and analgesic activity was
studied in rats. The activity was compared with the marketed Diclofenac gel. The analgesic and anti-inflammatory activity for the TDDS was not only higher than that produced by the marketed gel but the action was also sustained. The anti-inflammatory and analgesic activity produced by the transdermal patches was significantly higher as indicated by the student's 't' test at every hour of observation (p<0.01). From 4\textsuperscript{th} hour onwards activity produced was highly significant. (p<0.001). The magnitude of anti-inflammatory and analgesic activity produced by the transdermal patches of Diclofenac diethylammonium containing chamomile oil as enhancer was higher than that obtained by patches containing geraniol. In order to confirm the validity of the postulate that transdermal administration is superior to the oral route, the efficacy must be demonstrated therefore a study was conducted to assess the effectiveness of the TDDS (100mg) as against the conventional oral dose of (150 mg/day in two or three divided doses). Diclofenac diethylammonium in the TDDS in its slow release formulation (100mg) provides relief for at least 24 hours with a single application. Hence, the same therapeutic effect as the oral conventional dose is obtained with a lesser dose of drug through the TDDS. Also the drug administration through the TDDS has the advantage that it elicits lesser side effects than the oral administration and bypasses the first pass metabolism, which is very high for Diclofenac diethylammonium (40%).

Bioavailability studies were conducted in rabbits and in human volunteers. Excellent correlation was observed between the animal and human volunteers study. The concentration of the drug reached maximum between 6-8 h in both animal and in the human volunteers, a C\textsubscript{max} of 0.066 \(\mu\)g/ml (FD-1) and 0.0646 \(\mu\)g/ml (FD-4) was obtained for humans and 0.419\(\mu\)g/ml was obtained for animals (Rabbits), thereafter the concentration of drug reduced gradually but even at 24\textsuperscript{th} hour, measurable quantities of diclofenac persisted in both animals and humans. The area under curve (AUC) values (which more correctly define bioavailability) indicated an extent of drug availability of 889 \(\mu\)g.h.ml\(^{-1}\) (FD-1), 0.959 \(\mu\)g.h.ml\(^{-1}\) (FD-4) in human volunteers and 6.232 \(\mu\)g.h ml\(^{-1}\) in rabbits. The elimination rate constant was found to be 0.069/h (FD-1) and 0.05 (FD-4).
in human volunteers and 0.158/h in rabbits. The calculated parameters also indicate that half-life of elimination for diclofenac is prolonged from two hours (conventional tablets) to about ten hours in human volunteers and to 4.3 hours in rabbits, hence the drug will remains in the body for a longer period of time and thus exerts a sustained effect.

The NSAIDs administered topically lead to relatively high concentrations in the dermis. Concentrations achieved in the muscle tissue below the site of application are variable, but at least equivalent to that obtained with oral administration. But topically applied formulations do not have a sustained action and have to be applied frequently for relief from pain. The topical gels and creams available for diclofenac do not provide a sustained effect and even after occlusion a 300 mg emulsion gel applied over an area of 360 cm$^2$ provides mean plasma C$_{max}$ of 0.039 μg/ml. Ex-vivo studies suggest that occlusion can enhance NSAID skin penetration and the product formulation may have dramatic impact, not only on penetration rates but also on penetration depth. (Heyneman et al., 2000) Also the drug penetrates slowly and in small quantities, the bioavailability and plasma concentrations are generally low and variable. The TDDS system that was developed showed a sustained action with a dose much lower to the oral conventional dose since the transdermal route bypasses the first pass metabolism, which is very high for diclofenac (40 %). Since this dosage form can be applied at the site of action itself, the drug not only permeates the skin and reaches the systemic circulation, but also concentrates itself at the site of action, hence it is possible to achieve maximum pharmacological effects with minimum quantities of the drug, thus making it possible to reduce dose administered through this route with a concomitant reduction in the side effects associated with the drug.

Therefore the acrylate based pressure sensitive system can be developed satisfactorily for further development of the transdermal drug delivery system of diclofenac in a larger and commercial scale.