REVIEW OF LITERATURE
2.1 TRANSDERMAL DRUG DELIVERY SYSTEM

2.1.1 Introduction to transdermal drug delivery system

The treatment of acute or chronic illness has, hitherto, been accomplished by delivering drugs to the patient via a variety of pharmaceutical formulations, like tablets, capsules, injections, aerosols, creams, ointments, suppositories, liquids, etc., as carriers which are referred to as conventional drug formulations. These have to be often administered several times a day in order to achieve, and maintain, drug concentrations within the therapeutically effective range. However, drug levels tend to fluctuate causing several problems. Thus, the drug becomes ineffective when plasma concentrations fall below the minimum effective dose, or may induce adverse effects when the drug concentration overshoots the therapeutic window. Under these circumstances, the clinical use of such conventional drug formulations, which remain the primary pharmaceutical products prescribed and are available as non-prescribing drugs, is sometimes of questionable value from the viewpoints of efficacy and safety (Mishra et al., 1990). In recent years, several technological advances have resulted in the development of different types of novel drug delivery systems, which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic affectivity and targeting the drug delivery to a particular tissue. Out of various novel drug delivery systems available, the transdermal drug delivery system, which is also known as transdermal therapeutic system or transdermal patches, remains one of the most rapidly evolving areas in pharmaceutical research.
2.1.2 Definition and explanation of transdermal drug delivery system

Transdermal permeation or percutaneous absorption can be defined as the passage of a drug from the outside of the skin through its various layers into the bloodstream, and this term includes all topically administered drug formulations.

Any time there is systemic accumulation of a drug, unwanted side effects or toxic effects can occur.

2.1.3 Development of transdermal drug delivery systems

Several technical approaches have been used to provide rate control over the release and transdermal permeation of drugs. All such transdermal dosage forms have a basic structure comprising many layers, with each having a specific function.

1. An outer covering (barrier): This backing layer is the outer most layer of the transdermal system which prevents wetting of the system during use.

2. A drug Reservoir: The second layer contains a reservoir that supplies a continuous quantum of drug for the predetermined functional lifetime of the system.

3. Rate control polymeric membrane: This regulates the rate of drug delivery from the drug reservoir to the skin surface. However, some transdermal systems do not have a rate control membrane and the drug diffuses directly from the reservoir to the skin surface.

4. Adhesive layer: Which is applied to part or whole of the system/skin interface and it contains a small fraction of the drug to deliver the initial loading dose and
glues the system to the skin. The adhesive layer is covered with a protective release liner that protects the integrity of the dosage form, and is removed before the use of the transdermal system.

The activity of these systems is defined in terms of the rate of release of drug(s) from the system.

Transdermal drug delivery system provides rate controlled, continuous supply of the drug during a predetermined time interval. The drug so delivered diffuses through the skin and enters the systemic circulation, bypassing the liver and the rate of drug release from the transdermal device is normally a small fraction of the amount that the skin can possibly absorb. Hence, even if there are variations in skin permeability, a constant rate of drug input into the circulation is achieved.

2.1.4 Transdermal drug delivery system available in the market

The commercialization of transdermal patches for controlled drug delivery began two decades ago and has resulted in diverse products. Among them are nitroglycerine for angina (Hadgraft et al., 1993), scopolamine for prophylaxis or therapy of motion sickness (Shaw, 1983), nicotine for smoking cessation (Ho and Chien, 1993), estradiol for hormone replacement therapy (Tymes et al., 1990) testosterone for male hypogonadism (Kim et al., 2001), clonidine for hypertension (Toon et al., 1989).
2.1.5 Benefits and Limitations of transdermal drug delivery systems

Certainly, each dosage form has its unique place in medicine, but some attributes of the transdermal drug delivery system provide distinct advantages over the traditional methods of attaining systemic levels of drugs (Cleary, 1984; Kydonieus, 1987).

Advantages

1. The system avoids chemically hostile GI environment.

2. It doesn’t have GI distress or other physiologic contraindications of oral route.

3. It provides controlled administration of a therapeutically effective dose at a desired rate of delivery.

4. It allows effective use of drugs with short biological half-lives.

5. It allows administration of drugs with narrow therapeutic window.

6. It maintains drug concentration within an optimal therapeutic range even during prolonged therapy.

7. It leads to the reduction of adverse drug effects.

8. It maximizes the efficacy – dose relationship.

9. It minimizes the need for frequent drug administration.

10. It leads to better patient compliance due to reduction in the frequency of dosing.

11. It bypasses hepatic first pass metabolism.

12. It interrupts drug input promptly when toxicities occur. (easy termination of drug therapy by mere removal of patch).

13. It has better cost: benefit ratio.
14. Self-application is possible.

Disadvantages

1. Drugs that require high-blood level can not be administered.

2. Adhesive may not adhere well to all types of skin.

3. Drug or drug formulation may cause skin irritation or sensitization.

4. It is uncomfortable to wear.

5. System may not be economical (Cleary, 1984; Kydonieus, 1987).

6. Difficulty of permeation through human skin for some drugs, e.g., high molecular weight drugs.

7. Cutaneous metabolism may occur.

2.2 FUNDAMENTALS OF SKIN PERMEATION

2.2.1 Structure of the skin

The skin is one of the most extensive and readily accessible organ of the human body, covering an average of 2 m² surface area and receiving about one third of the total blood circulating at a given time, in an adult (Gale et al., 1999). The human skin is multi layered organ, the three major tissue layers being (1) the epidermis, (2) the dermis which is richly supplied by blood vessels and lymphatics which provide an efficient means of drug removal into the systemic pool. (3) The hypodermis (or subcutaneous fat).
2.2.2 Stratum corneum as the skin permeation barrier

The epidermis is composed of five layers with the stratum corneum (15 µm thick) forming the outermost layers consisting of many layers of compact, flattened, dehydrated and keratinized cells, which act as a physical protective barrier (fig.1). The constituents of these cells are proteins, water and lipids, in which, the latter are concentrated largely in the extracellular phase and serve to cement the structure together into a coherent membrane (Forslind et al., 1997; Chien, 1992). Before a topically applied drug can act either locally or systemically, it must penetrate the stratum corneum. This horny layer of the skin presents very peculiar physico-chemical properties, acting both as a barrier and a reservoir. It has been said that nothing penetrates freely through this layer but everything penetrates to a certain degree (Forslind et al., 1997; Chien, 1992). Beneath the stratum corneum, the living viable epidermis is highly hydrophilic in nature and the diffusional properties of this region are somewhat similar to those of an aqueous protein gel.

The phenomenon of percutaneous absorption (or skin permeation) of a drug molecule presented in a dermal delivery system can be visualized as consisting of a series of steps in sequence.

1. Diffusion of the drug molecule within the device.

2. Diffusion of a penetrant molecule onto the surface layers of stratum corneum.

3. Partitioning through it into the viable epidermis.

4. Diffusion through the viable epidermis.
5. Finally, at the papillary layer of the dermis, the molecule is taken up into the microcirculation for subsequent systemic distribution.

The viable tissue layers and the capillaries are relatively permeable, and the peripheral circulation is sufficiently rapid, so that for the great majority of penetrants, diffusion through the stratum corneum is often the rate limitation step. Hence the stratum corneum acts as a passive, but not an inert, diffusion medium. No active transport process has been shown to be involved in skin permeation, therefore the mechanism of permeation follows the diffusion principles described by Fick.

![Fig. 1: The structure of skin](image)

There are significant differences in the structure and chemistry of human stratum corneum from one region of the body to another that are reflected in the skin's permeability. For instance, plantar and palmar callus can be as thick as 400-600 μm compared to only 10-20μm for the back, arms, legs, and abdomen.
Drug molecules may diffuse through the skin by three different routes: the intact stratum corneum, the hair follicle region, and the sweat gland ducts. Recently a growing number of investigators are inclined to accept both routes, with the relative importance depending upon the characteristics of the penetrating molecules. In the initial transient diffusion stage, the drug molecules may penetrate the skin along the hair follicles or sweat ducts and are then absorbed through the follicular epithelium and the sebaceous glands. When a steady diffusion has been reached, diffusion through the stratum corneum becomes the dominant pathway.

For a systemically active drug to reach a target tissue remote from the site of drug administration on the skin surface, it must possess physico chemical properties that facilitate the sorption of drug by the stratum corneum, the penetration of drug through the viable epidermis, and also the uptake of drug by microcirculation in the dermal papillary layer (fig. 2). The rate of permeation ‘dQ/dt’ across various layers of skin tissue can be expressed mathematically as:

\[
\frac{dQ}{dt} = P_s(C_d - C_r)
\]

(1)

Where \(C_d\) and \(C_r\) are, respectively, the concentrations of a skin penetrant in the donor phase, e.g., the concentration of drug on the stratum corneum surface as delivered from transdermal drug delivery system, and in the receptor phase, e.g., systemic circulation; and \(P_s\) is the overall permeability coefficient of the skin tissues to the penetrant and is defined by:

\[
P_s = \frac{K_{s|d}D_{ss}}{h_s}
\]

(2)
Fig. 2: Drug release and absorption across skin tissues form the site of topical drug application.
Where $K_{w,d}$ is the partition coefficient for the interfacial partitioning of the penetrant molecule from a transdermal drug delivery system onto the stratum corneum; $D_{ss}$ is apparent diffusivity for the steady-state diffusion of the penetrant molecule through the skin tissues; and $h_s$ is the overall thickness of the skin tissues for penetration.

In order to achieve a constant rate of drug permeation, the drug concentration on the surface of the stratum corneum ($C_d$) should be consistently and substantially greater than the drug concentration in the body ($C_r$) i.e. $C_d >> C_r$. Therefore equation (1) can be reduced to

$$\frac{dQ}{dt} = P_s C_d$$ \hspace{1cm} (3)

Hence the rate of skin permeation ($dQ/dt$) becomes constant if the $C_d$ values remain fairly constant throughout the course of skin permeation. To maintain the $C_d$ at a constant value, it is necessary to deliver the drug at a rate $R_d$ that is either constant or always greater than the rate of skin absorption $R_a$; i.e., $R_d >> R_a$. By doing so, the drug concentration on the skin surface $C_d$ is maintained at a level equal to or greater than the equilibrium (or saturation) solubility of the drug in the stratum corneum $C^*_s$; i.e., $C_d \geq C^*_s$; and a maximum rate of skin permeation ($dQ/dt)_m$, as expressed by equation (4), is thus achieved:

$$\left(\frac{dQ}{dt}\right)_m = P_s C^*_s$$ \hspace{1cm} (4)

Thus, the magnitude of $(dQ/dt)_m$ is determined by the skin permeability coefficient of the drug ($P_s$) and its equilibrium solubility in the stratum corneum $C^*_s$. This indicates that skin permeation of drugs is stratum corneum limited.
2.3 FACTORS AFFECTING TRANSDERMAL PERMEATION

In the development of transdermal drug delivery systems and the subsequent transport of the drug(s) through the skin, a series of interrelated elements must be taken into consideration. These factors can be classified into four basic areas (Ranade, 1991; Sood and Jayaswal, 1987).

2.3.1 Skin structure and its properties

In respect of drug permeation, the most important tissue in this complex organ is the stratum corneum (SC) or horny layer, which usually provides the rate-limiting or slowest step in the penetration process.

Skin possesses several properties that regulate the rate of drug permeation. These are:

Lipid bilayer: The lipid film that is present in the surface of the skin acts as a protective layer, which prevents the removal of moisture from the skin and helps in maintaining the barrier function of the SC.

Skin temperature: Increase in the skin temperature results in increase in the rate of skin permeation due to rise in solubility of drug in skin tissues and increased dilation of skin vessels.

Skin hydration: Hydration of SC can enhance transdermal permeability. Skin hydration can be achieved simply by covering or occluding the skin with plastic sheeting leading to accumulation of sweat and condensed water vapour.
Cutaneous drug metabolism: Catabolic enzymes present in the viable epidermis may render a drug inactive by metabolism and thus affect the topical availability of the drug. Other properties that affect drug penetration through the skin are the integrity and thickness of the SC, density of sweat glands and follicles and the presence of injuries on the skin, which leads to increase in the permeability of the drug.

2.3.2 The penetrating molecule and its physical & chemical properties

a. Water-soluble electrolytes like sodium chloride, potassium chloride, and non-electrolytes like glucose, urea, etc., penetrate the skin freely.

b. Lipid soluble substances have the best chance of diffusing into the horny layer, especially when they are non-polar and of moderately low molecular weight.

c. Polymers and macromolecules of high molecular weight, like proteins and polysaccharides, penetrate poorly.

d. Partition coefficient: Drugs possessing both lipid and water solubilities are favorably absorbed through the skin. Transdermal permeability coefficient shows a linear dependency on partition coefficient. A lipid-water partition coefficient of 1 or greater is generally required for optimal transdermal permeation.

e. pH conditions: Application of solutions whose pH values are either very high or very low can be damaging to the skin. The flux of ionizable drugs can be affected by changes in pH that alter the ratio of charged and uncharged species and hence their transdermal permeability.
f. Penetrant concentration: Assuming membrane limited transport, increasing concentration of dissolved drug in the delivery system causes a proportional increase in its flux. Stability, binding affinity, and vehicle effects are the other factors affecting drug permeation.

2.3.3 The design of the delivery system carrying the penetrant

The composition of drug delivery system not only affects the rate of drug release, but also the permeability of stratum corneum by means of hydration. The barrier permeability can be enhanced by using penetration enhancers or decreased by addition of other solvents, for example, benzocaine permeation can be decreased by addition of polyethylene glycols of low molecular weight.

The release rate of the drug from the delivery system depends upon:

1. The solubility of drug in the vehicle, i.e. whether the drug molecules are dissolved or suspended in the delivery system.

2. The interfacial partition coefficient of the drug from the delivery system to the skin tissue.

3. pH of the vehicle.

2.3.4 The combination of skin, the penetrant, and the delivery systems as a whole

Although the process of penetrating the skin by drug has been simplified by representing it as one of simple unidirectional transport, in practice, the sequence is more complicated. Factors playing their part include:
1. Inhomogeneity of the stratified tissues.

2. The interruption of the stratum corneum, by hair follicles and sweat glands.

3. The division of basal cells, their transport through the horny layer and their loss from the surface.

4. Since drugs penetrate the skin under dynamic conditions, the medicament, vehicle components, and occlusive hydration effects may progressively change the skin barrier.

5. Sweat, sebum, and cellular debris may enter the product, changing its physico-chemical characteristics.

6. Volatile solvents may evaporate which will alter the chemical potential of the drug and may even develop supersaturated solutions.

7. For the type of transdermal delivery device that incorporates a rate-controlling membrane, the flux across this barrier should be low enough so that the underlying skin acts as a sink by reducing the resistance of the horny layer by using penetrant enhancers.

2.4 TECHNOLOGIES FOR DEVELOPING TRANSDERMAL DRUG DELIVERY SYSTEMS

Several technologies have been successfully developed to provide rate control over the release and skin permeation of drugs. Four different approaches have been utilized to manufacture transdermal patches: (Chien, 1987; Misra, 1995).
Polymer Membrane permeation-Controlled systems

In this system the drug reservoir is sandwiched between a drug impermeable metallic plastic laminate and a rate-controlling polymeric membrane, which may be microporous or non-porous, e.g., ethylene-vinyl acetate (EVA) copolymer, with a defined drug permeability property (Fig.3). The drug molecules are permitted to release only through the rate-controlling polymeric membrane. In the drug reservoir compartment, the drug solids are dispersed homogeneously in a solid polymer matrix (e.g., poly isobutylene), suspended in an unleachable, viscous liquid medium (e.g., silicone fluid) to form a paste like suspension, or dissolved in a releasable solvent (e.g., alkyl alcohol) to form a clear drug solution. On the external surface of the polymeric membrane a thin layer of drug compatible, hypoallergenic pressure-sensitive adhesive polymer, e.g., silicone adhesive, may be applied to provide intimate contact of the system with the skin surface. The rate of drug release from this system can be tailored by varying the composition of the drug reservoir formulation and the permeability coefficient and/or thickness of the rate controlling membrane and the adhesive.

The intrinsic rate of drug release \( \frac{dQ}{dt} \) from this system should be constant and defined by the following equation:

\[
\frac{dQ}{dt} = \frac{K_{m/r}K_{a/m}D_mD_m}{K_{m/r}D_mD_m + K_{a/m}D_mD_a}C_R
\]

Where \( C_R \) is the drug concentration in the reservoir compartment; \( K_{m/r} \) and \( K_{a/m} \) partition coefficients for the interfacial partitioning of drug from the reservoir to the membrane and from the membrane to the adhesive layer, respectively; \( D_m \) and \( D_a \) are
diffusion coefficients in the rate controlling membrane with thickness of $h_m$ and in the adhesive layer with a thickness of $h_a$ respectively. For a microporous membrane the porosity and tortuosity of the membrane should also be taken into account in calculation of the $D_m$ and $h_m$ values.

The constant release rate of the drug is the major advantage of membrane permeation controlled transdermal system. However, a rare risk also exists when an accidental breakage of the rate controlling membrane can result in dose dumping or a rapid release of the entire drug content. Examples of this system are mentioned in table 1.

![Cross-sectional view of polymer membrane permeation controlled transdermal drug delivery system](image)

**Fig. 3: Cross-sectional view of polymer membrane permeation controlled transdermal drug delivery system**
Table 1: Examples of membrane moderated transdermal controlled release products

<table>
<thead>
<tr>
<th>Drug</th>
<th>Trade name</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitroglycerine</td>
<td>Transderm Nitro</td>
<td>Angina pectoris</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>Transderm Scop</td>
<td>Motion Sickness</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Estraderm</td>
<td>Hormone treatment</td>
</tr>
<tr>
<td>Clonidine</td>
<td>Catapress- TTs</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>Duragesic</td>
<td>Opioid analgesic</td>
</tr>
<tr>
<td>Diphenhydramine</td>
<td>Zenol</td>
<td>Antihistamine</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>Zepolas</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Indomethin</td>
<td>Antiinflammatory</td>
</tr>
</tbody>
</table>

2.4.2 Polymer Matrix Dispersion controlled transdermal drug delivery system

In this system, the drug reservoir is prepared by homogeneously dispersing drug particles in a hydrophilic or lipophilic polymer matrix (Liquid polymer or a highly viscous base polymer). The resultant medicated polymer is then moulded into a medicated disc with a defined surface area and controlled thickness.

This drug reservoir-containing polymer disc is then pasted on to an occlusive base plate in a compartment fabricated from a drug impermeable plastic backing. The adhesive polymer is then spread along the circumference to form a strip of adhesive rim around the medicated disc. (fig. 4).
The rate of drug release in this system is defined by:

\[
\frac{dQ}{dt} = \left( \frac{L_0 C_p D_p}{2t} \right)^{1/2}
\]  

(2)

Where \( L_0 \) is the initial drug loading dose dispersed in the polymer matrix, \( C_p \) and \( D_p \) are solubility and diffusivity of the drug in the polymer, respectively.

The advantage of the matrix diffusion is the absence of dose dumping, since the polymer cannot rupture.

### 2.4.3 Adhesive diffusion controlled system

This is a simplified form of the membrane permeation controlled system. As represented in Fig (5), the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer, made up of either poly (isobutylene) or poly (acrylate), and then spreading the medicated adhesive, by solvent casting, onto a flat sheet of drug-impermeable metallic plastic backing, to form a thin drug reservoir layer. The
layers of non-medicated rate-controlling adhesive polymer, of constant thickness, are applied on the top of the drug reservoir layer to produce an adhesive diffusion controlled delivery system. The rate of drug release in this system is defined by:

$$\frac{dQ}{dt} = \frac{K_{\text{av}} D \alpha C_r}{h_\alpha}$$

Where, $K_{\text{av}}$ is partition coefficient for the interfacial partitioning of drug from the reservoir layer to the adhesive (a) layer.

Fig. 5: Cross sectional view of adhesive diffusion controlled transdermal drug delivery system
2.4.4 Microreservoir Dissolution-controlled transdermal drug delivery systems

This type can be considered a hybrid of the reservoir – and matrix dispersion-type drug delivery systems. Here, the drug reservoir is formed by first suspending the solids in an aqueous solution of a water-miscible drug solubilizer e.g., polyethylene glycol, and then homogeneously dispersing the drug suspension in a lipophilic polymer, e.g. silicone elastomers, by high energy dispersing technique, to form thousands of unleachable microscopic spheres of drug reservoirs (fig. 6). This thermodynamically unstable dispersion is quickly stabilized by immediately cross-linking the polymer chains in situ, which produces medicated polymer disk with a constant surface area and a fixed thickness. A transdermal therapeutic system is then produced by mounting the medicated disk at the centre of an adhesive pad. The rate of drug release from a microreservoir drug delivery system is defined by:

\[
\frac{dQ}{dt} = \frac{D_p D_r AK_p}{D_p h d + D_r h_p AK_p} \left[ BS_p - \frac{D_r S_i (1-B)}{h_i} \left( \frac{1}{K_i} + \frac{1}{K_m} \right) \right]
\]

Where \( A = a/b \), \( a \) is the ratio of the drug concentration in the bulk of elution solution over the drug solubility in the same medium; \( b \) = ratio of the drug concentration at the outer edge of the polymer coating membrane over the drug solubility in the
Fig. 6: Cross-sectional view of a microreservoir dissolution-controlled transdermal drug delivery system.

same polymer composition; B = ratio of the drug concentration at the inner edge of the interfacial barrier over the drug solubility in the polymer matrix. $K_I, K_m$ and $K_p$ are the partition coefficients for the interfacial partitioning of drug from the liquid compartment to the polymer matrix, from the polymer matrix to the polymer coating membrane, and from the polymer coating membrane to the elution solution (or skin), respectively; $D_I, D_p$ and $D_s$ are drug diffusivities in the liquid compartment, polymer coating membrane, and elution solution (or skin) respectively.

$S_1$ and $S_p$ - Solubilities of the drug in the liquid compartment and in the polymer matrix, respectively. $h_I, h_p$ and $h_d$ are the thickness of the liquid layer surrounding the drug particles, the polymer coating membrane around the polymer matrix, and the hydrodynamic diffusion layer surrounding the polymer coating membrane, respectively. $D_a$ is the diffusion coefficient in adhesive layer ($a$), $C_R$ is the drug concentration in the reservoir compartment ($R$).
2.5 OPTIMIZATION OF PERCUTANEOUS ABSORPTION

One of the major problems in delivering a drug into or through the skin is its inherent impermeability. The barrier properties of the skin give rise to a skin bioavailability of only a few percent for many conventional topical products. It would be desirable to produce formulations with significantly greater bioavailability. This may result from rational and optimized formulation design, the use of appropriate penetration enhancers and/or the use of physical methods for penetration enhancement viz. electrical or ultrasound (Stillwell, 1983; Nolan et al., 2003).

2.5.1 Chemical penetration enhancers

Some solvents can remove lipids from the SC. The barrier function is reduced when the lipids are modified in this way, although the effect has been shown to be reversible. Some transdermal products contain high concentrations of solvents such as ethanol that may be capable of altering the lipid content of the skin (Bommannan et al., 1991).

Kunta et al. (1997) studied the effect of four terpenes l-menthol, d-limonene, carvacrol and linalool on percutaneous absorption of propranolol hydrochloride across excised hairless mouse skin. The terpenes were very effective in promoting the skin transport of propranolol. A significant concentration effect was observed only with linalool. The permeation of propranolol across mouse skin from the hydrogel patch formulation containing l-menthol was significantly higher than that from a control without the terpene.
An inspection of Fick's 1st law of diffusion shows that two major effects can be obtained if a formulation excipient permeates into the SC. It may intercalate into the structured lipids of the skin where it can disrupt the packing. The effect may render them more fluid thereby increasing the diffusion coefficient of the permeant. This has been demonstrated using differential scanning calorimetry (DSC) and measuring the effect on phase transition temperature (Cornwell et al., 1996), ESR studies (Gay et al., 1989), FTIR (Golden et al., 1986) investigations.

The molecular characteristic that typifies an enhancer that disrupts the chain lipids is a polar head group with a long alkyl chain (C_{10} to C_{14} appear optimal, Bouwstra et al., 1989; Okamoto et al., 1988).

Compounds such as the nonionic surfactants have such properties and Brij 36T has been shown to be an effective enhancer (Walters et al., 1988). Oleic acid also acts by disrupting the skin lipid but appear to form pools in the lipids rather than distribute homogeneously (Ongpipattanakul et al., 1991).

The second way in which excipients can modify skin permeability is to shift the solubility parameter of the skin in the direction of that of the permeant. The solubility of the permeant in the outer layers of the skin will be increased and this in turn, improves the flux, simple solvent type molecules such as propylene glycol, ethanol, transcutol® and N-methyl pyrrolidone are thought to act in this way. For example, it is well known that propylene glycol permeates the skin, therefore, it must be distributed in the SC (Potts et al., 1991).
The mechanism of permeation through skin is a partition diffusion process and follows the Fick's law of diffusion. The major barrier to the drug penetration is the SC. The factors that affect drug release include the physicochemical properties of the vehicles (e.g. viscosity) and the solubilities of the drug and the vehicle. The interaction of the vehicle with the SC may vary from simply occluding the skin to extracting lipid components from the SC (Wiechers, 1989).

One way to extend the range of drugs, which may be administered transdermally, is to incorporate penetration enhancers into formulations. The safest and most widely used penetration enhancer is water. Increased hydration reduces the barrier property of skin (Williams & Barry, 1992).

Azone was the first molecule specifically designed as a skin penetration enhancer; for enhancing the skin transport of both hydrophilic and lipophilic drugs by reducing the order of the intercellular lipids (Williams & Barry, 1992).

Metronidazole has an enhanced permeability if skin is pretreated with propylene glycol (Wotton et al., 1985). If enhancement strategies include both an effect on diffusion and an effect on the solubility, a multiplicative result is predicated. Synergy between these approaches has been shown for numerous systems including metronidazole (Azone plus propylene glycol).

The effect is also possible when supersaturation is combined with a lipid fluidizer e.g. for flurbiprofen (increased degree of saturation plus oleic acid (Pellet et al., 1997).
Terpenes have an excellent potential to be used as permeation enhancers (Trease and Evans, 1989). The three essential oils (eucalyptus, peppermint and turpentine oil) were found to increase the permeation of 5-fluorouracil through excised rat skin. Eucalyptus oil was found to be most active, causing 60 fold increase, while peppermint and turpentine oil showed 48 & 28 fold increase respectively. Mode of action of these enhancers may be due to a combined process of partition and diffusion, the latter being dominant.

d-limonene (20%) was found effective in enhancing the permeation of ketoprofen significantly (Huang et al., 1996).

The penetration index of piroxicam from piroxicam gel after one hour pretreatment with 10% cardamom oil in alcohol/pH7.4 buffer was 34.9 times higher than that from untreated rabbit abdominal skin.

Further studies revealed that the increase in permeation was due to the presence of the monoterpenes, terpineol & acetylterpineol (Yamahara et al., 1989)

N-methyl-2-pyrrolidone (NMP) has been widely used to enhance the skin absorption of ibuprofen and flurbiprofen (Akhter and Barry, 1985). By the use of NMP, the flux of the ibuprofen increased 16 times and that of flurbiprofen increased 3 times through cadaver skin.

Goodman and Barry (1988) studied the effect of 2% azone in propylene glycol (PG) on the permeation of 5-fluorouracil (5-FU). Azones promoted the absorption of 5FU by almost 100 fold, but in combination with tween 20, the effect was less pronounced.
The enhancing effect of pyrrolidone derivatives on the transdermal penetration of 5-FU, triamcinolone acetonide, indomethacin and flurbiprofen were studied by Sasaki et al. (1991).

Pyrrolidone derivatives were found to enhance the flux of penetrants in skin by increasing the solubility of penetrants in the SC.

Chi et al. (1995) investigated the effect of fatty acids and urea in PG vehicle on the skin permeation of flurbiprofen through rat skin.

The mixture of oleic acid and urea showed a significantly higher permeation rate than oleic acid alone.

Ghosh et al. (1992) studied the comparison between hairless mouse and human cadaver skin and effect of n-decylmethyl sulf oxide on the permeability of metoprolol tartrate. Skin permeation rate across human cadaver skin was found to be lower than that of hairless mouse.

Stott et al. (2001) studied the transdermal permeation of propranolol through human skin in the presence of fatty acid (lauric, capric) penetration enhancers. There was no clear difference in permeation rates of the fatty acids compared with the β-blockers suggesting that the permeating species was the intact addition compound.

Reddy et al. (1992) conducted the pharmacokinetic and pharmacodynamic studies of piroxicam gel after oral and transdermal administration to healthy male rats. They reported the improved effect of β-cyclodextrin on bioavailability & anti-inflammatory activity of piroxicam.
2.6 INVITRO SKIN PERMEATION

The invitro skin permeation studies form different transdermal delivery systems can be evaluated using a two compartment diffusion cells. One compartment contains the drug component & the other contains a receptor solution. Excised skin specimen separates the two compartments with SC side facing the donor compartment (Frantz, 1990).

The Frantz diffusion cell (Frantz, 1990) and modified Keshary Chien cells (Keshary and Chien, 1984) are the most commonly used diffusion cells to perform invitro permeation studies.

The skin permeation studies are performed by withdrawing the samples at predetermined time intervals from the side arm sampling port and assaying the drug concentration in the samples by a sensitive analytical method. The equal amount of receptor phase is added into the receptor compartment to maintain uniform volume during the experiment.

A number of animal models have been used to perform invitro skin permeation studies. Ideally human skin is the most appropriate membrane for performing invitro permeation studies (Skelly et al., 1987; Behl et al., 1990). Due to the scarcity and difficulty in controlling the gender, race, age and skin condition of the donor, a number of animal models such as rat, hairless mouse, shed skin etc. (Catz and Friend, 1990; Catz et al., 1988; Buyuktimkin et al., 1995; Roy et al., 1994) have been used as possible models for human skin. The criterion for selection should be the correlation between permeation rate using human skin and animal skin.
Synthetic membranes such as polydimethylsiloxane cellulose acetate, polyurethane have also been used to study invitro permeation release Kinetics, however they cannot predict accurately the permeabilities through the human skin (Williams and Barry, 1992; Bronaugh et al., 1982).

The receptor solution used in diffusion cells should accept permeating drugs and provide the water, biochemical and ions needed for the skin membrane to function in the permeation experiment at the proper pH & osmotic strength (Skelly et al., 1987; Smith, 1990). Typically aqueous phases are used with polar drugs and ethanolic phases with lipophilic drugs. It is important that the concentration of the penetrant in the receptor solution remains low at all times, to prevent significant back diffusion. In general the thermodynamic activity should never exceed 10% of that in the donor formulation, in order to maintain an adequate diffusion gradient.

Other important factors that can effect invitro skin permeation are temperature, solubility and stirring (Friend, 1992; Skelly et al., 1987). Control of receptor solution temperature is important to minimize variations in experimental conditions. The temperature should be kept at normal physiological conditions, since temperature elevation may lead to increased hydration of the skin. It is known that a rise of 10°C in temperature can produce a 2 to 3 fold increase in permeation (Frantz, 1990). Solubility and stirring are important to allow the permeant to be taken up and transported away from the skin after it has passed through, avoiding a concentration build up within or below the skin. Stirring of the receptor solution is also important to provide a homogeneous receptor solution (Frantz, 1990; Friend, 1992). The concentration of the permeant in the receptor solution should remain low (less than
10%) compared with its solubility in the solution (Skelly et al., 1987). If the 
permeant is relatively insoluble, solubility enhancing components can be added to 
receptor solution, however their effects on the skin must be considered.

2.7 SELECTION OF RATE CONTROLLING MEMBRANE

In this type of system, the drug reservoir is entirely encapsulated in a shallow 
compartment moulded from a drug impermeable backing laminate and a rate 
controlling polymeric membrane, which may be porous or nonporous. e.g. ethylene 
vinyl acetate copolymer, ethylene vinyl alcohol-copolymers.

Due to the saturation of drug storage period, there is an active agent present in the 
membrane. Thus there is no additional contribution to the lag time for the diffusion 
across the SC.

The drug release from these systems can be controlled by changing the polymer 
composition, the permeability coefficient and/or thickness and composition of rate 
controlling membrane.

Roy and Manoukian (1995) studied the transdermal permeability of ketorolac 
tromethamine through microporous membranes (3M™ Co -Tran™). The skin flux of 
Ketorolac was found same with and without a microporous membrane. They 
concluded that microporous membrane offered practically no diffusional resistance 
for transport of the drug and vehicles due to the fact that pore size of the 
microporous membrane was larger enough to allow free transport of the drug and 
vehicles.
A reservoir type of transdermal delivery system of testosterone was developed by Kim et al. (2001) using ethanol water co solvent system as the vehicle. The permeation rate of testosterone through the ethylene vinylacetate membrane was observed to increase as the vinylacetate content in the copolymer increased.

Sun et al. (1997) prepared composite membranes by casting a linear poly (2-hydroxy ethyl methacrylate) (PHEMA) solution onto polyester non woven supports, and then the supported PHEMA within the membranes was cross linked by a diisocyanate cross linking agent to form a network structure.

The usefulness of a film forming material isolated from the roots of salacia macrosperms to serve as a rate controlling membrane for the reservoir type patches was studied by Arra et al. (1998) using isosorbide 5-mononitrate as a model drug.

Important properties of 3M™ Co-Tran™ 9702 membrane, 3M™ Scotchpak™ 9733 backing and 3M™ Scotchpak™ 1022 release liner are;

3M™ Co-Tran™ 9702 membrane (Controlled caliper ethylene vinyl acetate (EVA) membrane)

The intact (continuous) membrane is translucent and has a very uniform (50.8μm) caliper. Its surface texture improves both web handling and bonding between the membrane and the adhesive. The vinyl acetate content of the membrane is 9%. It is heat sealable.
3M™ Scotchpak™ 9733 backing (polyester film laminate)

The laminate consists of polyester and a medium density polyethylene/ethylene vinyl acetate heat seal layer. It is translucent, occlusive, conformable and heat sealable.

3M™ Scotchpak™ 1022 release liner (Fluoropolymer coated polyester film)

It is transparent, occlusive and has got an excellent chemical stability.

2.8 GELLING AGENTS

To increase the viscosity of the reservoir solution in membrane moderated transdermal drug delivery system, often viscosity building agents (polymers) e.g. hydroxypropyl methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), carbopol, sodium carboxy methyl cellulose are incorporated into the drug solution. HPMC has been widely used in the design of reservoir systems for hydrocortisone (Elkattan et al., 2000), testosterone (Kim et al., 2001). A nonionic cellulose polymer derivative HPMC is less hydrophilic than methylcellulose, therefore it was selected for the design of reservoir system because it can be easily solubilized in binary solvent mixture, PG: IPA (30:70 %v/v)

2.8.1 Hydroxypropyl methyl cellulose (HPMC) (Wade and Jweller, 1994)

It is mixed alkyl hydroxyalkyl cellulosic ether and may be regarded as the propylene glycol ether of methylcellulose.

Synonyms: Methocel, Metolose, Pharmacat, HPMC.
Empirical formula: \( \text{C}_8\text{H}_{13}\text{O}_6 - (\text{C}_{10}\text{H}_{18}\text{O}_6)_n - \text{C}_8\text{H}_{12}\text{O}_5 \)

Chemical name: Cellulose, 2-hydroxypropyl methyl ether

Grades: Methocel- E5, E15, E 50, E4M, F50, K4M, K100, K15M, K100M

Description: An odorless, tasteless, white or creamy-white fibrous or granular powder.

Molecular weight: Approx. 86,000

Density (bulk): 0.25 – 0.70 g/cm³

pH: 6.0 – 8.0 (1% aqueous solution)

Solubility: Soluble in cold water, forms a viscous colloidal solution, insoluble in alcohol, ether and chloroform, but soluble in mixtures of methyl alcohol and methylene chloride. Certain grades are soluble in aqueous acetone, mixtures of methylene chloride and isopropyl alcohol and other organic solvents.

Stability: Very stable in dry conditions. Solutions are stable at pH 3.0 – 11.0. Aqueous solutions are liable to be affected by microorganisms.

Incompatibilities: Extreme pH conditions, oxidizing materials.

Safety: Human and animal feeding studies have shown HPMC to be safe.

Applications: It is a suspending, viscosity increasing and film forming agent. It is also used as a tablet binder and as an adhesive ointment ingredient.
2.9 INVIVO EVALUATION

Human beings are the ideal models for carrying out pharmacokinetic and pharmacodynamic evaluation of transdermal drug delivery system. The clinical studies serve as the standard against which all the animal models for determining percutaneous absorption should be judged.

Various species have been used to carry out invivo evaluation of TDS viz. rabbit, mouse, rat (Guy and Hadgraft, 1989a; Guy et al., 1989b).

In pharmacokinetic studies the relationship between a given response and plasma concentration of the drug is determined by two factors, whether concentration is directly or indirectly related to response and whether the drug substance interacts with the receptor in a reversible or irreversible manner (Gibaldi and Perrier, 1982).

The plasma concentration of antiarrhythmics is directly related to the response of interaction between drug and the receptor is reversible. The plasma concentration of coumarin anticoagulants is not directly related to the response. In case of anticancer drugs there is an irreversible interaction between the drug and the receptor.

Where plasma concentration is not directly related to the elicited response, pharmacodynamic response, is used as a parameter to assess the pharmacological activity of the drug within the system e.g. anti-inflammatory activity of non steroidal anti inflammatory drugs (NSAID’S) by rat paw edema (Pandey et al., 2000).
Rheumatic diseases have affected mankind since ages and are one of the commonest inflammatory conditions in developing countries. Rheumatoid arthritis forms a major prototype of rheumatic disease and is a common cause of disability. It is a chronic systemic inflammatory disorder that may affect many tissues and organs e.g. skin, blood vessels, heart, lungs, and muscles—but principally attacks the joints, producing a nonsupportive proliferative synovitis that often progresses to destruction of the articular cartilage and ankylosis of the joints. Although the etiopathogenesis of RA remains uncertain, it is currently believed that it involves diverse and complex factors such as genetic background, rheumatic factor (circulating antibodies), immune complexes, complement activation, lymphocytes, arachidonic acid metabolites, free oxygen radical, etc. (Nuki et al., 1999). Hence the exposure of an immunogenetically susceptible host to an arthritogenic microbial antigen triggers the initial acute arthritis. But it is the continuing auto immune reaction, the activation of CD4 + helper T cells, and the local release of inflammatory mediators and cytokines that ultimately destroys the joint.

About 10% of the world’s population is afflicted by RA, women three to five times more often than men. The peak incidence is in the twenties and forties but no age is immune.

2.10.1 Osteoarthritis (OA)

Also known as degenerative joint disease, the term osteoarthritis implies an inflammatory disorder, in which, the inflammatory cells maybe present. The disease is considered to be an intrinsic one, affecting the cartilage—particularly the articular
cartilage – in which biochemical and metabolic alterations results in its breakdown. Osteoarthritis joins heart disease and cancer as one of the dividends of growing older. In the great majority of instances, it appears insidiously, without apparent initiating cause, as an aging phenomenon (idiopathic or primary osteoarthritis). In these cases, the disease is usually oligoarticular (affects few joints) but may be generalized, in about 5% of cases, OA may appear in younger individuals having some predisposing conditions, such as previous macro traumatic or repeated micro traumatic injuries to joint; a congenital developmental deformity of a joint(s), or some underlying systemic disease such as diabetes, ochronosis, hemochromatosis, or marked obesity. In these settings the disease is called secondary osteoarthritis and often involves one or several predisposed joints. Gender has some influence on distribution. The knees and hands are more commonly affected in women and the hips in men. Chondrocytes play a primary role in the process and constitute the cellular basis of the disease. Other mediators, such as prostaglandin derivatives and IL-6, also have a role in the cascade of matrix degradation (Bones, 1999).

2.10.2 Therapeutic strategies for rheumatic diseases

The conventional drug treatment of rheumatoid arthritis involves two primary goals first, the relief of pain, which is often the presenting symptom and the major continuing complaint of the patient, and second, the slowing or in theory- arrest of the tissue – damaging process.

Reduction of inflammation with nonsteroidal anti-inflammatory drugs (NSAIDs) often result in relief of pain for significant period. Furthermore, most of the nonopioid analgesics (aspirin, etc.) also have anti-inflammatory effects, so they are appropriate for the treatment of both acute and chronic inflammatory conditions.
The glucocorticoids (prednisone, etc.) also have powerful anti-inflammatory effect and when first introduced were considered to be the ultimate answer to the treatment of inflammatory arthritis through the inhibition of phospholipase A2, the enzyme responsible for the liberation of arachidonic acid from membrane lipids and the selective inhibition of the expression of COX-2 enzyme (Kujubu and Herschman, 1992; Winn et al., 1993). Unfortunately, the toxicity associated with chronic corticosteroid therapy (Fractures, infections, cataracts, etc.) inhibits their use except in the control of acute flare-up of joint disease.

Another important group of agents are characterized as slow-acting antirheumatic drugs (SAARDs) or disease-modifying antirheumatic drugs (DMARDs).

These drugs might arrest or at least slow the progression of bone and cartilage destruction by modifying the disease itself. The effects of disease-modifying therapies may take 6 weeks to 6 months to become evident, i.e., they are slow acting compared with NSAIDs. These therapies include methotrexate, azathioprine, penicillamine, Hydroxy chloroquine and chloroquine, organic gold compounds, etc. Unfortunately, they may also be more toxic than the NSAID’s (Fries, 1993; Furst, 1990). Therefore, the NSAID’s have assumed a major role in the treatment of rheumatic diseases particularly rheumatoid arthritis and osteoarthritis.

2.10.3 Nonsteroidal anti-inflammatory drugs (NSAIDs)

The clinical effects of non-steroidal anti-inflammatory drugs (NSAID’s) are based on the inhibition of the enzyme cyclooxygenase (COX), which catalyzes the rate-limiting step in the formation of prostanoids, prostaglandins (PGs), and thromboxane A2 (Tx A2) from arachidonic acid. (fig. 7) (Moncada and Vane, 1979).
Membrane - bound phospholipids

Phospholipase A2

Arachidonic acid

COX-1 / NSAIDs, ASA

COX-2 / Coxibs

PGG2

PGH2

Tissue specific isomerasers

PGD2

PGE2

PGF2

PGI2

TXA2

Fig. 7: Prostaglandin and thromboxane synthesis COX = Cyclooxygenase; Coxibs = COX-2 inhibitors PG = prostaglandin; TXA2 = Thromboxane A2; NSAID = Non steroidal anti-inflammatory drug; ASA = aspirin
Prostaglandins (PGs) are ubiquitous compounds that mediate a variety of physiologic and pathologic processes. Under normal physiologic conditions, PGs play an essential role in homeostasis, renal physiology, Gestation and parturition. The production of PGs is induced at sites of inflammation, where they are involved in propagation of inflammation, pain and fever. The long term therapy with non specific NSAID's (which inhibit both COX; COX-1 and COX -2) is frequently limited by their adverse effects, particularly those caused by erosion of gastric mucosal protection ranging form dyspepsia to life threatening perforation, ulcers, obstructions, and bleeds (Warner et al., 1999).
OBJECTIVES

AND

PLAN OF WORK
Transdermal delivery affords an improved approach to the administration of drug by maintaining a therapeutic but constant concentration of drug in the blood for a desired period of time, usually between 1 and 7 days.

The advantages are that the system

1. Avoids chemically hostile GI environment.
2. Does not have GI distress or other physiologic contraindications of oral route.
3. Permits use of drugs with short biological half life.
4. Avoids first pass effect.
5. Allows administration of drugs with narrow therapeutic window.
6. Effective maintenance of steady state drug concentration.
7. Minimizes inter and intra patient variation.
8. Improves patient compliance.
9. Enhances therapeutic efficacy.
10. Interrupts drug input promptly when toxicities occur.

3.1 THE MAIN OBJECTIVES OF THE PRESENT STUDY WERE

1. To develop a stable, reproducible and patient non-infringing drug delivery system.
2. Reduction of side effects due to optimization of the blood concentration time profile.
3. Extended duration of activity that allows greater patient compliance owing to elimination of multiple dosing schedules.
OBJECTIVES AND PLAN OF WORK

4. To obviate specific problems associated with the drugs e.g. gastro-intestinal tract irritation, low absorption, first pass effect, formation of metabolites that cause side effects and short half life necessitating frequent dosing.

3.2 RATIONALE OF THE STUDY

Although some drugs have inherent side effects that cannot be eliminated in any dosage form, many drugs exhibit undesirable behaviors that are specifically related to a particular route of administration. One recent effort at eliminating some of the problems of traditional dosage forms is the transdermal delivery system.

Flurbiprofen, a potent non-steroidal anti-inflammatory drug has been used extensively for the treatment of rheumatoid arthritis and its related disorders (Brogden et al., 1979).

It is more potent inhibitor of prostaglandin biosynthesis than ibuprofen, indomethacin and aspirin both invitro and in-vivo (Crook and Collins, 1975; Brogden, et al., 1979). Flurbiprofen has a potent action against the migration of leucocytes into inflamed sites (Adams, 1977). It is a potent inhibitor of second phase human platelet aggregation induced in-vivo by adenosine phosphate, adrenaline, collagen and thrombin.

Although flurbiprofen has potent pharmacological activities with oral administration, it has also unwanted problems such as systemic side effects and gastrointestinal irritation.

Considering the fact that flurbiprofen is usually used for a long period, it is desirable to reduce these side effects while maintaining its therapeutic blood concentration.
OBJECTIVES AND PLAN OF WORK

Among several attempts to achieve this goal transdermal preparations such as gels, ointments and creams have been intensively studied (Kitagawa et al., 1993; Kyuki et al., 1984; Masumoto et al., 1982).

However, it was difficult to get an effective blood concentration by transdermal delivery of flurbiprofen due to its intrinsically poor skin permeability. Therefore it is indispensable to employ penetration enhancers to increase the skin permeation rate of flurbiprofen in order to maintain an effective blood level.

3.3 CHOICE OF THE DRUG

Transdermal delivery systems have been developed for local and systemic administration of drugs. Relationship between skin permeability and physicochemical properties of drugs have been investigated to clarify the mechanisms of skin permeation, as well as to predict plasma drug concentrations after applying transdermal delivery.

Skin permeation consists of sequential physical and biological processes. The partition and diffusion of drugs are recognized as important physical processes. Diffusion in skin is determined by the molecular weight of drug. Smaller molecules permeate the skin more rapidly than larger molecules.

The influence of partition has been evaluated by correlating skin permeability with drug lipophilicity such as the logarithm of n-octanol / buffer partition coefficient.

Passive transdermal absorption often exhibits a parabolic relationship with the maximum at intermediate lipophilicity, which is explained by a change in the rate limiting step for skin permeation. For hydrophilic drugs the partition into stratum corneum is the rate limiting step of skin permeation, whereas for lipophilic drugs the partition between stratum corneum and viable epidermis becomes important some
OBJECTIVES AND PLAN OF WORK

generalizations on chemical structure of drugs and their skin permeation (Mishra et al., 1990) can be made.

(a) Water-soluble electrolytes like sodium chloride, potassium chloride and non-electrolytes like glucose, urea etc. penetrate the skin freely.

(b) Lipid soluble substances have best chance of diffusing into the horny layer, especially when they are non polar and of moderately low molecular weight.

(c) Polymers and macromolecules of high molecular weight like proteins and polysaccharides penetrate poorly.

For putative selection of a drug candidate for transdermal delivery there are some basic prerequisites pertaining to the physicochemical and pharmacokinetic properties of the drug molecule, namely, it should have low molecular weight, low dose, balanced oil-water partition coefficient, relatively short biological half-life, low melting point, non irritant to skin, high first pass metabolism etc.

Flurbiprofen was selected as a candidate for drug delivery because

(a) It has a low molecular weight i.e. 244.25.

(b) It has a low melting point i.e. 114 to 117°C.

(c) It has an elimination half-life between 3 and 4 hours and plasma half-life is 3.5 hours.

(d) It causes unwanted problems such as systemic side effects and gastrointestinal irritation (Brogden et al., 1979; Teixeira et al., 1984).

(e) It is used for a long period.

(f) It undergoes extensive first pass metabolism (Kaiser et al., 1986).

(g) It has high log p (lipophilic character) value of 4.16 (Hadgraft et al., 2000).
3.4 FLURBIPROFEN – A PROFILE

The substituted phenylalkanoic acid, a major group of non-steroidal anti-inflammatory agent, were first developed in the Research Department of the Boots Company Limited, Nottingham, England and were found to be active in the rheumatic disease in the early 1960. Flurbiprofen (Ansaid, UpJohn) was introduced in the United Kingdom in 1977.

Flurbiprofen is 2-(2-fluro-4-biphenyl) propionic acid with the following structure:

Empirical formula : $C_{13}H_{13}FO_2$

Molecular weight : 244.25

Melting range : 114 to 117°C

Flurbiprofen is a white to cream crystalline powder practically insoluble in water. It is soluble in 3 parts of ethanol (96%), in 4 parts of chloroform, in 4.5 parts of ether. It dissolves in solutions of alkali hydroxides and carbonates (British Pharmacopoeia, 1988).

Flurbiprofen exhibits pH dependent solubility (Mizushima et al., 1975). Its solubility versus pH is in excellent agreement with the theoretical profile which assumes an intrinsic solubility of $5.0 \times 10^{-5}$ M for the free acid and pKa of 4.22±0.03. The mean molar ionic activity coefficient is estimated to be 0.9 at an ionic strength of 0.01 (Anderson and Conradi, 1985).
Flurbiprofen behaves as a typical monoprotic weak acid having a slope of one in the plot of log solubility versus pH. Solubility at pH 7.2 is 15 mg/ml. (Bill and Snider, 1987).

It is stable in the solid state for at least 29 days at 70°C as determined by infrared spectroscopy. In solution the rotation of the drug and its D and L isomer are stable (Smith et al., 1989). Flurbiprofen is stable under normal conditions as tablets, capsules, oral liquids and suppositories (Adams and Buckler, 1983).

Flurbiprofen is one of the most potent member of phenylalkanoic acid series. It is an orally effective, well-tolerated drug possessing analgesic and anti-inflammatory properties (Adams et al., 1977; Glenn et al., 1973). Its mode of anti-inflammatory action is believed to be through inhibition of prostaglandin biosynthesis, which in turn prevents sensitization of tissue to pain mediators such as histamine, 5-hydroxy tryptamine and kinins (Buckler and Adams, 1979; Smith et al., 1987). It is more potent inhibitor of prostaglandin biosynthesis (Crook and Collins, 1975) than ibuprofen, indomethacin and aspirin both in-vitro and in-vivo (Brogden et al., 1979).

As a non selective inhibitor, flurbiprofen suppresses both PGE₂ and PGF₂ probably through inhibition of endoperoxidase.

Flurbiprofen has a potent action against the migration of leucocytes into inflamed sites (Adams, 1977). It is a potent inhibitor of second phase human platelet aggregation induced in-vivo by adenosine phosphate, adrenaline, collagen and thrombin, being active in concentration as low as 10⁻⁷ mol/l. It can modify changes in cell membrane permeability under some experimental condition but only
stabilizes erythrocyte membrane in high concentration. It has little effect on lysosomal enzyme release (Ringrose et al., 1975).

Flurbiprofen appears to be readily absorbed after oral administration (Crook and Collins, 1975; Salisbury et al., 1986). Peak plasma concentration is about 12\(\mu\)g/ml after a 100 mg dose and is attained 1.5 to 3 hr after ingestion. A peak serum drug concentration of 7.9 \(\mu\)g/ml was observed at 1.5 hrs. A peak mean drug concentration of 2.1 \(\mu\)g/ml was seen in synovial fluid at 3 hrs. The apparent elimination half-lives from serum and synovial fluids were estimated to be 3 and 5 hrs respectively (Chalmers et al., 1977; Kean et al., 1992). Plasma half-life is 3.5 hrs (Clark, 1986).

Flurbiprofen is at least 99% bound to human serum albumin at therapeutic concentration. Flurbiprofen may bind to red blood cells. Area under plasma drug concentration versus time curve increase with increasing dose of drug administered. Long term administrations of Flurbiprofen neither induce nor inhibit its own metabolism (Cardoe et al., 1975).

Flurbiprofen exist as racemate and is used as such. Although the activity of 2-APA’s is mainly due to their S-enantiomers, information on the pharmacokinetics of flurbiprofen is usually based on the measurement of total concentration of S and R flurbiprofen (Jamali and Berry, 1988). As compared with the R enantiomer, S flurbiprofen has a smaller volume of distribution (7.23 \(\pm\) 1.9 versus 8.41 \(\pm\) 3.00) and clearance (1.23 \(\pm\) 0.34 versus 1.47 \(\pm\) 0.50 h) but an equal half life (4.21 \(\pm\) 1.2 versus 4.18 \(\pm\) 1.3 h).
Between 65 to 85% of Flurbiprofen and its metabolites were present as glucuronide and sulphate conjugate (Risdall et al., 1978; Crompton and Adams, 1973). More than 95% of each daily dose was excreted in the urine within 24 hrs. 20-25% of a dose is recovered in the urine unchanged and its principal metabolites are devoid of pharmacological activity. Half-life of elimination is about 3.5 during repeated doses (Cardoe, 1977).

Many controlled studies have established the clinical efficacy of flurbiprofen in rheumatoid arthritis, Osteoarthritis and ankylosing spondylitis. The thermo graphic index of inflamed rheumatoid joints and morning stiffness were both significantly improved by flurbiprofen (Bacon et al., 1977). Post operative gynecologic pain and dysmenorrhea respond favorably to flurbiprofen (Delia, 1982). It appears to provide good adjunctive pain control in painful intractable cancer (Sunshine, 1986).

Abdominal discomfort, dyspepsia and constipation have been most frequently reported side effects of flurbiprofen. Other occasionally or rarely reported adverse effects include edema of lips and eye region, skin irritation and rash, dry mouth, palpitation and diarrhoea.

Flurbiprofen has a low acute oral toxicity, the LD_{50} in mice and rats being approximately 750 and 600 mg/kg respectively. No carcinogenic and teratogenicity was detected in life span of study (Sheldrake et al., 1977).
Flurbiprofen is not recommended for use in patients with peptic ulcers. Care should be taken when patients have a history of asthma and in prescribing the drug to patients with renal impairment (Henrich, 1983).

The concomitant administration of flurbiprofen and aspirin results in decreased bioavailability in patients with rheumatoid arthritis (Brooks and Khong, 1977). Beta adrenoceptor blocking drugs like atenolol and propranolol may differ in the degree to which their hypotensive effects are attenuated by flurbiprofen (Webster, 1984). A delay in absorption of digoxin has been seen with the drug, (Rau et al., 1982). Effects of diuretics such as furosemide are reduced by the drug (Rawles, 1982). The usual initial dose of Flurbiprofen is 150-200 mg in 3 or 4 divided doses. The usual strength in which it is available is 50 mg and 100 mg.

Flurbiprofen is listed in British Pharmacopoeia (British Pharmacopoeia, 1988) and The Extra Pharmacopoeia (Reynolds, 1993). British Pharmacopoeia (B.P.) has specified identification of the drug on the basis of infra red (I.R.) spectra and color reaction with chromic-sulfuric acid mixture. It can also be identified by color test like Lieberman’s test and Marquis test (Clark, 1986). Thin layer chromatography, GC/HPLC and mass spectroscopy are the other methods for the identification.

Flurbiprofen B.P. is assayed by acid base titration. Flurbiprofen tablet B.P. is assayed by powdering the tablet, extracting the powder and measuring ultraviolet (UV) absorbance. Polarographic determination of flurbiprofen in pharmaceutical dosage forms has been reported (Kanoute et al., 1985).
A variety of methods have been reported for the estimation of flurbiprofen in biological fluids. Flurbiprofen in pH buffer of pH 7.4 exhibits maximum at 247nm. Direct spectrophotometric stereo specific HPLC assay of flurbiprofen in biological specimens are reported (Berry and Jamali, 1988). Johnson and Wilson (1986) have reported HPLC method for the estimation of flurbiprofen in plasma and breast milk.

Adam and Bothwell (1987) and Albert et al. (1984) have reported simultaneous determination of flurbiprofen and its metabolite in physiological fluids using liquid chromatography with fluorescence detection.

Gas chromatography-mass spectrometry with selective ion monitoring has also been reported for determination of flurbiprofen in human plasma (Kawahara et al., 1981).

Gas-liquid chromatography (Kawahara and Masumura, 1975; Kaiser, 1974) and isocratic liquid chromatography (Omile et al., 1986) are also used.
3.5 PLAN OF WORK

The following plan of work was envisaged:

I. Characterization of flurbiprofen

1. Pharmacopoeial tests
   i. Physical characteristics of the drug
   ii. Identification tests
   iii. Assay

2. Non Pharmacopoeial tests

II. Analytical methodology

1. Determination of UV absorption spectra

2. Preparation of calibration curve for preliminary invitro skin permeation study

3. Selection of method for determination of flurbiprofen in invitro skin permeation study.

4. Method development and validation for determination of flurbiprofen in rat plasma and in excised skin samples.
III. Working of the assemblies and methods employed

1. Fabrication of apparatus for invitro drug skin permeation studies

2. Invitro skin permeation studies

3. Calculation of steady state plasma concentration

4. Preparation of flurbiprofen formulations

5. In vitro drug permeation studies of medicated viscous systems

6. Fabrication of the patch

7. Invitro permeation study of patch formulations

IV. Stability studies

V. Interaction studies

VI. Histological studies

VII. In vivo evaluation of patch formulations

1. Measurement of plasma concentration of flurbiprofen in albino rats after application of transdermal patch formulations

2. Comparison of the pharmacokinetic parameters obtained after transdermal application of the patch formulations containing penetration enhancers with that obtained with transdermal patch formulation without enhancer and further compare them with orally administered flurbiprofen.

VIII. Skin accumulation studies

IX Pharmacodynamic studies
EXPERIMENTAL
4.1 MATERIALS AND INSTRUMENTS

4.1.1 Materials

Flurbiprofen : Wings Pharmaceuticals, New Delhi

Ibuprofen : Wings Pharmaceuticals, New Delhi

Acetophenone : Wings Pharmaceuticals, New Delhi

Hydroxy propyl methyl cellulose : Wings Pharmaceuticals, New Delhi

Monobasic sodium-Phosphate (AR) : S.D. Fine Chemicals Pvt. Ltd. Boisar-401501

Dibasic sodium phosphate (AR) : BDH, INDIA

Sodium Chloride (AR) : BDH, INDIA

Ammonia solution : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501

Isopropyl alcohol : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501

Propylene glycol : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501

Lemon oil : Nice Chemicals, New Delhi

Turpentine oil : Nice Chemicals, New Delhi

NA-102 : Nice Chemicals, New Delhi

Water (HPLC) : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
EXPERIMENTAL

Acetonitrile (HPLC) : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Glacial acetic acid (AR) : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Methanol (HPLC) : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Ortho phosphoric acid (AR) : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Trypsin : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Benzene : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Perchloric acid : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Sulphuric acid : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Sodium hydroxide : Merck, India Ltd.
Formaldehyde : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Tri ethyl amine : S.D. Fine Chemicals Pvt. Ltd., Boisar-401501
Scotch Pak™ 1022 release liver : 3M™, St. Paul, USA.
EXPERIMENTAL

Co - Tran™ 9702 membrane : 3M™, St. Paul, USA.
Scotchpak™ 9733 backing : 3M™, St. Paul, USA.
Laminated aluminum foil : Oscar Pharmaceuticals, Okhla, New Delhi

4.1.2 Instruments

Double beam U.L. spectrophotometer : λ-20 by Perkin Elmer, Germany
FTIR spectrophotometer : Perkin Elmer, Germany
Electrical balance : Sartorius, India
pH meter : HI 84240, microcomputer pH meter, Italy
Melting point apparatus : Scientific Apparatus, India
Magnetic stirrer : Metrex, Delhi
Oven : Widsons Scientific Works, Delhi
Keshary Chien cell : Locally fabricated
Centrifuge : Remi Equipments Delhi
Micropipettes : Accupipet, Germany
High pressure liquid chromatography : Shimadzu Series
Plethysmometer : Ugo Basile, Italy
Electric water bath : Locally fabricated
4.2 CHARACTERIZATION OF FLURBIPROFEN

The drug was characterized as per the specifications of British Pharmacopoeia (1988).

(A) Pharmacopoeial test

I. Physical characteristics of the drug

1. Nature : Crystalline powder
2. Colour : White
3. Odour : Slight
4. Taste : Bitter
5. Melting point : 115°C
6. Solubility : Solubility of the drug in various solvents was determined and is shown in table 2.

Table 2: Solubility of flurbiprofen in different solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol (96%)</td>
<td>1 in 3 parts</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1 in 4 parts</td>
</tr>
<tr>
<td>Ether</td>
<td>1 in 4.5 parts</td>
</tr>
<tr>
<td>Sodium hydroxide solution</td>
<td>Freely soluble</td>
</tr>
</tbody>
</table>
II. Identification test

1. The Infra red spectrum of the drug in potassium bromide pellet was determined on Perkin Elmer infrared spectrometer and compared to that of standard flurbiprofen (Fig 8 & 9).

![Fig. 8: I.R. Spectrum of Reference flurbiprofen (British Pharmacopoeia, 1988)](image)

![Fig. 9: I.R. Spectrum of flurbiprofen sample](image)
2. The light absorption in the range of 200 to 400 nm of 0.001% w/v solution in 0.1 M sodium hydroxide was measured on Perkin Elmer \( \lambda-20 \) U.V. Spectrophotometer.

3. 0.5 ml of chromic sulphuric acid mixture was heated in a small test tube in a water bath for 5 minutes. The solution wets the sides of the tube readily and there is no greasiness. 2 to 3 mg of drug added to the test tube and heated in a water bath for five minutes, the solution does not wet sides of the tube and does not pour easily from the tube.

III. Assay

About 0.5 g of accurately weight sample of the drug was dissolved in 100 ml of ethanol (96%) previously neutralized to phenolphthalein solution and titrated with 0.1 N sodium hydroxide using phenolphthalein solution as indicator. A blank titration was carried out in the same manner omitting the sample.

The percentage of flurbiprofen in the sample was calculated using the factor given below.

\[ \text{1 ml of 0.1 N Sodium hydroxide is equivalent to 0.02443 g of flurbiprofen.} \]

(B) Non pharmacopoeial test

Following non pharmacopoeial tests were performed on the sample of flurbiprofen to confirm its identity.

1. **Colour test**

   a) Sample was treated with Libermann reagent (10% w/v Na\(_2\)O\(_2\) in H\(_2\)SO\(_4\)) and was warmed slightly. The color of the solution was observed.

   b) A drop of Marquis regent [Formaldehyde solution: sulphuric acid (1:9)] was added to a sample on a white tile and color of the solution was observed. The results are given in table 3.
Table 3: Results of Characterization of flurbiprofen sample

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Pharmacopoeial Specifications</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Identification</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(i) Infra red absorption spectrum in potassium bromide pellet</td>
<td>Concordant with the reference spectrum of Flurbiprofen</td>
<td>Infra red spectrum of sample complies with pharmacopoeial specification</td>
</tr>
<tr>
<td></td>
<td>(ii) Absorbance of 0.001% w/v solution in 0.1 M NaOH at 247 nm</td>
<td>About 0.8</td>
<td>0.811</td>
</tr>
<tr>
<td></td>
<td>(iii) Two mg drug + 0.5 ml of chromic-sulphuric acid mixture. Heat on</td>
<td>The solution does not wet the sides of the tube and does not pour easily from the tube.</td>
<td>The solution initially wets the sides of the tube. After addition of drug the solution does not wet the sides and pour easily from the tube.</td>
</tr>
<tr>
<td></td>
<td>water bath for 5 minutes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Percent drug content</td>
<td>Not less than 99% and not more than 100.5% calculated with reference to dried substance.</td>
<td>99.87%</td>
</tr>
</tbody>
</table>

Non pharmacopoeial tests

| 1.     | Sample + Marquis reagent                                           | Red colour                                                                                     | Red colour                                                                                     |
| 2.     | Sample + Libermann’s reagent                                        | Brown colour                                                                                    | Brown colour                                                                                    |

Conclusion:

On the basis of above tests, it could be confirmed that the drug sample of flurbiprofen was an authentic sample.
4.3 ANALYTICAL METHODOLOGY

4.3.1 UV absorption spectra

A 0.002 w/v% solution of flurbiprofen was prepared in isotonic sodium phosphate buffer (IPB) of pH 7.4 and scanned for UV absorption in the range of 200 to 400 nm. The UV absorption spectra exhibited max at 247 nm (fig. 10).

![UV absorption spectrum of flurbiprofen in IPB of pH 7.4](image)

Fig. 10: U.V. absorption spectrum of flurbiprofen in IPB of pH 7.4

The IPB was prepared according to (US Pharmacopoeia, 1995)

- Stock solution of monobasic sodium phosphate (NaH$_2$PO$_4$)
  
  (8 grams of NaH$_2$PO$_4$/ litre)

- Stock solution of dibasic sodium phosphate (Na$_2$HPO$_4$)
  
  (9.47 grams of Na$_2$HPO$_4$/ litre)

<table>
<thead>
<tr>
<th>Monobasic sodium phosphate</th>
<th>Dibasic sodium phosphate</th>
<th>PH</th>
<th>Sodium chlorides (g/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>80</td>
<td>7.4</td>
<td>0.44</td>
</tr>
</tbody>
</table>
4.3.2 Compassion of UV absorption spectra before and after storage of flurbiprofen in IPB of pH 7.4

Drug solution was prepared in IPB of pH 7.4 & divided into two portions. UV spectra were obtained for the following:

(i) Portion stored for 24 hrs. in the laboratory conditions after covering the container with aluminum foil.

(ii) Portion stored for 24 hours in laboratory and exposed to light.

Fig. 11: U.V. absorption spectra of flurbiprofen samples

(i) Protected from light
(ii) Exposed to light for 24 hours in laboratory conditions
As shown in the fig. 11 no change in λmax was observed after storing the drug sample in IPB of pH 7.4 for 24 hours at different places in the laboratory. Thus in-vitro skin permeation studies could be performed in IPB of pH 7.4 for 24 hours.

4.3.3 Calibration curve for preliminary in-vitro skin permeation study

Calibration curve of flurbiprofen in IPB of pH 7.4 was prepared by dissolving 5 mg of the drug in 100 ml of IPB of pH7.4. Serial dilutions in the concentration range of 0.5 to 12 μg/ml were made from the stock solution and the absorbance of these solutions were determined at λmax 247 nm using IPB of pH 7.4 as blank.

Table 4: Absorbance values of flurbiprofen in isotonic phosphate buffer of pH 7.4 at λmax 247 nm

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration (x) (μg/ml)</th>
<th>Mean* (± S.D.) absorbance</th>
<th>Regressed value of absorbance (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.5</td>
<td>0.0410 ± 0.0020</td>
<td>0.0409</td>
</tr>
<tr>
<td>2.</td>
<td>1.0</td>
<td>0.0765 ± 0.0021</td>
<td>0.0782</td>
</tr>
<tr>
<td>3.</td>
<td>2.0</td>
<td>0.1582 ± 0.0083</td>
<td>0.1529</td>
</tr>
<tr>
<td>4.</td>
<td>4.0</td>
<td>0.2953 ± 0.0173</td>
<td>0.3023</td>
</tr>
<tr>
<td>5.</td>
<td>6.0</td>
<td>0.4631 ± 0.0002</td>
<td>0.4517</td>
</tr>
<tr>
<td>6.</td>
<td>8.0</td>
<td>0.5931 ± 0.0173</td>
<td>0.6011</td>
</tr>
<tr>
<td>7.</td>
<td>10.0</td>
<td>0.7504 ± 0.0010</td>
<td>0.7505</td>
</tr>
<tr>
<td>8.</td>
<td>12.0</td>
<td>0.9040 ± 0.0270</td>
<td>0.8999</td>
</tr>
</tbody>
</table>

* Results are the mean of three experiments

Regression equation; absorbance y = 0.0035 ± 0.0747 × concentration

Coefficient of correlation ‘r’ = 0.9999584.
The absorbance values corresponding to each concentration were then statistically evaluated and plotted as a standard graph, between absorbance on y-axis and concentration on x-axis. The regression values are shown in table 4 and calibration curve is shown in Fig. 12. The graph obeyed the beer-Lambert's law in the selected concentration range.

![Graph](image.png)

Fig. 12: Calibration curve of flurbiprofen in isotonic sodium phosphate buffer of pH 7.4 at $\lambda_{max}$ 247 nm

4.3.4 Method for the measurement of flurbiprofen in transdermal patches, in-vitro drug release studies, stability studies and percentage recovery studies

In-vitro samples were analyzed by reverse phase and HPLC method (US Pharmacopoeia, 1995).
The HPLC system consisted of Shimadzu series SCL-10AVP model LC-10 ATVP binary pumps operated in isocratic mode, fitted with a 20μl loop injector and a Shimadzu UV detector model SPD-10AVP. Mobile phase consisted of 1.4 grams of monobasic sodium phosphate in 570 ml of water and 430 ml of acetonitrile adjusted to pH 3 with phosphoric acid, pumped at 2ml/minute. Mobile phase was filtered through a 0.45 μm millipore membrane filter and degassed before use.

HPLC was performed on C18 column with 5μm particles protected by pre column cartridge. Elements were monitored at 254nm.

Acetophenone (0.8 μl/ml in mobile phase) was used as an internal standard. Under these conditions flurbiprofen eluted at 3.975 minutes & IS at 1.292 minutes.

Fig. 13: The representative HPLC chromatogram for standard preparation for the assay of invitro release study samples containing I.S. (Acetophenone)
Fig. 13 shows the representative HPLC chromatograms for standard preparation for the assay of invitro release study samples containing acetophenone.

The amount of flurbiprofen (in mg) permeated into the receptor medium were quantitated by the formula:

\[ \frac{WV}{10} \times \frac{R_v}{R_s} \]

W= quantity in milligrams (mg) of flurbiprofen used to prepare the standard preparation.

V = Volume in milliliter (ml) of internal standard solution used.

R_v & R_s = Ratio of flurbiprofen peak response to acetophenone peak response obtained from the assay preparation & the standard preparation respectively.

Standard preparation was prepared by adding 10 ml of internal standard solution to 30 mg of flurbiprofen. Portion of the stock solution was diluted with 20 volumes of mobile phase and mixed. 20µl was injected into the system. Table 5 shows the ratio of flurbiprofen peak response to acetophenone response obtained from the standard preparation (R_s) for HPLC analysis of invitro drug release study samples and stability study samples.

Table 5: Ratio of flurbiprofen peak response to acetophenone (IS) response obtained from the standard preparation (R_s) for HPLC analysis of flurbiprofen in invitro drug release study, stability studies and percent recovery studies from patch formulations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Retention time</th>
<th>Mean (± SD) peak area</th>
<th>Peak area ratio of flurbiprofen / IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetophenone peak</td>
<td>1.292</td>
<td>124146 ± 0.023</td>
<td></td>
</tr>
<tr>
<td>Flurbiprofen peak</td>
<td>3.975</td>
<td>1607080 ± 0.033</td>
<td>12.945</td>
</tr>
</tbody>
</table>
4.3.5 Method development and validation for determination of flurbiprofen in rat plasma

Various methods have been reported for the analysis of flurbiprofen in biological samples (Knadler and Hall, 1989; Geisslinger et al., 1992; Askholt and Nielsen, 1986; Hira et al., 1997). Our intention was to develop a relatively simple assay using standard chromatographic equipment. With this method, the limit of quantification was comparable or even better than the reported methods. The applicability of the method was proved in the pharmacokinetic and skin accumulation studies of flurbiprofen transdermal drug delivery systems in albino rats. The method combines simple manipulation and a wide use of instruments with no special assembly required. The mobile phase used was very simple and column friendly, pumped at very low flow rate so as to prevent high back up pressure thereby increasing the column life. The same method was used for the analysis of drug in excised rat skin with slight modification.

4.3.5.1 HPLC instrumentation and chromatographic conditions

The HPLC system consisted of Shimadzu series, Model LC-ATVP binary pumps operated in binary mode, a manual injector fitted with a 20 µl loop and a Shimadzu UV detector Model SPD-10A VP. HPLC was performed on a C18 Shim-Pack CLC-ODS (250cm-4.6mm I.D.) reversed phase column with 5 µm particles.

The mobile phase consisted of methanol and 1% v/v phosphoric acid in water (80:20%v/v) pumped at 0.5 ml/min. Detector was operated in dual mode i.e. at two
wavelengths, 254 nm for flurbiprofen and 220 nm for IS. Under these conditions, flurbiprofen eluted at 9.5 min. and IS at 11.7 min. Interference from endogenous compounds in plasma samples was not observed.

4.3.5.2 Preparation of Stock solutions

The stock solutions of flurbiprofen and IS (1 mg/ml) were prepared in mobile phase. Working standard solutions of flurbiprofen (0.1, 0.25, 0.5, 1.0, 1.5, 2.5, 5.0, 10.0 µg/ml) were prepared by diluting stock solution with mobile phase. The stock solution of IS was diluted with mobile phase to prepare its working standard solution i.e. 50 µg/ml. All the stock solutions were stored at 4°C from which working standards were prepared a fresh whenever needed.

4.3.5.3 Preparation of plasma samples

A 100 µl plasma samples were prepared by addition of 200 µl of IS solution followed by addition of 100 µl of 30% perchloric acid to precipitate protein. The samples were vortex mixed for 1 minute to remove trapped or bound drug from the precipitate. The samples were extracted with 5ml of benzene for 30 minutes and then centrifuged at 3000rpm for 5 minutes. After centrifugation 4ml of the organic phase was removed and evaporated to dryness under nitrogen. The residue was reconstituted in 200 µl of mobile phase by vortex mixing. Samples where further diluted wherever necessary. 20µl was injected into the system.
4.3.5.4 Standardization and assay validation

4.3.5.4.1 Calibration and linearity

Calibration curves were constructed using eight standard concentrations (0.1-10 μg/ml) in plasma. To 80 μl of blank plasma, 20 μl of corresponding flurbiprofen solution in mobile phase, 200 μl of IS and 100 μl of 30% perchloric acid were added. The samples were subjected to the extraction procedure and analysed as described in the preparation of plasma samples. Curves were obtained daily for 3 days by plotting the peak area ratio of flurbiprofen to IS against the corresponding flurbiprofen concentration. The calibration curves were evaluated by linear regression analysis and concentrations of unknown samples were calculated from the calibration plot.

4.3.5.4.2 Intra day and inter day precision and accuracy

To determine assay precision and variability, quality control samples (0.1, 1.0 and 5.0 μg/ml) were prepared as described in the preparation of calibration curve.

The intra day precision and accuracy were assessed by analysis of three quality control samples, extracted and analysed 10 fold on a single day. Inter assay precision and accuracy was determined by analysing the samples 6 fold on 3 different days.

Precision expressed as coefficient of variation (%CV) and accuracy were calculated.

The limit of quantification (LOQ) was determined as the lowest concentration with a coefficient of variation < 20% and a within day accuracy of between 85 and 110% of nominal concentration.
4.3.5.3 Extraction recovery

Extraction recovery was determined for flurbiprofen at three different concentrations (0.1, 1.0 and 5.0 µg/ml) and for the IS at its working concentration (50 µg/ml). Water standards for the recovery determinations were prepared by adding 20 µl of flurbiprofen standard solution to 80 µl of water, followed by 200 µl of IS solution and 100 µl of 30% perchloric acid. The samples were vortex mixed for 1 minute and extracted with 5ml of benzene for 30 minutes. The samples were centrifuged at 3000rpm for 5 minutes. After centrifugation 4ml of the organic phase removed and evaporated to dryness under nitrogen. The residue was reconstituted in 200 µl of methanol and further diluted wherever necessary. 20 µl was injected into the system. Recoveries were calculated by comparing the areas of the standards in rat plasma to the water standards.

% recovery = Mean peak area of extracted sample / Mean peak area of water standard × 100

4.3.5.4 Freez thaw stability

Quality control samples were subjected to three freeze thaw cycles consisting of a thaw to reach room temperature and then refreezing (-20°C) overnight. These were then defrosted and analysed.

4.3.5.5 Result and discussion

The present method was developed to determine the concentration of flurbiprofen in rat plasma by an HPLC method using standard equipment. The representative HPLC chromatograms illustrating the detection of flurbiprofen (0.1 µg/ml) and internal standard (50 µg/ml) in rat plasma at 254nm and 220nm are shown in fig. 14.
Fig. 14: Representative HPLC Chromatograms illustrating the detection of flurbiprofen (0.1 µg/ml) and internal standard (50 µg/ml) in rat plasma at
(a) 254 nm
(b) 220 nm
The UV-Vis absorption spectrum of flurbiprofen indicated the presence of an analytically useful absorption band with a maximum at 247nm. Ibuprofen was chosen as internal standard as it posseses similar properties with respect to extraction.

An essential step in extraction of drug from plasma is protein precipitation with 30% perchloric acid prior to liquid-liquid extraction with benzene. Protein precipitation was also performed with phosphoric acid, 1M sulphuric acid but 30% perchloric acid gave better extraction recoveries. The results from the recovery experiments are summarized in table 6.

Table 6: Extraction recoveries of flurbiprofen and IS from rat plasma, n*= 3

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Mean (±SD) Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flurbiprofen</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>98.96 ± 10.64</td>
</tr>
<tr>
<td>1.0</td>
<td>94.15 ± 7.18</td>
</tr>
<tr>
<td>5.0</td>
<td>97.12 ± 15.40</td>
</tr>
<tr>
<td>IS</td>
<td></td>
</tr>
<tr>
<td>50.0</td>
<td>93.42 ± 17.07</td>
</tr>
</tbody>
</table>

* Results are the mean of three experiments

Average recoveries of flurbiprofen and the IS were found to be 96.74 ± 2.43% and 93.42 ± 17.07% respectively. The recoveries obtained are sufficient for a sensitive measurement of flurbiprofen. The ratio of methanol and water containing phosphoric acid were varied to give satisfactory separation of flurbiprofen and IS from endogenous plasma components. It was found that with methanol and 1% v/v phosphoric acid in the ratio of 80:20 %, the retention times of flurbiprofen and internal standard were 9.5 and 11.7 respectively.
Flurbiprofen is a weakly acidic drug, pKa 4.13, which is highly protein bound and in the unionised form, likely to be highly lipid soluble due to its biphenyl moiety.

For initial sample preparation, protein precipitation followed within one minute Vortex mixing sufficiently liberated the bound drug to precipitated proteins.

IS showed λmax at 220nm due to which the UV detector was adjusted in dual mode.

4.3.5.5.1 Calibration linearity

Calibration curves were constructed, using eight standard concentrations in plasma, daily for three days by plotting the peak area ratios of flurbiprofen to IS against the corresponding nominal concentration. Linear calibration curves were generated by linear regression analysis as shown in table 7 & fig. 15.

Table 7: Mean peak area ratio values of Flurbiprofen / IS in mobile phase for HPLC analysis of flurbiprofen in rat plasma

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration of flurbiprofen (x) μg/ml</th>
<th>Mean (± S.D.) peak area ratio (n=3)</th>
<th>Regressed value of peak ratio (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.1</td>
<td>0.0452 ± 0.001</td>
<td>0.0472</td>
</tr>
<tr>
<td>2.</td>
<td>0.25</td>
<td>0.0454 ±0.004</td>
<td>0.0499</td>
</tr>
<tr>
<td>3.</td>
<td>0.5</td>
<td>0.0527 ± 0.020</td>
<td>0.0545</td>
</tr>
<tr>
<td>4.</td>
<td>1.0</td>
<td>0.0619 ± 0.002</td>
<td>0.0636</td>
</tr>
<tr>
<td>5.</td>
<td>1.5</td>
<td>0.0711 ± 0.001</td>
<td>0.0727</td>
</tr>
<tr>
<td>6.</td>
<td>2.0</td>
<td>0.0803 ± 0.001</td>
<td>0.0818</td>
</tr>
<tr>
<td>7.</td>
<td>5.0</td>
<td>0.1623 ± 0.023</td>
<td>0.1364</td>
</tr>
<tr>
<td>8.</td>
<td>10.0</td>
<td>0.2162 ± 0.010</td>
<td>0.2274</td>
</tr>
</tbody>
</table>

Coefficient of correlation ‘r’ = 0.992862

Regression equation

Area ratio = 0.0454 + 0.0182 x concentration
The regression equation for flurbiprofen was \( y = (0.0454 \pm 0.001914) + (0.0182 \pm 0.0001732)x \), \( r = 0.9928 \pm 0.010072 \), where 'x' is the drug concentration in \( \mu g/ml \) and 'y' is the ratio of the area of drug peak to IS peak. Lowest limit of quantitation (LOQ) was selected as the lowest concentration of standard curve.

Fig.15: Calibration curve for the estimation of flurbiprofen in rat plasma

4.3.5.5.2 Assay precision and accuracy

The precision and accuracy of the plasma assay were determined by three drugs of quality control analysis. Inter day and intra day variability is shown in table 8.
Table 8: Intra and inter day precision and accuracy for the determination of flurbiprofen in rat plasma

<table>
<thead>
<tr>
<th>Concentration added (µg/ml)</th>
<th>Number of samples (n)</th>
<th>Mean (±SD) Concentration found (µg/ml)</th>
<th>CV (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>0.093 ± 0.004</td>
<td>4.301</td>
<td>93.0</td>
</tr>
<tr>
<td>1.0</td>
<td>10</td>
<td>0.965 ± 0.010</td>
<td>1.036</td>
<td>96.5</td>
</tr>
<tr>
<td>5.0</td>
<td>10</td>
<td>4.732 ± 0.162</td>
<td>3.423</td>
<td>94.6</td>
</tr>
<tr>
<td>Inter assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>18</td>
<td>0.091 ± 0.005</td>
<td>5.494</td>
<td>91.0</td>
</tr>
<tr>
<td>1.0</td>
<td>18</td>
<td>0.925 ± 0.043</td>
<td>4.648</td>
<td>92.5</td>
</tr>
<tr>
<td>5.0</td>
<td>18</td>
<td>4.688 ± 0.331</td>
<td>7.060</td>
<td>93.7</td>
</tr>
</tbody>
</table>

At all concentrations intra and inter assay variabilities were below 8%. The intra day precision of the assay was 7.904 or better. Assay accuracy was found to be within 9% of nominal value. The limit of quantification (LOQ) was 0.10 µg/ml. The LOQ was sufficient to support the pharmacokinetic studies.

4.3.5.5.3 Freez thaw stability

Quality control samples (n=5 at each concentration) were subjected to three freeze thaw cycles. Results of the analysis are presented in table 9. Three freeze thaw cycles had no effect on the stability of the analytes as the accuracy of the samples was within 4% of expected values.
Table 9: Freez thaw stability of flurbiprofen in rat plasma

<table>
<thead>
<tr>
<th>Nominal concentration (µg/ml)</th>
<th>Mean (± SD) found concentration (µg/ml)</th>
<th>CV(%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.096 ± 0.011</td>
<td>11.458</td>
<td>96.0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.962 ± 0.120</td>
<td>12.474</td>
<td>96.2</td>
</tr>
<tr>
<td>5.0</td>
<td>4.890 ± 0.526</td>
<td>10.756</td>
<td>97.8</td>
</tr>
</tbody>
</table>

4.3.6 Calibration curve for measurement of drug in excised rat skin samples

It was prepared by slight modification of the HPLC method developed in our laboratory.

Chromatographic conditions were same as mentioned in section 4.3.5.

Calibration curve was made by using seven concentration ranges from 0.1 - 10 µg/ml. Calibration curve was obtained by adding 100 µl of IS to 100 µl of the corresponding flurbiprofen concentration and 20µl of the resulting solution was directly injected into the system. Peak area ratio of flurbiprofen to internal standard was plotted against the corresponding flurbiprofen concentration. The regression values are presented in table 10 and calibration curve is shown in fig. 16.
Table 10: Mean peak area ratio of flurbiprofen to IS in mobile phase for HPLC analysis of flurbiprofen in excised skin samples

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Concentration of flurbiprofen (x) (µg/ml)</th>
<th>Mean* (± S.D.) peak area ratio</th>
<th>Regressed value of peak area ratio of (y)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.1</td>
<td>0.0381 ± 0.022</td>
<td>0.0260</td>
</tr>
<tr>
<td>2.</td>
<td>0.25</td>
<td>0.0430 ± 0.001</td>
<td>0.0428</td>
</tr>
<tr>
<td>3.</td>
<td>0.5</td>
<td>0.0864 ± 0.001</td>
<td>0.0709</td>
</tr>
<tr>
<td>4.</td>
<td>1.0</td>
<td>0.1093 ± 0.032</td>
<td>0.1271</td>
</tr>
<tr>
<td>5.</td>
<td>2.5</td>
<td>0.2827 ± 0.015</td>
<td>0.2955</td>
</tr>
<tr>
<td>6.</td>
<td>5.0</td>
<td>0.5652 ± 0.007</td>
<td>0.5763</td>
</tr>
<tr>
<td>7.</td>
<td>10.0</td>
<td>1.1481 ± 0.025</td>
<td>1.1378</td>
</tr>
</tbody>
</table>

* Results are the mean of three experiments

Regressed equation: Area ratio = 0.0148 + 0.1123 × concentration.

Coefficient of correlation (r) = 0.99953
4.3.7 Assay method for flurbiprofen in transdermal patches

Transdermal patch was cut into small pieces and the pieces were grounded in mortar and flurbiprofen was extracted with mobile phase. The contents were filtered through Whatmann number 42 filter paper.

The solution was diluted with 20 volumes of mobile phase and mixed. 20 µl was injected into the system after filtering the solution through millipore membrane filter. 0.2 ml of the resulting solution was mixed with 0.2 ml of IS (acetophenone). The area ratio of flurbiprofen peak to IS peak was recorded and the quantity of flurbiprofen was calculated according to the method 4.3.4.
The drug content was calculated as follows:

\[ \text{Drug content (mg)} = \text{concentration (mg/ml)} \times \text{dilution factor} \]

Drug content and % recovery of flurbiprofen from transdermal patches is shown in table 11.

**Table 11: Drug content and percent recovery of flurbiprofen from transdermal patches**

<table>
<thead>
<tr>
<th>Transdermal patch</th>
<th>Mean* area ratio ( R_v / R_s )</th>
<th>Drug content (mg)</th>
<th>Mean* (±SD) % recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>1.6652 (D.F = 100)</td>
<td>99.91</td>
<td>99.91 ± 0.86</td>
</tr>
<tr>
<td>T-2</td>
<td>1.6583 (D.F = 100)</td>
<td>99.50</td>
<td>99.50 ± 0.93</td>
</tr>
<tr>
<td>T-3</td>
<td>1.6650 (D.F = 100)</td>
<td>99.90</td>
<td>99.90 ± 0.08</td>
</tr>
</tbody>
</table>

* Results are the mean of three experiments

Drug content in one patch = 100 mg

Patch T-1 contains NA-102 as penetration enhancer
Patch T-2 contains turpentine oil as penetration enhancer
Patch T-3 contains lemon oil as penetration enhancer.

4.3.8 Analysis of in vitro preliminary skin permeation studies

1 ml sample of the receptor medium was withdrawn at different time intervals and replaced immediately with an equal volume of fresh isotonic phosphate buffer of pH 7.4. The sample was filtered through Whatmann filter paper number 42 and diluted whenever needed. The samples were analyzed spectrophotometrically at 247 nm. The concentration of drug was read from the calibration curve prepared according to the
method 4.3.3. The cumulative amount of drug permeated was calculated by the following equation.

\[
\frac{\text{Concentration (µg/ml) \times Dilution factor \times Volume of receiver compartment}}{\text{Area of permeation } \times 1000}
\]

Cumulative amount of drug permeated (mg/cm²)

4.3.9 Analysis of in-vitro drug release study samples

0.2 ml sample of the receptor medium was withdrawn at different time internals and replaced immediately with an equal volume of fresh IPB of pH 7.4.

Sample was mixed with an equal volume of IS (acetophenone).

It was diluted with 20 volumes of mobile phase and mixed.

20 µl of the resulting solution was injected into the HPLC system after filtering the solution through 0.45 µm membrane filter (Millipore). The quantity in mg of flurbiprofen permeated was calculated according to the method 4.3.4.

The cumulative amount of drug permeated was calculated in the same manner as mentioned in section 4.3.8.

4.3.10 Analysis of in-vivo study samples

After the application of patch, a blood sample of 250 µl was collected at 0.5, 1, 2, 4, 6, 8, 12 & 24 hours post dose from the tail vein of the rat. Collected rat blood samples were immediately transferred into polypropylene tubes containing small
amount of disodium EDTA and centrifuged at 300 rpm for 5 minutes to obtain plasma. The plasma samples were stored at -20°C till analysis by HPLC method.

A 100 µl of plasma samples were prepared by addition of 200 µl of IS (ibuprofen) and 100 µl of 30% v/v perchloric acid. The samples were vortex mixed for 1 minute to remove trapped or bound drug from the precipitate. The samples were extracted with 5 ml of benzene for 30 minutes and then centrifuged at 3000 rpm for 5 minutes. After centrifugation, 4 ml of the organic phase was removed and evaporated to dryness under nitrogen. The residue was reconstituted in 200 µl of mobile phase by vortex mixing. Samples were further diluted wherever necessary, 20 µl was injected into the system.

Area ratio of flurbiprofen to IS peaks obtained for different samples was noted. Concentration of the drug was calculated from the calibration curve of the drug prepared according to method 4.3.5.

4.3.11 Analysis of stability study samples

Patch formulations stored in humidity chamber maintained at 40°C / 75% RH, were withdrawn at 0, 15, 30, 45, 60 & 90 days. The patches were cut into smaller pieces and dissolved in mobile phase by continuous shaking for 2 hours.

0.2 ml of the resulting solution was mixed with 0.2 ml of IS (acetophenone). It was diluted with 20 volumes of mobile phase and mixed. 20 µl was injected into the HPLC system after filtering through 0.45 µm membrane filter. The quantity in mg of flurbiprofen remaining in the patch was calculated according to the method 4.3.4.
4.3.12 Analysis of excised skin sample

Flurbiprofen concentration in excised skin was determined by HPLC analysis. Full thickness skin of rat over which transdermal patch was applied was removed. To 100 μl aliquot of the supernatant obtained from homogenization of the excised skin in 30 ml of mobile phase was added 100 μl of IS and vortex mixed. 20 μl of the resulting solution was injected into the HPLC system, concentration of the drug remaining in the patch was calculated form the calibration curve of drug prepared according to method 4.3.6.
4.4 WORKING OF THE ASSEMBLIES AND METHODS EMPLOYED

4.4.1 Fabrication of apparatus for in-vitro drug skin permeation studies

In-vitro drug skin permeation studies were carried out in modified Keshary Chien cell.

The assembly consisted of two chambers:

1. The upper cylindrical chamber (donor compartment), which was open from above.

2. The lower cylindrical chamber (receiver compartment), containing a sampling port and had Teflon coated magnetic needle at the base. The capacity of the receiver compartment was 40 ml. The area of diffusion between the two half cells was 8.86 cm$^2$ (fig. 17).

Hooks were made on the sides of both the chambers so that the two chambers formed one single unit without any leakage once the rubber bands were secured over these hooks.

The receptor compartment was maintained at 37 °C by an electric bath.

The donor compartment was maintained at the ambient temperature of 25 ± 2 °C.
Fig 17: Sketch diagram of modified Keshary Chien diffusion cell.
4.4.2 Preparation of rat skin

A number of animal models have been reported for performing invitro skin permeation studies e.g. rat (Catz and Friend, 1990), rabbit (Hirovonen et al., 1993), hairless mouse (Catz and friend, 1990), Shed snake skin (Buyuktimkin, et al., 1995), human cadaver skin (Roy et al., 1994). Albino rats were selected because of their easy availability. The protocol for the preparation of whole skin was approved by Institutional Animal Ethical Care Committee. Rats were sacrificed by excess ether inhalation. The dorsal skin of animal was shaved with the help of animal hair clipper and full thickness skin was surgically removed from each rat. The skin specimen was cut into appropriate sizes after carefully removing subcutaneous fat and washing with IPB of pH 7.4, wrapped in aluminum foil and stored at -20 °c till it was used.

4.4.3 In vitro skin permeation studies

Invitro skin permeation across rat skin was conducted with vertical Keshary Chien diffusion cell. The experimental set up is shown in figure17. The freshly excised full thickness skin was mounted on the diffusion cell with the stratum corneum side facing the donor compartment. The area of diffusion for all invitro experiments was 8.86 cm² and the capacity of the receiver compartment was 40 ml. The skin was equilibrated for 6 hours with the receptor medium. A blank sample of 1 ml was withdrawn from the receptor compartment and analyzed to ensure that the diffusion cells did not have any residual absorbance. The buffer solution was replaced after every 30 minutes. The 6 hour sample showed no absorbance indicating the complete stabilization of the skin.
The receptor solution (IPB of pH 7.4) was then introduced into the magnetically stirred receptor compartment maintained at 37°C by an electric water bath.

The donor compartment was maintained at the ambient temperature of 25±20°C.

The donor compartment, which faced the stratum corneum surface, contained a 1% w/v solution (5ml) of flurbiprofen in the vehicles with or without skin permeation enhancer and was covered with Para film. Table 12 and 13 shows the working formulae of flurbiprofen for performing invitro permeation studies.

Samples (1 ml) were withdrawn from receptor compartment for 24 hours at regular intervals and analyzed for drug content by UV spectrophotometry method at 247nm using IPB of pH 7.4 as blank as mentioned in section 4.3.8. The receptor volume was immediately replaced with fresh receptor medium. Sampling port was covered with Para film to prevent the evaporation of receptor medium.

**Table 12:** Working formulae for performing invitro permeation study of flurbiprofen in various solvents and their binary combinations through rat skin

<table>
<thead>
<tr>
<th>Drug solution (1%w/v) in</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>N6</th>
<th>N7</th>
<th>N8</th>
<th>N9</th>
<th>N10</th>
<th>N11</th>
<th>N12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG: IPA (v/v)</td>
<td>100:0</td>
<td>70:30</td>
<td>50:50</td>
<td>30:70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG: IPB (v/v)</td>
<td>0:100</td>
<td>70:30</td>
<td>50:50</td>
<td>30:70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPA: IPB (v/v)</td>
<td>100:0</td>
<td>70:30</td>
<td>50:50</td>
<td>30:70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

87
Table 13: Formulae for performing invitro skin permeation studies of flurbiprofen in optimized solvent mixture (PG: IPA; 30:70\%v/v) containing various penetration enhancers

<table>
<thead>
<tr>
<th>Composition of the drug solution</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>P7</th>
<th>P8</th>
<th>P9</th>
<th>P10</th>
<th>P11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flurbiprofen (%w/v)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>NA-102 (%w/v)</td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turpentine oil (%w/v)</td>
<td></td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lemon oil (%w/v)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG:IPA (30:70% v/v), q.s.</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

4.4.4 Calculation of the steady state plasma concentration

In vitro permeation data can be used to find the steady state plasma concentration by using the following formula.

\[ C_{ss} = \frac{\text{Flux} \times \text{Surface area}}{\text{Systemic clearance after IV administration} \times \text{body weight}} \]

Surface area = 9 cm\(^2\)

Body weight of the patient = 60 Kg

Clearance = 0.35 ml/minute/Kg (Hardman and Limbard, 1996)

**Formulation P3**

Flux = 198.88 μg/cm\(^2\)/hour
C_{ss}=1.42 \mu g/ml

Formulation P7

Flux= 241.37 \mu g/cm^2/hour

C_{ss}=1.72 \mu g/ml

Formulation P10

Flux=210.3 \mu g/cm^2/hour

C_{ss}=1.50 \mu g/ml

Since the achieved steady state plasma concentrations are less than the required steady state plasma concentration of flurbiprofen (3 - 5 \mu g/ml). The drug load was increased to 100mg for incorporation in transdermal patches (Hadgraft, 1996).

4.4.5 Preparation of flurbiprofen formulation

A reservoir type transdermal delivery system of Flurbiprofen was developed. Transdermal system of flurbiprofen comprised of 3 basic components.

1. Drug impermeable backing membrane to avoid evaporation of solvent system

2. Drug reservoir viscous solution consisting of flurbiprofen in PG: IPA (30: 70 %v/v) co solvent system containing a skin permeation enhancer and thickening agent

3. Rate controlling membrane
EXPERIMENTAL

The formulations were prepared by soaking method. Penetration enhancer and drug were solubilized in the co solvent system by stirring. Then the polymer was added slowly with constant stirring and weight was made by addition of sufficient quantity of co solvent system. The system was kept overnight for complete swelling of polymer. The composition of the formulation is given in table 14.

Table 14: Composition of viscous formulations of flurbiprofen

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A-1</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>0.1</td>
</tr>
<tr>
<td>Methocel</td>
<td>0.04</td>
</tr>
<tr>
<td>NA-102</td>
<td>0.25</td>
</tr>
<tr>
<td>Turpentine oil</td>
<td></td>
</tr>
<tr>
<td>Lemon oil</td>
<td></td>
</tr>
<tr>
<td>PG:IPA q.s.</td>
<td>4</td>
</tr>
</tbody>
</table>

4.4.6 Invitro drug permeation studies of medicated viscous system

The formulations were subjected to invitro permeation studies across the rat skin using Keshary Chien diffusion cells as mentioned above except that 4 grams of flurbiprofen viscous system was used as donor phase in place of flurbiprofen.
solution. 0.2 ml of the receptor medium was withdrawn at different time intervals and replaced immediately with an equal volume of fresh IPB of pH 7.4. Samples were mixed with equal volume of internal standard, acetophenone, followed by dilution with 20 volumes of mobile phase. 20 µl of the resulting solution was injected into the HPLC system (US Pharmacopoeia, 1995) after filtering through 0.45 µm membrane filter (Millipore) and analysis was performed as mentioned in section 4.3.9.

4.4.7 Fabrication of the patch

Transdermal patches of flurbiprofen were fabricated by encapsulating the flurbiprofen reservoir viscous solution within a shallow compartment molded from a drug impermeable backing and a rate controlling membrane. The ethylene vinyl acetate (EVA) (3M™, USA) rate controlling membrane was placed over on the release liner fluropolymer coated polyester film (3M™, USA) and then polyester film laminate (3M™, USA) backing membrane was placed on it. The composite was heat sealed and cut to appropriate size. The flurbiprofen reservoir viscous solution was dispersed into the device using a syringe. The device was heat sealed again to close the unsealed side of the device ensuring no reservoir leaking out of the device. Then each patch was packed in laminated aluminum foil. The components of the patch formulations are shown in table 15.
Table 15: Flurbiprofen transdermal patch components

<table>
<thead>
<tr>
<th>Components</th>
<th>T-1</th>
<th>T-2</th>
<th>T-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reservoir solution</td>
<td>A-2</td>
<td>B-2</td>
<td>C-2</td>
</tr>
<tr>
<td>Backing film</td>
<td>3M™ Scotch pak™ 9733</td>
<td>3M™ Scotch pak™ 9733</td>
<td>3M™ Scotch pak™ 9733</td>
</tr>
<tr>
<td>Microporous membrane</td>
<td>3M™ Co-Tran™ 9702</td>
<td>3M™ Co-Tran™ 9702</td>
<td>3M™ Co-Tran™ 9702</td>
</tr>
<tr>
<td>Release liner</td>
<td>3M™ Scotch pak™ 1022</td>
<td>3M™ Scotch pak™ 1022</td>
<td>3M™ Scotch pak™ 1022</td>
</tr>
<tr>
<td>Peripheral micropore adhesive</td>
<td>3M™</td>
<td>3M™</td>
<td>3M™</td>
</tr>
</tbody>
</table>

4.4.8 In vitro permeation study of patch formulations

In vitro permeation study of patch formulations were carried out using the same experimental set up as mentioned above except that a transdermal patch was placed in intimate contact with rat skin secured with the help of micropore adhesive (3M, USA) which was placed over backing membrane. The samples were analyzed by HPLC as mentioned in section 4.3.9.

4.4.9 Stability studies

Stability studies were carried out to determine the effect of the presence of polymer or formulation additives on stability of the drug and also to determine the physical stability of the formulations under accelerated storage conditions of temperature and humidity. Stability studies were carried out according to ICH guidelines (Singh,
1999). Sufficient replicates of the optimized patch formulations were stored in closed petri dishes lined internally with aluminum foil. They were stored in humidity chamber maintained at 40°C / 75% RH for 90 days. Samples were withdrawn at specified time intervals and analyzed for drug content by HPLC method as outlined in section 4.3.11. The logarithms of percent drug remaining were plotted against time in days (fig.33). The slope of the straight line was determined and degradation rate constant was deduced using the following equation.

Slope = \(-K/2.303\)

Where \(K\) is degradation rate constant.

4.4.10 Interaction study

In order to investigate any interaction between drug and polymer or other additives or due to conditions of the formulation process, the following interaction studies were carried out.

1. I.R. Spectral analysis: I.R. spectra of the pure drug, dried medicated patches and placebo patches were recorded using the carbon tetrachloride solubilization method and the spectra were compared.

2. U.V. Spectral analysis: UV spectra of the pure drug, transdermal patches and placebo patches were taken after dissolving them in IPB of pH 7.4. The solutions were filtered and scanned between 200-400nm. The spectra were then compared to detect any shift in \(\lambda_{\text{max}}\).
4.4.11 Histological study

Transdermal patch formulations were applied for 24 hours on the excised skin mounted on the diffusion cell. The transdermal patch formulations were removed, the rat skin was wiped off with tissue paper and fixed with 10% v/v formalin solution in saline for at least 72 hours before routine processing. The skin was sectioned vertically and each section was dehydrated and embedded in paraffin wax. Tissues were divided into small pieces and stained with hematoxylin and eosin. All sections were then examined under a microscope (Alol et al., 1993). Skin not treated with any formulation served as a control.

4.4.12 Pharmacokinetic studies

The study had been approved by local animal ethics committee (ICPCA). Project number 94 was assigned to the animal requisition form.

Albino rats weighing between 250 ± 10 grams were used for this study. The rats were lightly anesthetized with ether prior to dosing. The hair of the dorsal area was removed carefully with clipper. The transdermal patch containing 1 mg of the drug was applied on the dorsal area of 9 cm² gently with the help of micropore adhesive.

After the application of the patch, blood sample of 250 µl was collected at 0.5, 1, 2, 4, 6, 8, 12 and 24 hours post dose from the tail vein of the rat. The blood was collected in polypropylene tubes containing EDTA and centrifuged at 3000rpm for 5 minutes to obtain plasma. The plasma was stored at -20 °c prior to analysis by HPLC method developed in our laboratory as given in section 4.3.10.
A 100μl plasma samples were prepared by addition of 200μl of internal standard ibuprofen and 100μl of 30 % v/v perchloric acid. The samples were vortex mixed for 1 minute to remove trapped or bound drug from the precipitate. The samples were extracted with 5 ml of benzene for 30 minutes and the centrifuged at 3000rpm for 5 minutes. After centrifugation 4ml of the organic phase was removed and evaporated and dried under nitrogen. The residue was reconstituted in 200μl of mobile phase by vortex mixing. Samples were further diluted wherever necessary. 20 μl was injected into the system. HPLC system consisted of Shimadzu series binary pumps operated in binary mode, a manual injector fitted with a 20 μl loop and a Shimadzu UV detector. HPLC was performed on C 18 (250 cm-4.6mm I.D) reverse phase column as mentioned in the section 4.3.10. The mobile phase consisted of methanol and 1% v/v phosphoric acid in water (80: 20 v/v) pumped at 0.5 ml/minute. Detector was operated in dual mode i.e. at 2 wavelengths 254 nm for flurbiprofen and at 220nm for internal standard ibuprofen. Under these conditions flurbiprofen eluted at 9.5 min. and internal standard at 11.7 minutes. The concentration of the flurbiprofen was read from the calibration plot constructed between peak area ratios of flurbiprofen to ibuprofen versus corresponding flurbiprofen concentration. The area under the plasma concentration time curve (AUCo-α) was determined to compare the amount of drug absorbed through the skin after the transdermal application of test formulation with orally administered flurbiprofen. The maximum plasma drug concentration (Cmax) and the time to reach the maximal concentration (Tmax) were determined from the concentration time curve.
4.4.13 Skin accumulation studies

Following the administration of transdermal patch skin accumulation studies were performed (Sasaki et al., 1987). Full thickness skin of rat over which transdermal patch was applied was removed. To 100μl aliquot of the supernatant obtained from homogenization of the excised skin in 30 ml of mobile phase was added 100 μl of internal standard ibuprofen and vortex mixed. 20 μl of the resulting solution was injected into the HPLC system and HPLC was performed by slight modification of the method developed in our laboratory as mentioned in section 4.3.12.

4.4.14 Pharmacodynamic studies. (Carrageenin paw edema inflammation model)

The topical antiinflammatory efficacy was evaluated by the carrageenin induced inflammation edema in rats weighing between 250 ± 10 grams. The activity of the drugs in the various vehicles with or without penetration enhancer was evaluated by measuring the change in paw volume with a Plethysmometer (Ugo Basile, Italy).

Left hind paws of each rat were marked, just beyond tibiotarsal junction, so that every time the paw is dipped upto the fixed mark to ensure constant paw volume. Animals were divided into several groups, each group comprising of 4 rats, initial paw volume of each rat was noted.

Formulations were applied on the dorsal area of 9 cm² gently with the help of micropore adhesive, one hour prior to the carrageenin injection. The paw volume was noted at 0, 1, 2, 4, 6, 8 and 24 hours.
Acute inflammation was produced by injecting 0.1 ml of 1% w/v carrageenin solution in the sub plantar region of the left hind paw one hour after treatment with drug.

The percentage swelling in paw was calculated by the following formula.

\[
\% \text{ age swelling} = \frac{b-a}{a} \times 100
\]

Where \(a\) = paw volume before producing the edema

\(b\) = paw volume measured hourly after producing the edema

4.4.15 Data Analysis

4.4.15.1 Determination of permeation parameters in invitro release studies

The steady state flux was determined from the slope of the linear portion of a cumulative amount permeated versus time plot. The lag time (\(T_{\text{lag}}\)) was determined by extrapolating the linear portion of the cumulative amount permeated versus time curve to the abscissa.

Permeability values were calculated by the following equation;

\[
\text{Permeability coefficient} = \frac{J}{C_d}
\]

Where \(J\) is steady state flux (\(\mu g/cm^2/hr\)), \(C_d\) is the concentration in donor compartment.

Enhancement ratio of the flux \((E_{\text{ren}})\) can be expressed as;
\[ E_{\text{pen}} = \frac{P_{\text{treatment}}}{P_{\text{control}}} \]

Where \( P_{\text{treatment}} \) is the flux of transdermal patch containing enhancer and \( P_{\text{control}} \) is the flux of control group i.e. transdermal patch without enhancer.

4.4.15.2 Pharmacokinetic analysis

The following pharmacokinetic parameters were computed for each formulation:

1. Time to reach maximum concentration (\( T_{\text{max}} \))

2. Peak plasma concentration (\( C_{\text{max}} \))

3. Area under the concentration time curve from 0 to 24 (\( \text{AUC}_{0-24} \)) and from 24 hours to infinity (\( \infty \)), (\( \text{AUC}_{0-\infty} \)), by using the linear trapezoidal rule.

Statistical analysis was performed using ‘t’ test and Analysis of Variance (ANOVA) followed by Students Newman Keuls Multiple Comparison tests.
RESULTS
AND
DISCUSSION
RESULTS AND DISCUSSION

Transdermal pharmaceutical products provide the considerable advantage of a non-invasive parental route for drug therapy, avoidance of first pass gut and hepatic metabolism, potentially decreased side effects and relative ease of drug input termination in problematic cases. The rate controlled transdermal dosage form can provide a precise regulation of drug concentration in plasma and thus a high degree of safety and selectivity of action for some drugs (Chien, 1992).

The major barrier to the percutaneous delivery is the impermeability of the stratum corneum. One method employed to decrease this diffusional barrier is to use penetration enhancers. These are materials, which usually interact with some components of the stratum corneum, thereby increasing drug permeation through the tissue.

Several solvents either alone or in combination with other solvents are being investigated to overcome the low permeability of drugs through the skin. The vehicle should permit easy partition of the drug into the skin. These solvent systems may modify the skin structure e.g. some solvents can remove lipids from the stratum corneum (Kim et al., 1996). The barrier function is reduced when the lipids are modified in this way, although the effect has been shown to be reversible. Some topical and transdermal products contain high concentrations of solvents such as ethanol that may be capable of altering the lipid of the skin (Bommannan et al., 1991). It has been demonstrated in various studies that by carefully changing the proportion of the solvents in cosolvent system, transdermal permeation of drugs could be increased to a therapeutically significant level. (Berner et al., 1989)
5.1 INVITRO PRELIMINARY SKIN PERMEATION STUDIES

In the present study, the effects of solvents isotonic phosphate buffer (IPB) of pH 7.4, isopropyl alcohol (IPA) and propylene glycol (PG) and their binary combinations on the invitro skin permeability of flurbiprofen was investigated in order to optimize the solvent system to develop a reservoir type of transdermal drug delivery system of flurbiprofen.

In vitro preliminary skin permeation studies were carried out in Keshry Chien cell. The receptor solution consisted of IPB of pH 7.4 maintained at 37 ± 2°C. the donor compartment contained 5ml of 1 % w/v solution of flurbiprofen in the vehicles with or with out skin penetration enhancers.

The effect of different solvent systems on invitro permeation of flurbiprofen through rat skin is shown in table 16 &17 and figures 18 – 20.
Table 16: Mean* in-vitro permeation of flurbiprofen through rats skin from various solvents and their binary combinations

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>N4</th>
<th>N5</th>
<th>N6</th>
<th>N7</th>
<th>N8</th>
<th>N9</th>
<th>N10</th>
<th>N11</th>
<th>N12</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>4.80±0.2</td>
<td>-</td>
<td>-</td>
<td>24.94±7.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.90±0.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>12.57±0.2</td>
<td>9.95±0.1</td>
<td>4.83± δ</td>
<td>59.82±6.3</td>
<td>2.71±2.0</td>
<td>6.93±4.2</td>
<td>17.81±1.9</td>
<td>2.10±5.3</td>
<td>4.83±6.8</td>
<td>1.81±5.3</td>
<td>1.2±1.8</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>35.76±0.1</td>
<td>37.16±2.2</td>
<td>19.94±6.1</td>
<td>131.74±5.2</td>
<td>-</td>
<td>16.61±5.9</td>
<td>46.82±6.6</td>
<td>46.82±2.2</td>
<td>9.95±6.2</td>
<td>8.76±5.2</td>
<td>19.32±1.8</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>67.68±0.2</td>
<td>59.82±1.2</td>
<td>29.91±2.3</td>
<td>159.84±2.2</td>
<td>8.76±4.3</td>
<td>25.67±2.2</td>
<td>76.75±5.0</td>
<td>71.92±4.5</td>
<td>19.93±0.5</td>
<td>17.81±5.0</td>
<td>47.74±3.0</td>
<td>1.81±2.2</td>
</tr>
<tr>
<td>4</td>
<td>99.58±0.9</td>
<td>88.53±0.2</td>
<td>51.96±5.3</td>
<td>191.58±5.2</td>
<td>20.94±3.6</td>
<td>43.21±6.3</td>
<td>121.47±1.0</td>
<td>101.83±4.4</td>
<td>29.91±1.2</td>
<td>23.86±6.0</td>
<td>80.97±3.2</td>
<td>16.91±2.3</td>
</tr>
<tr>
<td>5</td>
<td>43.9±11</td>
<td>114.83±0.3</td>
<td>89.73±3.3</td>
<td>299.75±2.2</td>
<td>19.93±11.2</td>
<td>43.81±5.9</td>
<td>166.29±5.2</td>
<td>149.86±2.9</td>
<td>39.89±5.0</td>
<td>28.69±6.2</td>
<td>106.95±5.5</td>
<td>26.8±8.6</td>
</tr>
<tr>
<td>6</td>
<td>165.81±3.3</td>
<td>144.74±3.3</td>
<td>129.93±2.1</td>
<td>333.91±2.3</td>
<td>27.49±13.3</td>
<td>54.38±11.3</td>
<td>216.37±1.2</td>
<td>226.61±3.0</td>
<td>53.48±5.2</td>
<td>41.69±3.9</td>
<td>134.15±5.4</td>
<td>45.62±11.3</td>
</tr>
<tr>
<td>7</td>
<td>201.61±2.9</td>
<td>174.97±1.2</td>
<td>169.82±2.2</td>
<td>359.89±3.2</td>
<td>25.37±12.5</td>
<td>53.77±8.8</td>
<td>221.78±4.3</td>
<td>247.79±2.7</td>
<td>64.97±3.8</td>
<td>49.84±2.0</td>
<td>169.82±4.4</td>
<td>51.96±6.1</td>
</tr>
<tr>
<td>8</td>
<td>234.47±5.7</td>
<td>204.88±5.1</td>
<td>199.73±1.1</td>
<td>423.66±1.2</td>
<td>33.52±9.0</td>
<td>56.19±6.9</td>
<td>234.18±4.0</td>
<td>297.95±14.1</td>
<td>76.75±7.1</td>
<td>59.82±7.1</td>
<td>193.68±10.7</td>
<td>60.43±3.4</td>
</tr>
<tr>
<td>9</td>
<td>261.56±6.5</td>
<td>234.79±1.1</td>
<td>234.79±4.2</td>
<td>559.93±12.2</td>
<td>34.74±5.7</td>
<td>69.80±7.4</td>
<td>241.44±4.7</td>
<td>354.76±12.2</td>
<td>84.90±88.8</td>
<td>69.80±26.2</td>
<td>233.91±2.2</td>
<td>75.8±3.3</td>
</tr>
<tr>
<td>10</td>
<td>301.69±6.4</td>
<td>264.70±10</td>
<td>284.94±3.3</td>
<td>642.14±1.3</td>
<td>35.03±7.4</td>
<td>84.90±44.4</td>
<td>250.81±64.0</td>
<td>413.68±95.5</td>
<td>99.71±15.6</td>
<td>74.92±6.7</td>
<td>250.81±1.0</td>
<td>78.2±6.1</td>
</tr>
<tr>
<td>12</td>
<td>366.95±2.2</td>
<td>324.83±1.2</td>
<td>364.72±1.0</td>
<td>704.99±63.2</td>
<td>66.77±9.2</td>
<td>108.78±5.4</td>
<td>281.92±23.4</td>
<td>537.88±25.3</td>
<td>124.79±17.5</td>
<td>94.88±12.2</td>
<td>312.75±10.9</td>
<td>112.71±2.3</td>
</tr>
<tr>
<td>16</td>
<td>500.88±1.3</td>
<td>439.98±2.2</td>
<td>509.77±1.1</td>
<td>1099.95±14</td>
<td>125.6±8.8</td>
<td>193.68±2.4</td>
<td>423.66±9.1</td>
<td>784.76±2.3</td>
<td>174.97±10.3</td>
<td>124.79±0.2</td>
<td>430.90±1.3</td>
<td>152.8±9.1</td>
</tr>
<tr>
<td>20</td>
<td>640.61±3.4</td>
<td>559.93±2.1</td>
<td>659.95±2.2</td>
<td>1479.80±22</td>
<td>186.75±6.3</td>
<td>273.77±1.1</td>
<td>-</td>
<td>1026.82±1.3</td>
<td>210.0±12.2</td>
<td>159.84±0.3</td>
<td>548.76±2.0</td>
<td>200.95±3.6</td>
</tr>
<tr>
<td>24</td>
<td>763.41±3.4</td>
<td>675.88±0.9</td>
<td>804.72±6.2</td>
<td>1920.38±5.5</td>
<td>249.30±5.5</td>
<td>355.06±1.6</td>
<td>826.16±4.6</td>
<td>1269.48±1.2</td>
<td>280.43±14.6</td>
<td>187.88±0.9</td>
<td>662.69±2.0</td>
<td>244.76±11.3</td>
</tr>
</tbody>
</table>

*Results are the mean of three observations
Fig. 18: The effect of IPA: PG solvent mixture on the invitro penetration of flurbiprofen through rat skin
### RESULTS AND DISCUSSION

Table 17: Mean permeation parameters of 1 %w/v flurbiprofen in various vehicles through excised rat skin.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Solvent mixture (%v/v)</th>
<th>Mean (±SD) flux (μg/cm²/hr)</th>
<th>Permeability coefficient (cm/hr)</th>
<th>Mean (±SD) Lag time (hours)</th>
<th>Cumulative percentage of flurbiprofen permeated</th>
<th>E_pen</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 (Control)</td>
<td>PG: IPA 100:0</td>
<td>32.61 ± 3.2</td>
<td>3.26X10⁻³</td>
<td>0.9 ± 0.4</td>
<td>13.53</td>
<td>1</td>
</tr>
<tr>
<td>N2</td>
<td>70:30</td>
<td>29.88 ± 0.3</td>
<td>2.99X10⁻³</td>
<td>1.1 ± 0.1</td>
<td>11.99</td>
<td>0.9</td>
</tr>
<tr>
<td>N3</td>
<td>50:50</td>
<td>37.66 ± 1.4*</td>
<td>3.77X10⁻³</td>
<td>2.2 ± 0.4*</td>
<td>14.26</td>
<td>1.2</td>
</tr>
<tr>
<td>N4</td>
<td>30:70</td>
<td>98.88 ± 3.1*</td>
<td>9.89X10⁻³</td>
<td>4.6 ± 0.3*</td>
<td>34.03</td>
<td>3.0</td>
</tr>
<tr>
<td>N5 (Control)</td>
<td>PG: IPB 0:100</td>
<td>15.33 ± 0.4</td>
<td>1.53X10⁻³</td>
<td>7.7 ± 0.2</td>
<td>4.42</td>
<td>1</td>
</tr>
<tr>
<td>N6</td>
<td>70:30</td>
<td>20.54 ± 0.4*</td>
<td>2.05X10⁻³</td>
<td>6.8 ± 0.4*</td>
<td>6.29</td>
<td>1.3</td>
</tr>
<tr>
<td>N7</td>
<td>50:50</td>
<td>41.35 ± 6.5*</td>
<td>4.14X10⁻³</td>
<td>3.9 ± 0.2*</td>
<td>14.64</td>
<td>2.7</td>
</tr>
<tr>
<td>N8</td>
<td>30:70</td>
<td>60.78 ± 1.2*</td>
<td>6.08X10⁻³</td>
<td>3.2 ± 0.9*</td>
<td>22.50</td>
<td>4.0</td>
</tr>
<tr>
<td>N9</td>
<td>IPA: IPB 0:100</td>
<td>15.3 ± 0.4</td>
<td>1.53X10⁻³</td>
<td>7.7 ± 0.2</td>
<td>4.42</td>
<td>1</td>
</tr>
<tr>
<td>N10</td>
<td>100:0</td>
<td>11.92 ± 0.6</td>
<td>1.19X10⁻³</td>
<td>1.6 ± 0.5*</td>
<td>4.97</td>
<td>0.8</td>
</tr>
<tr>
<td>N11</td>
<td>70:30</td>
<td>8.64 ± 1.4</td>
<td>8.64X10⁻³</td>
<td>1.4 ± 0.4*</td>
<td>3.33</td>
<td>0.6</td>
</tr>
<tr>
<td>N12</td>
<td>50:50</td>
<td>29.10 ± 1.5*</td>
<td>2.91X10⁻³</td>
<td>1.3 ± 0.2*</td>
<td>11.74</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>30:70</td>
<td>11.11 ± 0.7</td>
<td>1.97X10⁻³</td>
<td>2.5 ± 0.4*</td>
<td>4.34</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Two tailed student t’ test was used to determine the significant difference in flux and lag time of flurbiprofen between control and treatment groups. * P< 0.05.
The skin flux increased as the amount of IPA increased from 30 to 70% in PG: IPA binary solvent system. A significant \( p < 0.05 \), t test) drop in the skin flux was observed as concentration of IPA was increased to 100%. The only known plasticizer for the keratin's present in the skin is water (Moellgaard and Hoelgaard, 1983). Absolute IPA dehydrates stratum corneum thereby causing a significant drop in flux (table 17).

The lag time of flurbiprofen in the PG/ IPA binary combination ranged from 0.9 to 4.6 hours. The lag time of formulation N4 was significantly higher than the control formulation N1. IPA enhanced the penetration of flurbiprofen in formulation N4 (E pen) by 3 times (table 17).

The flux of flurbiprofen increased significantly \( p < 0.05 \), t test) as the amount of PG increased from 0 -30 % with penetration enhancement of 4. Further increase in concentration of PG caused decrease in the flux. The lag time of flurbiprofen in the PG: IPB binary solvent mixture ranged from 0.9 -7.7 hours. The lag time of formulation N8 was significantly less than that in the purified IPB (T lag of 7.7 hrs).
PG has been shown to enhance sorption by its cosolvency effect and by carrier mechanism (Hoelgaard and Moelgaard, 1985). At higher concentration, PG extracts water from the lipid bilayer of stratum corneum and thereby leading to an increase in the barrier property of stratum corneum (Panchagnula et al., 2001; Goates and Knutson, 1994).

The increase in the penetration enhancement of 1.9 times was obtained when amount of IPA was increased from 0 to 50% in IPA: IPB solvent mixture (table 17). However a sharp decrease in the skin flux was observed when concentration of IPA was further increased. Also a significant decrease (p<0.05, t test) in the lag time was
observed with the addition of IPA in IPA: IPB binary solvent mixture. The increase in flux at lower concentration of IPA in IPA: IPB solvent mixture may be due to the fact that at lower concentration only lipoidal pathway gets affected. Flurbiprofen being a lipophilic compound, its flux increases at lower concentration of IPA.

![Graph showing the effect of IPA: IPB solvent mixture on the in vitro permeation of flurbiprofen through rat skin.](image)

**Fig. 20:** The effect of IPA: IPB solvent mixture on the in vitro permeation of flurbiprofen through rat skin

The flux of formulations N4 (PG: IPA; 30:70%v/v), N8 (PG: IPB; 30:70%v/v) and N11 (IPA: IPB; 50:50%v/v) was 98.88 μg/cm²/hour, 60.78μg/cm²/hour and 29.07 μg/cm²/hour respectively. The percentage of flurbiprofen permeated across the rat skin from the formulations N4, N8 and N11 was 34.03%, 22.50% and 11.74 % respectively.
RESULTS AND DISCUSSION

Among the three binary solvent mixtures, PG: IPA (30:70) showed the highest permeation rate of 98.88 μg/cm²/hour. The flux and lag time of formulations N4, N8, and N11 were compared by students Newman Keuls Multiple comparison test as shown in table 18.

Table 18: Results of students Newman Keuls multiple comparison test

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Flux</th>
<th>Lag time</th>
</tr>
</thead>
<tbody>
<tr>
<td>N4 versus N11</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>N8 versus N4</td>
<td>P&lt;0.001</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>N11 versus N8</td>
<td>P&lt;0.001</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

It was found that the flux of formulation N4 was significantly higher (p<0.05) than N8 and N11 formulations. Thus N4 formulations were selected for further studies.

5.2 PERMEATION OF FLURBIPROFEN FROM THE PG: IPA (30:70%V/V) SOLVENT MIXTURE CONTAINING DIFFERENT PENETRATION ENHANCERS

The transdermal permeation rate of flurbiprofen across the rat abdominal skin from formulation N4 was 98.88 μg/cm²/hour. The steady state plasma concentration obtained with this permeation rate will be approximately 0.71 μg/ml. The required steady state plasma concentration was 3-5 μg/ml. To increase the permeation flux of flurbiprofen, so that required steady state plasma concentration could be achieved, penetration enhancers were incorporated into formulations.
Terpenes are receiving much attention as penetration enhancers (Williams and Barry, 1991). Terpenes are derived from plant essential oils and combine good penetration enhancing abilities with low skin irritancy and low systemic toxicity. A number of terpenes viz. menthol, 1-8- cineol, d- limonene, nerolidol, geraniol, thymol, cyminine, carvone, acetylterpineol, α- bisabolol have been shown to increase transdermal permeation rate of hydrophilic and lipophilic drugs (Cornwell and Barry, 1991; Gao and Singh, 1998). Present study investigated the penetration enhancing effect of lemon oil and turpentine oil on the transdermal permeation rate of flurbiprofen through rat skin. Lemon oil and turpentine oil has been shown to enhance the permeation rate of drugs and are free from toxic effects (Yamane et al., 1995).

Penetration enhancement effect of NA-102, a fixed oil, on the transdermal permeability flux of flurbiprofen through rat skin was also investigated.

The cumulative amount of flurbiprofen permeated across the rat skin from PG: IPA (30:70%v/v) solvent mixture containing different penetration enhancers is shown in table 19.
## RESULTS AND DISCUSSION

Table 19: Mean* in-vitro permeation of flurbiprofen through rat skin from PG: IPA (30:70%v/v) containing different concentration of permeation enhancers

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Mean* (±S.D) cumulative amount of flurbiprofen permeated (μg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
</tr>
<tr>
<td>0.5</td>
<td>8.44±3.4</td>
</tr>
<tr>
<td>1</td>
<td>24.15±4.6</td>
</tr>
<tr>
<td>2</td>
<td>76.16±11.9</td>
</tr>
<tr>
<td>3</td>
<td>116.03±8.2</td>
</tr>
<tr>
<td>4</td>
<td>230.25±49.9</td>
</tr>
<tr>
<td>5</td>
<td>340.86±11.2</td>
</tr>
<tr>
<td>6</td>
<td>479.86±5.4</td>
</tr>
<tr>
<td>7</td>
<td>513.09±6.6</td>
</tr>
<tr>
<td>8</td>
<td>641.83±19.2</td>
</tr>
<tr>
<td>9</td>
<td>688.98±12.9</td>
</tr>
<tr>
<td>10</td>
<td>801.99±6.3</td>
</tr>
<tr>
<td>12</td>
<td>1096.93±5.3</td>
</tr>
<tr>
<td>16</td>
<td>1709.16±9.2</td>
</tr>
<tr>
<td>20</td>
<td>2300.23±13.2</td>
</tr>
<tr>
<td>24</td>
<td>3026.68±15.2</td>
</tr>
</tbody>
</table>

* Results are the mean of three experiments
As shown in the figure 21 and table 20, the skin flux of flurbiprofen increased significantly (p<0.001) as the amount of NA-102 was increased to 5%. No increase in flux could be observed, as the concentration of NA-102 was increased further (p>0.05).

The maximum transdermal permeation rate of 198.88 µg/cm²/hour was obtained with 5% NA-102 in PG: IPA (30:70%v/v) solvent mixture. The lag time of flurbiprofen ranged from 4.4 to 2.0 hours. The magnitude of the flux enhancement factor in the presence of 5% NA-102 was 2.01 times higher than that of PG: IPA (30:70) binary solvent mixture. This implies that NA-102 had the ability to modify the barrier properties of stratum corneum, leading to an increase in drug diffusion. The lag time at 5% concentration of NA-102 was significantly shorter (p<0.001) than the PG: IPA (30:70%v/v) solvent mixture.
### RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Penetration enhancer</th>
<th>Mean (±SD) Flux (µg/cm²/hour)</th>
<th>Permeability coefficient (cm/hour)</th>
<th>Mean (±SD) Lag time (hours)</th>
<th>Cumulative percentage of drug permeated</th>
<th>E&lt;sub&gt;pen&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>N4</td>
<td>-</td>
<td>98.88 ± 3.1</td>
<td>9.89 x 10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>4.6 ± 0.3</td>
<td>34.03</td>
<td>1.0</td>
</tr>
<tr>
<td>P-1</td>
<td>NA-102</td>
<td>152.27 ± 4.2</td>
<td>0.15 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4.9 ± 0.4</td>
<td>53.63</td>
<td>1.5</td>
</tr>
<tr>
<td>P-2</td>
<td>NA-102</td>
<td>155.93 ± 19.9</td>
<td>0.16 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>2.3 ± 0.2</td>
<td>59.01</td>
<td>1.6</td>
</tr>
<tr>
<td>P-3</td>
<td>NA-102</td>
<td>198.92 ± 3.6</td>
<td>0.20 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>2.0 ± 0.6</td>
<td>75.31</td>
<td>2.0</td>
</tr>
<tr>
<td>P-4</td>
<td>NA-102</td>
<td>187.92 ± 24.4</td>
<td>0.19 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>4.4 ± 0.5</td>
<td>74.90</td>
<td>1.9</td>
</tr>
<tr>
<td>P-5</td>
<td>Turpentine oil</td>
<td>210.69 ± 7.9</td>
<td>0.21 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>2.2 ± 0.7</td>
<td>79.94</td>
<td>2.1</td>
</tr>
<tr>
<td>P-6</td>
<td>Turpentine oil</td>
<td>229.15 ± 3.4</td>
<td>0.23 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>3.4 ± 0.3</td>
<td>84.27</td>
<td>2.3</td>
</tr>
<tr>
<td>P-7</td>
<td>Turpentine oil</td>
<td>241.37 ± 14.3</td>
<td>0.24 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.9 ± 1.1</td>
<td>93.99</td>
<td>2.4</td>
</tr>
<tr>
<td>P-8</td>
<td>Turpentine oil</td>
<td>234.71 ± 6.0</td>
<td>0.23 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.5 ± 0.2</td>
<td>94.22</td>
<td>2.4</td>
</tr>
<tr>
<td>P-9</td>
<td>Lemon oil</td>
<td>156.03 ± 7.4</td>
<td>0.16 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.6 ± 0.4</td>
<td>61.34</td>
<td>1.6</td>
</tr>
<tr>
<td>P-10</td>
<td>Lemon oil</td>
<td>210.23 ± 6.5</td>
<td>0.21 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.5 ± 0.4</td>
<td>84.10</td>
<td>2.1</td>
</tr>
<tr>
<td>P-11</td>
<td>Lemon oil</td>
<td>210.77 ± 7.8</td>
<td>0.21 x 10&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>1.1 ± 0.3</td>
<td>84.74</td>
<td>2.1</td>
</tr>
</tbody>
</table>
The cumulative amount of flurbiprofen permeated over 24 hours was found to be 4249.98μg/cm² from the formulation containing 5% NA-102 in PG: IPA (30:70%v/v) solvent mixture. 75.31% of the drug was found to permeate across the rat skin at the end of 24 hours from the formulation P3 containing 5% NA-102 in PG: IPA (30:70%v/v).

As shown in figure 22 and table 20, a synergistic effect on the skin permeation rate of flurbiprofen was observed by adding turpentine oil to the co-solvent system (PG: IPA; 30:70%v/v). The magnitude of the flux enhancement factor with turpentine oil was 2.4 at 5%v concentration. There was no significant (p>0.05, students Newman Keuls test) change in the flux and lag time at concentrations higher than 5%. The lag time at 5% concentration of turpentine oil was 1.9 hrs, significantly (P<0.001, students Newman Keuls test) shorter than the binary solvent vehicle.

![Graph showing effect of turpentine oil](image_url)

**Fig. 22:** Effect of turpentine oil in PG: IPA (30:70%v/v) solvent mixture on the *in vitro* permeation of flurbiprofen through rat skin.
The cumulative amount of flurbiprofen permeated over 24 hours was found to increase from 4511.06 \mu g/cm^2 to 5303.97 \mu g/cm^2 as the concentration of turpentine oil was increased from 1 to 5% with corresponding increase in the permeability coefficient from 0.021 cm/hour to 0.024 cm/hour. Almost 93.99% of the drug was found to permeate across the rat skin over 24 hours from formulations with 5% turpentine oil in PG: IPA (30:70%v/v).

The effect of different concentration of penetration enhancer Lemon oil on the penetration of flurbiprofen from PG: IPA (30:70%v/v) solvent mixture across rat skin was investigated.

![Fig. 23: Effect of lemon oil in PG: IPA (30:70%v/v) solvent mixture on the invitro permeation of flurbiprofen through rat skin](image-url)
As shown in figure 23 the flux of flurbiprofen increased significantly (p<0.001, Students Newman Keuls test) with the addition of lemon oil. The maximum increase in flux was obtained with 3% lemon oil. The maximum transdermal permeability flux of flurbiprofen was 210.33 µg/cm²/hour from the formulation P10 containing 3% lemon oil. The cumulative amount of flurbiprofen permeated over 24 hours was found to increase from 3461.51 µg/cm²/hour to 4745.82 µg/cm²/hour from the formulations containing 1% and 3% lemon oil respectively and the corresponding permeability coefficient values were 0.016 cm/hour and 0.021 cm/hour. With the incorporation of 5% lemon oil there was insignificant (p>0.05, Students Newman Keuls test) increase in the flux.

The increase in the penetration enhancement of 2.1 times was obtained at 3% concentration of lemon oil. A significant drop (p<0.001, students Newman Keuls test) in the lag time was observed with the addition of lemon oil.

Co application of terpenes in PG/water solvent system has been shown to increase drug flux significantly (Yamane et al., 1995; Kurihara-Bergstom et al., 1990).

High diffusion and increased lipid disruption in the stratum corneum might have caused the increase in the flux.

Among the three different penetration enhancers, turpentine oil was the most effective, increasing the flux from 98.88 µg/cm²/hr to 241.37 µg/cm²/hr at a concentration of 5% with an E_p of 2.4. The three optimized formulations (P-3, P-7 & P-10) decreased the lag time significantly as compared to the solvent mixture PG: IPA (30:70%v/v).
The order of the enhancement effect was 5% turpentine oil, 3% lemon oil and 5% NA-102 (Table 20 & Figures 24 and 25).

There was a significant difference in flux of the formulations P-7 & P-3 (p<0.001, Students Newman Keuls test) and P-7 & P-10 (p<0.01, Students Newman Keuls test). However there was no significant difference in the flux of formulations P-10 & P-3 (p>0.05, Students Newman Keuls test) No significant change in lag time of the three optimized formulations could be observed. Based on the above results, formulation P-3, P-7 & P-10 were selected for further studies.
RESULTS AND DISCUSSION

5.3 EFFECT OF THICKENING (GELLING) AGENT

The transdermal flux of flurbiprofen across the rat skin from formulations P-3, P-7 and P-10 was 198.88 \( \mu \text{g}/\text{cm}^2/\text{hour} \), 241.37 \( \mu \text{g}/\text{cm}^2/\text{hour} \) and 210.23 \( \mu \text{g}/\text{cm}^2/\text{hour} \) respectively.

The steady state plasma drug concentration corresponding to this flux was 1.42 \( \mu \text{g}/\text{ml} \), 1.72\( \mu \text{g}/\text{ml} \) and 1.50 \( \mu \text{g}/\text{ml} \) respectively. To increase the steady state plasma concentration of the drug, the permeation rate of the drug needed further enhancement. In an ideal system, there is a linear relationship between the rate of diffusion and the concentration of diffusant. The maximum flux occurs when the

Fig. 25: Comparison of the invitro flux of flurbiprofen through rat skin
concentration reaches the solubility limit (Hadgraft, 1996). Thus 100 mg of the drug was incorporated into the transdermal drug delivery system intended to release drug for 24 hours with an objective of achieving steady state blood plasma levels comparable to oral therapy with reduced number of application and improved bioavailability. Hydroxypropyl methylcellulose (HPMC, Methocel) was selected as gelling agent for the ease of fabrication of the patch. HPMC has been used extensively as viscosity building agent in the design of reservoir systems for testosterone (Kim et al., 2001) and hydrocortisone (El-Kattan et al., 2000). Being less hydrophilic than methylcellulose, HPMC could be easily solubilized in PG: IPA (30:70%v/v) solvent mixture. HPMC gel formulations with 1% w/w of HPMC were too thin whereas HPMC gel formulations with 3%w/w HPMC were too thick. 2% w/w HPMC gel formulations were found to possess optimum consistency and were easy to handle during the fabrication of the patch.
Table 21: Mean* invitro permeation profile of flurbiprofen through rat skin from PG: IPA (3:70%v/v) solvent system containing penetration enhancers with varying quantity of HPMC

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Mean* (±S.D) cumulative amount of flurbiprofen permeated (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>266.8±1.7</td>
</tr>
<tr>
<td>2</td>
<td>525.5±1.6</td>
</tr>
<tr>
<td>4</td>
<td>912.9±1.1</td>
</tr>
<tr>
<td>6</td>
<td>1474.9±0.6</td>
</tr>
<tr>
<td>8</td>
<td>1951.7±1.7</td>
</tr>
<tr>
<td>12</td>
<td>2868.2±7.1</td>
</tr>
<tr>
<td>16</td>
<td>4260.0±6.7</td>
</tr>
<tr>
<td>20</td>
<td>5766.6±6.3</td>
</tr>
<tr>
<td>24</td>
<td>7525.1±1.1</td>
</tr>
</tbody>
</table>

*Results are the mean of three experiments
Fig. 26: Effect of HPMC in PG: IPA (30:70% v/v) solvent mixture containing 5% NA-102 on the invitro permeation of flurbiprofen through rat skin.

The cumulative amount of flurbiprofen permeated across the rat skin from formulations A-1, A-2 and A-3 over the period of 24 hours was 7525.1 µg/cm², 6717.8 µg/cm² and 5570.2 µg/cm² respectively containing 1% w/w, 2% w/w and 3% w/w of HPMC as shown in table 21 and figure 26. The transdermal permeation rate of flurbiprofen was 362.3 µg/cm²/hour, 389.2 µg/cm²/hour and 329.7 µg/cm²/hour from the formulations A-1, A-2 and A-3 respectively (table 22). There was a significant drop in flux in formulations A-3 containing 3% w/w of HPMC as compared to formulation A-2 (table 21).
**RESULTS AND DISCUSSION**

Table 22: Mean permeation parameters of flurbiprofen in PG: IPA (30:70% v/v) containing different penetration enhancers and varying quantity of HPMC

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean (± SD) Flux (μg/cm²/hour)</th>
<th>Mean (± SD) Lag time (hours)</th>
<th>Cumulative percentage of drug permeated</th>
<th>Results of Students Newman Keuls multiple comparison test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Formulation code</td>
</tr>
<tr>
<td>A-1</td>
<td>362.3±0.6</td>
<td>4.3±0.5</td>
<td>66.67</td>
<td>A-3</td>
</tr>
<tr>
<td>A-2</td>
<td>389.2±0.1</td>
<td>6.3±0.3</td>
<td>59.52</td>
<td>A-2</td>
</tr>
<tr>
<td>A-3</td>
<td>329.7±1.2</td>
<td>7.2±0.6</td>
<td>49.35</td>
<td></td>
</tr>
<tr>
<td>B-1</td>
<td>513.6±2.1</td>
<td>3.7±0.1</td>
<td>92.52</td>
<td>B-3</td>
</tr>
<tr>
<td>B-2</td>
<td>399.2±1.0</td>
<td>3.3±0.6</td>
<td>88.61</td>
<td>B-2</td>
</tr>
<tr>
<td>B-3</td>
<td>492.6±3.6</td>
<td>5.8±0.2</td>
<td>78.31</td>
<td></td>
</tr>
<tr>
<td>C-1</td>
<td>373.4±2.3</td>
<td>2.2±0.1</td>
<td>72.31</td>
<td>C-3</td>
</tr>
<tr>
<td>C-2</td>
<td>409.9±0.0</td>
<td>3.5±0.2</td>
<td>71.16</td>
<td>C-2</td>
</tr>
<tr>
<td>C-3</td>
<td>378.3±2.8</td>
<td>5.4±0.1</td>
<td>60.96</td>
<td></td>
</tr>
</tbody>
</table>

There was no significant difference in flux (p>0.05 students Newman Keuls test) of the flurbiprofen at 2% and 3% w/w HPMC concentration in formulations B-2 & B-3 and C-2 & C-3 (Table 22). Lag time was increased significantly (P<0.01, students Newman Keuls test) at 3% w/w concentration as shown in figure 27 and figure 28. However there was a considerable difference in the cumulative percentage of drug permeated at the two concentration levels of HPMC.
Fig. 27: Effect of HPMC in PG: IPA (30:70%v/v) solvent mixture containing 5% turpentine oil on the invitro permeation of flurbiprofen through rat skin

At the end of 24 hrs 92.52%, 88.61 and 78.31% of the initial drug loading was found to permeate from the formulations B-1, B-2 and B-3, containing HPMC in 1%, 2%, 3% concentration respectively.

Similarly, 72.3%, 71.2%, and 60.9% of the initial drug loading was found to permeate from the formulations C-1, C-2 and C-3, containing 1%, 2% and 3% w/w HPMC. The cumulative amount of drug permeated at the end of 24 hours was 8161.1 µg/cm², 8031.1 µg/cm² and 6879.9 µg/cm² from formulations C-1, C-2 and C-3 respectively.
Fig. 28: Effect of HPMC in PG: IPA (30:70%v/v) solvent mixture containing 3% lemon oil on the invitro permeation of Flurbiprofen through rat skin

Formulations containing 2 %w/w HPMC were easy to handle during the fabrication of the patch and thus further studies were carried out using 2%w/w of HPMC in the reservoir solution.
5.4 IN VITRO SKIN PERMEATION OF FLURBIPROFEN PATCH FORMULATIONS THROUGH RAT SKIN

In order to design a liquid reservoir transdermal patch, the effect of microporous membrane on the invitro permeation rate was evaluated.

Three formulations A-2, B-2 and C-2 containing 5% NA-102, 5% turpentine oil and 3% lemon oil in the PG: IPA (30:70%v/v) with 2% w/w HPMC were encapsulated in a shallow compartment moulded from a drug impermeable polyester backing film laminate and EVA rate controlling membrane. The fabricated patch formulations were subjected to invitro permeation studies across the rat skin to evaluate the effect of EVA membrane on the transdermal permeation rate of flurbiprofen. The cumulative amount of flurbiprofen permeated from patch formulations across the rat skin is shown in table 23.
Table 23: Mean* in-vitro permeation profile of flurbiprofen transdermal patch formulations through rat skin

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Mean* (±S.D) cumulative amount of flurbiprofen permeated (μg/cm²)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order rate constant (K₀) moles litre⁻¹ hour⁻¹</td>
<td>First order rate constant (K₁) hour⁻¹</td>
<td>Zero order rate constant (K₀) moles litre⁻¹ hour⁻¹</td>
<td>First order rate constant (K₁) hour⁻¹</td>
</tr>
<tr>
<td>0.5</td>
<td>42.9±27.9</td>
<td>-</td>
<td>1.6480</td>
<td>0.0166</td>
</tr>
<tr>
<td>1</td>
<td>104.3±6.4</td>
<td>101.1±19.3</td>
<td>103.4±25.5</td>
<td>0.9241</td>
</tr>
<tr>
<td>2</td>
<td>214.0±9.4</td>
<td>306.5±7.5</td>
<td>253.7±11.7</td>
<td>0.9480</td>
</tr>
<tr>
<td>4</td>
<td>404.5±5.4</td>
<td>904.7±9.2</td>
<td>647.0±42.5</td>
<td>0.8960</td>
</tr>
<tr>
<td>6</td>
<td>702.9±5.6</td>
<td>1406.3±3.9</td>
<td>1057.0±12.5</td>
<td>1.0380</td>
</tr>
<tr>
<td>8</td>
<td>1405.4±3.9</td>
<td>1841.5±9.6</td>
<td>1747.6±21.2</td>
<td>1.5565</td>
</tr>
<tr>
<td>12</td>
<td>2628.0±2.6</td>
<td>3842.4±14.6</td>
<td>3217.2±11.1</td>
<td>1.9403</td>
</tr>
<tr>
<td>16</td>
<td>3943.1±3.9</td>
<td>5014.0±1.8</td>
<td>4604.5±11.2</td>
<td>2.1835</td>
</tr>
<tr>
<td>20</td>
<td>5105.6±7.4</td>
<td>7703.8±6.7</td>
<td>6325.1±17.2</td>
<td>2.2618</td>
</tr>
<tr>
<td>24</td>
<td>6511.1±6.2</td>
<td>9506.1±6.5</td>
<td>7612.2±10.3</td>
<td>2.4037</td>
</tr>
<tr>
<td>Mean</td>
<td>1.5724</td>
<td>0.0189</td>
<td>2.2557</td>
<td>0.0311</td>
</tr>
<tr>
<td>SD±</td>
<td>0.64</td>
<td>0.01</td>
<td>0.86</td>
<td>0.02</td>
</tr>
<tr>
<td>CV%</td>
<td>40.40</td>
<td>53.97</td>
<td>38.21</td>
<td>68.49</td>
</tr>
</tbody>
</table>

*Results are the mean of three experiments
The diffusion flux of flurbiprofen across EVA membrane / skin composite was found to be 323.6 µg/cm²/hour, 523.2 µg/cm²/hour and 353.2 µg/cm²/hour from formulations T-1, T-2 and T-3 respectively (table 24 and figure 29).

Fig. 29: Cumulative amount of flurbiprofen Permeated from patch formulations (across EVA membrane/rat skin composite)

The cumulative percentage of drug permeated from patch formulations T-1, T-2 and T-3 was found to be 57.69%, 84.22% and 67.44% respectively (fig.30).
RESULTS AND DISCUSSION

Fig. 30: Invitro skin permeation profile of flurbiprofen patch formulations
(across EVA membrane/rat skin composite)

Table 24: Mean permeation parameters of flurbiprofen transdermal patch formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean (±SD) Flux ($\mu g/cm^2$)</th>
<th>Mean (±SD) Lag time (hours)</th>
<th>Cumulative percentage of drug permeated</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1</td>
<td>323.6 ±6.4*</td>
<td>4.1 ±0.3*</td>
<td>57.69</td>
</tr>
<tr>
<td>T-2</td>
<td>523.1±8.7</td>
<td>4.3±0.6</td>
<td>84.22</td>
</tr>
<tr>
<td>T-3</td>
<td>353.2±2.5*</td>
<td>3.3±0.2</td>
<td>67.44</td>
</tr>
</tbody>
</table>

(* P<0.05, students Newman Keuls multiple comparison test)
The flux and lag time of the flurbiprofen was not significantly altered in patch formulation T-2 (p>0.05, students Newman Keuls test) as compared to viscous formulation B-2 (fig. 31 and table 23). These results suggest that the patch formulations did not modulate the skin permeation rate through rat skin. The flux of the flurbiprofen was significantly altered in patch formulations T-1 and T-3 (p<0.05, students t' test) as compared to viscous formulations A-2 and C-2 respectively (fig. 31). These results suggest that the patch formulations modulated the skin permeation rate through rat skin.

Fig. 31: Effect of EVA membrane on the invitro permeation of flurbiprofen through rat skin
The decrease in permeability rate might have occurred due to the low vinyl acetate content of the EVA membrane which may cause decreased water vapour permeability (Kim et al., 2001). However there was no significant effect of EVA membrane on the transdermal permeation rate of flurbiprofen across the rat skin. This might be due to the higher enhancing potential of turpentine oil. On storage significant portion of the drug migrates from the reservoir system to the membrane.

In vitro permeation data were fitted to different equations and kinetic models to explain the release kinetics of flurbiprofen from patch formulations (fig. 30 & 32 and table 25).

Table 25: Kinetic models to explain the release kinetics of flurbiprofen

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order</th>
<th></th>
<th>First order</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$r^2$</td>
<td>$r$</td>
<td>$r^2$</td>
</tr>
<tr>
<td>T-1</td>
<td>0.9932</td>
<td>0.9864</td>
<td>0.9797</td>
<td>0.9598</td>
</tr>
<tr>
<td>T-2</td>
<td>0.9894</td>
<td>0.9790</td>
<td>0.9391</td>
<td>0.8820</td>
</tr>
<tr>
<td>T-3</td>
<td>0.9944</td>
<td>0.9889</td>
<td>0.9754</td>
<td>0.9514</td>
</tr>
</tbody>
</table>

The best fit with the highest coefficient of correlation ($r = 0.9932$, 0.9894 and 0.9944 for T-1, T-2 and T-3 respectively) and coefficient of determination ($r^2 = 0.9864$, 0.9790 and 0.9889 for T-1, T-2 and T-3 respectively) was shown by zero order drug release. The facts were further confirmed by the coefficient of variation, which were lesser for zero order rate constant.
RESULTS AND DISCUSSION

1 %

5.5 STABILITY STUDIES

Stability studies were carried out according to ICH guidelines (Singh, 1999) to establish the structural integrity of the reservoir formulation. The HPLC chromatograms showed neither any additional or interfering peaks nor any change in the retention time of flurbiprofen indicating that the drug is stable in the formulations. The studies revealed no significant changes in the physical appearance of the reservoir formulations.

Fig. 32: Log percent of drug remaining versus time plot for optimized formulations
Table 26: Mean* drug content of flurbiprofen patch formulations stored at 40 ± 0.5 °C / 75 ± 5% RH

<table>
<thead>
<tr>
<th>Patch formulation</th>
<th>Time (days)</th>
<th>Mean*(± SD) amount of drug present (mg)</th>
<th>% of drug remaining</th>
<th>Log % drug remaining</th>
<th>Degradation rate constant K (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>99.9</td>
<td>99.94 ± 3.3</td>
<td>1.9997</td>
<td>2.15 x 10⁻⁴</td>
</tr>
<tr>
<td>T1</td>
<td>15</td>
<td>99.5</td>
<td>99.53 ± 5.7</td>
<td>1.9980</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.2</td>
<td>99.20 ± 3.6</td>
<td>1.9965</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>98.9</td>
<td>98.95 ± 4.5</td>
<td>1.9954</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>98.7</td>
<td>98.70 ± 11.2</td>
<td>1.9943</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>98.1</td>
<td>98.06 ± 13.3</td>
<td>1.9915</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>0</td>
<td>99.7</td>
<td>99.72 ± 2.0</td>
<td>1.9988</td>
<td>2.38 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>99.4</td>
<td>99.39 ± 1.3</td>
<td>1.9973</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.0</td>
<td>99.01 ± 16.7</td>
<td>1.9957</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>98.9</td>
<td>98.87 ± 12.9</td>
<td>1.9951</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>98.3</td>
<td>98.29 ± 21.2</td>
<td>1.9894</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>97.6</td>
<td>98.84 ± 7.6</td>
<td>1.9884</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>0</td>
<td>99.9</td>
<td>99.93 ± 5.4</td>
<td>1.9997</td>
<td>1.57 x 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>99.6</td>
<td>99.62 ± 3.4</td>
<td>1.9983</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>99.4</td>
<td>99.40 ± 6.8</td>
<td>1.9974</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>98.9</td>
<td>98.97 ± 6.0</td>
<td>1.9955</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>98.8</td>
<td>98.76 ± 4.0</td>
<td>1.9946</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>98.5</td>
<td>98.47 ± 14.5</td>
<td>1.9933</td>
<td></td>
</tr>
</tbody>
</table>

* Results are the mean of three observations.
The degradation constant of transdermal patch formulations T-1, T-2 and T-3 was found to be $2.15 \times 10^{-4}$ day$^{-1}$, $2.38 \times 10^{-4}$ day$^{-1}$ and $1.57 \times 10^{-4}$ day$^{-1}$ respectively (table 26 and figure 33). Since there was no significant change in drug content (less than 5%), a tentative shelf life of 24 months may be given to the formulations. The percentage mean recovery was found to be 99.91 ± 0.86, 99.50 ± 0.93 and 99.90 ± 0.08 respectively from patch formulations T-1, T-2 and T-3 as shown in table number 11.

![Figure 33: Log percent of drug remaining versus time plot for optimized formulation kept at 40°C/75%RH](image)

Fig. 33: Log percent of drug remaining versus time plot for optimized formulation kept at 40°C/75%RH
5.6 INTERACTION STUDIES

In order to further ascertain any interaction between drug and the polymer or other additive, IR and UV spectral analysis of flurbiprofen was carried out.

Fig. 34: I.R. spectra of (a) placebo (b) transdermal patch T-1
Fig. 35: I.R. spectra of (a) placebo (b) transdermal patch T-2
Fig. 36: I.R. spectra of (a) placebo (b) transdermal patch T-3
Fig. 37: U.V. absorption spectra of (a) transdermal patch T-1 (b) placebo

Fig. 38: U.V. absorption spectra of (a) transdermal patch T-2 (b) placebo
As shown in figures 34 to 39, IR and UV spectra of the optimized formulations T-1, T-2 and T-3 showed similar absorption spectra to that of the pure drug. These peaks were absent in case of placebo formulation. It can be concluded that there was no interaction between the drug and the polymer or other additives.
5.7 HISTOLOGICAL STUDIES

A comparative study of penetration enhancing capacities of 5% turpenine oil, 3% lemon oil and 5% NA-102 in PG: IPA (30:70% v/v) with that of PG: IPA (30:70% v/v) solvent mixture for flurbiprofen on rat skin was performed and further compared with the untreated control group. The influence of penetration enhancer and solvents on the anatomical structure of the skin is discussed with the aid of light microscopic findings.

Histological findings on skin biopsies from albino rats taken after treatment with skin penetrance enhancers are shown in figures 40 to 44.

Control sections show normal rat skin with uniformly layered stratum corneum, a 2-cell thick epidermis and loosely textured collagen in dermis (fig. 40).

Fig.40: Histological findings on normal skin biopsies from albino rats
RESULTS AND DISCUSSION

PG: IPA (30: 70 %v/v) treated skin section shows clear disruption of stratum corneum organization, mild increase in epidermal thickness and a dermis within normal limits. (Figs 41 a &b)

Fig.41: Histological findings on skin biopsies from albino rats taken after treatment with PG: IPA (30: 70 %v/v)
Skin treated with 5% NA-102 in PG: IPA (30:70%v/v) shows extensive disruption of stratum corneum with condensation of the normally stratified corneal layers and a definite increase in epidermal thickness from normal 2-3 cells to 4-5 cells. Dermis does not show significant change. (Figure 42 a&b)

Fig.42: Histological findings on skin biopsies from albino rats taken after treatment with 5% NA-102 in PG: IPA (30:70%v/v)
RESULTS AND DISCUSSION

Skin treated with 5% turpentine oil in PG: IPA (30:70%v/v) shows disruption of normal stratification in stratum corneum, epidermis is not thickened. Dermis has a mildly increased number of inflammatory cells. (Figure 43 a & b)

Fig.43: Histological findings on skin biopsies from albino rats taken after treatment with 5% turpentine oil in PG: IPA (30:70%v/v)
Skin treated with 3% lemon oil in PG: IPA (30:70%v/v) shows stratum corneum reduced and normal stratification lost. Epidermis is irregularly thickened. Dermal inflammatory cells slightly increased. (Figure 44 a & b)

Fig.44: Histological findings on skin biopsies from albino rats taken after treatment with 3% lemon oil in PG: IPA (30:70%v/v)
It can be concluded, within the experimental conditions of this study, that 5% turpentine-oil, 5% NA-102 and 3% lemon oil showed superior absorption enhancing properties on rat skin as compared to solvent treated and normal control groups, with negligible skin irritation, and hence they were found to be well tolerated skin penetration enhancers.

5.8 PHARMACOKINETIC STUDIES

The plasma concentration of flurbiprofen in albino rats following oral administration and transdermal application of flurbiprofen formulations is shown in table 27.

Table 27: Mean* (±SD) plasma concentration of flurbiprofen in albino rats following oral administration of flurbiprofen and application of transdermal patch formulations

<table>
<thead>
<tr>
<th>Times (hours)</th>
<th>Mean* (± S.D) plasma concentration of flurbiprofen (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oral administration</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>9.40±6.3</td>
</tr>
<tr>
<td>1</td>
<td>26.26±3.5</td>
</tr>
<tr>
<td>2</td>
<td>20.55±8.3</td>
</tr>
<tr>
<td>4</td>
<td>9.84±6.3</td>
</tr>
<tr>
<td>6</td>
<td>3.63±5.2</td>
</tr>
<tr>
<td>8</td>
<td>0.60±4.7</td>
</tr>
<tr>
<td>12</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>5.11±0.1</td>
</tr>
</tbody>
</table>

*Results are mean of 4 observations
Transdermal patches of 9 cm² area, containing 1 mg of the drug were applied on the dorsal side of the rats. Figure 45 shows the plasma flurbiprofen concentration versus time profiles following the application of the patch formulation. Patch formulation containing no enhancer acted as the control.

The time to reach maximum was 8 hours for all the formulations. The $C_{\text{max}}$ of the patch formulations T1, T2 and T3 were 17.86, 30.82 and 22.03 μg/ml respectively. The $C_{\text{max}}$ of all the formulations was significantly (P< 0.01, ANOVA followed by Dunnet's t test) higher than the control patch formulation (C max=13.08). The $C_{\text{max}}$ of the T1, T2 & T3 was 1.37, 2.36 and 1.68 times higher than the control patch formulation, respectively. The area under the plasma concentration time curve
(AUC) of the patch formulations T1, T2 & T3 was 1.28, 1.87 & 1.61 times higher than the control patch formulation respectively (table 28).

Table 28: Mean (±SD) pharmacokinetic parameters of flurbiprofen after transdermal application and oral administration in albino rats

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (μg/ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;(0-α)&lt;/sub&gt;</th>
<th>Relative bioavailability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral administration</td>
<td>26.26 ± 6.1</td>
<td>1.0</td>
<td>83.61 ± 28.2</td>
<td>-</td>
</tr>
<tr>
<td>Plain patch formulation (control)</td>
<td>13.08 ± 1.6</td>
<td>8.0</td>
<td>248.09 ± 13.8</td>
<td>2.97</td>
</tr>
<tr>
<td>T-1</td>
<td>17.86 ± 0.4*</td>
<td>8.0</td>
<td>317.66 ± 12.6**</td>
<td>3.80</td>
</tr>
<tr>
<td>T-2</td>
<td>30.82 ± 2.1**</td>
<td>8</td>
<td>464.98 ± 11.9**</td>
<td>5.56</td>
</tr>
<tr>
<td>T-3</td>
<td>22.03 ± 1.8**</td>
<td>8.0</td>
<td>398.31 ± 11.9**</td>
<td>4.76</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01, ANOVA followed by Dunnet's t test was applied to compare T-1, T-2 and T-3 with control formulation.

The area under the plasma concentration time curve (AUC) decreased in the following order T2 > T3 > T1 > Control > oral. The pharmacokinetic parameters of flurbiprofen after the oral administration were significantly different from the parameters obtained after the transdermal application of patch formulations. After the oral administration of flurbiprofen the C<sub>max</sub> of the drug reached within 1 hour and a sharp decline was observed.

The increase in the AUC<sub>(0-α)</sub> of flurbiprofen after application of transdermal patch formulations was significantly higher than orally administered flurbiprofen, which
indicates the improved bioavailability of flurbiprofen through transdermal drug delivery route. The bioavailability of flurbiprofen with reference to orally administered flurbiprofen was found to increase by 2.97, 3.80, 5.56 and 4.76 times when transdermal patch formulations plain patch formulation, T-1, T-2 and T-3 respectively, were applied (table2S).

The results were consistent with invitro skin permeation studies were T2 provided higher flux and cumulative percentage drug permeation.

Flurbiprofen plasma levels of greater than 5 μg/ml were achieved immediately and were maintained till the last sample. It has been previously reported that plasma levels of approximately 5 μg/ml were achieved in albino rats after 2 hours of application of transdermal patch containing 2 mg of drug applied over 14 cm² area and were maintained till 10 hours (Sasaki et al., 1987). In other previously reported study flurbiprofen levels of 43.9 μg/ml were achieved in rats at 1.8 hours after the application of 500μl of 1 % flurbiprofen containing 5 % of oleic acid and 5 % urea in propylene glycol, on the dorsal area of 9 cm² (Chi et al., 1995). However the plasma levels of the drug dropped sharply and at 12 hrs less than 10 μg/ml of the drug was detected in the plasma. In present study, due to the application of skin penetration enhancers in binary solvent mixture, higher plasma levels of flurbiprofen were achieved with the application of transdermal patches containing 1 mg of drug.
RESULTS AND DISCUSSION

5.9 SKIN ACCUMULATION STUDY

Approximately 5.76 µg/cm², 18.29 µg/cm², 5.75 µg/cm² and 3.44 µg/cm² of drug was recovered from the transdermal patches T-1, T-2, T-3 & Control patch formulation respectively as shown in table 29 and figure 46.

Table 29: Skin accumulation of flurbiprofen transdermal patches after 24 hours of application

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean* (±S.D) amount (µg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>5.76±0.7</td>
</tr>
<tr>
<td>T2</td>
<td>18.29±2.5</td>
</tr>
<tr>
<td>T3</td>
<td>5.75±1.6</td>
</tr>
<tr>
<td>T4</td>
<td>3.44±1.0</td>
</tr>
</tbody>
</table>

* Results are the mean of 4 observations

The turpentine oil provided the highest skin accumulation among the three enhancers studied.
Fig. 46: Skin accumulation of flurbiprofen after 24 hours of application of transdermal patch formulations in vivo

Propylene glycol has been found to cause increased drug partitioning into the stratum corneum and terpenes are known for their increased lipid disruption in stratum corneum (Yamane et al., 1995). Since control patch formulations contained only PG: IPA, lesser amount of drug was recovered from them as compared to the patches with penetration enhancers.
5.10 PHARMACODYNAMIC STUDIES

Antiinflammatory studies were carried out in rats by carrageenin induced paw edema method.

Table 30: Effect of the swelling edema in hind paw of rats produced by carrageenin injection

<table>
<thead>
<tr>
<th>Times (hours)</th>
<th>Control (untreated)</th>
<th>Plain patch flurbiprofen</th>
<th>Orally administered flurbiprofen</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>5.3±3.2±5.8</td>
<td>73.72±7.32</td>
<td>71.62±7.6</td>
<td>60.20±5.5</td>
<td>65.78±14.8</td>
<td>101.12±8.7</td>
</tr>
<tr>
<td>1</td>
<td>63.26±7.9</td>
<td>58.58±3.3</td>
<td>82.43±8.3</td>
<td>19.38±4.3</td>
<td>21.05±14.0</td>
<td>68.53±26.0</td>
</tr>
<tr>
<td>4</td>
<td>81.63±11.3</td>
<td>55.55±6.7</td>
<td>28.37±9.2</td>
<td>9.18±16.6</td>
<td>6.58±21.3</td>
<td>53.93±25.9</td>
</tr>
<tr>
<td>6</td>
<td>51.73±20.3</td>
<td>141.41±10.3</td>
<td>18.91±6.6</td>
<td>8.16±14.0</td>
<td>2.63±17.7</td>
<td>44.94±13.3</td>
</tr>
<tr>
<td>8</td>
<td>44.6±4.6</td>
<td>35.35±13.2</td>
<td>24.32±11.1</td>
<td>6.12±10.6</td>
<td>31.31±6.4</td>
<td>10.11±15.4</td>
</tr>
<tr>
<td>24</td>
<td>43.6±9.0</td>
<td>29.29±11.2</td>
<td>47.29±12.0</td>
<td>1.02±11.2</td>
<td>1.1±7.7</td>
<td>1.12±5.4</td>
</tr>
</tbody>
</table>

*Results are the mean of 4 observations

The tested formulation showed significantly improved antiinflammatory activity as compared to the control formulation as shown in figure 47 and table 30.
It has been shown in the previously reported study that the flurbiprofen gel formulations (Loganathan et al., 2001) containing 1%w/w flurbiprofen with 15% DMSO showed a maximum of 73.15% inhibition of edema. In present study, due to the increased plasma levels of the drug, the percentage swellings was suppressed significantly and at the end of 24 hours were almost brought back to the normal values. These results further support the accuracy of invitro permeation and pharmacokinetic studies. It can be concluded that the penetration enhancer lead to enhanced penetration of the drug and improved the efficacy of the formulation as compared to the control formulation.