WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 13, 1483-1513. Research Article ISSN 2277– 7105

SCREENING OF NATURAL PERMEATION ENHANCERS (ESSENTIAL OILS) IN FORMULATION AND DEVELOPMENT OF TRANSDERMAL PATCHES

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Article Received on
12 August 2022,

Revised on 02 Sept. 2022,
Accepted on 22 Sept. 2022,

DOI: 10.20959/wjpr202213-25599

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ABSTRACT

The drug delivery by oral route is so far most convenient and accepted route of drug delivery but now a day's considerable interest has been increased in delivery of drugs through skin to the systemic circulation and for local effect. However, the outer most layer of the human skin, stratum corneum; possess the formidable barrier to drug penetration thereby reducing bio-availability. Most of the drugs do not have an ability to penetrate the stratum corneum. Owing to the side effects of chemical penetration enhancers interest has been aroused among researchers to find out natural sources as penetration enhancers to improve the bio-availability of drugs so that they can be delivered through skin. A popular approach for improving transdermal drug delivery involves the use of penetration enhancers (sorption promoters or accelerants) which penetrate into skin to reversibly reduce the barrier resistance. The potential mechanisms of action of penetration enhancers include disruption of intercellular

lipid and/or keratin domains and tight junctions. This results in enhanced drug partitioning into tissue, altered thermodynamic activity/solubility of drug etc. Synthetic chemicals (solvents, azones, pyrrolidones, surfactants etc.) generally used for this purpose are rapidly losing their value in transdermal patches due to reports of their absorption into the systemic circulation and subsequent possible toxic effect upon long term application. Essential oils are included in the list of Generally Recognized As Safe (GRAS) substances and have low irritancy potential. The current work was aimed to screen percutaneous permeation enhancement of various natural permeation enhancers (Essential oils) for transdermal delivery of an antifungal drug (Itraconazole) as they have minimum chances of side effects and have improved efficacy compared to chemical enhancers. The patches were formulated using different natural permeation enhancers (Essential oils such as pepper oil, clove oil & Ginger oil) in 2 different ratios. The physicochemical evaluation of the patches was performed for suitability. In vitro permeation studies were performed using Goat skin as the permeating membrane in Franz diffusion cell. The result indicated that maximum release was obtained at 6% of clove oil and it was concluded that that the problems of Itraconazole on oral administration like dissolution rate limited absorption of drug, gastric side effects, ulcer and bleeding can be overcome by applying Itraconazole topically in the form of transdermal patch & natural permeation enhancers (Essential oils) are suitable for effective delivery of drug *via* transdermal route.

KEYWORDS: Transdermal, HPMC, EC, Eudragit, Itraconazole, Permeation enhancers, Pepper oil, Clove oil, Ginger oil.

INTRODUCTION

At present, the most common form of delivery of drugs is the oral route. While it has advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic metabolism and the tendency to produce rapid blood level spikes, leading to a need for high and/or frequent dosing, which can be cost prohibitive and nconvenient.[1]

To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs by more precise (i.e site specific), spatial and temporal placement within the body thereby reducing both the size and number of doses.[2]

New drug delivery systems are also essential for the delivery of novel, genetically engineered pharmaceuticals (i.e. peptides, proteins) to their site of action, without incurring significant immunogenicity or biological inactivation. One of the methods most often utilized has been transdermal delivery- meaning transport of therapeutic substances through the skin for systemic effect. Closely related is percutaneous delivery, which is transport into target tissues, with an attempt to avoid systemic effects.[3]

Both topical and transdermal drug products are intended for external use. However, topical dermatologic products are intended for localized action on one or more layers of the skin (e.g., sunscreens, keratolytic agents, local anesthetics, antiseptics and anti-inflammatory agents). Although some medication from these topical products may unintentionally reach systemic circulation, it is usually in sub-therapeutic concentrations, and does not produce effects of any major concern except possibly in special situations, such as the pregnant or nursing patient. On the other hand, transdermal drug delivery systems use the percutaneous route for systemic drug delivery, but the skin is not the primary target organ.[4]

Percutaneous Drug Absorption

Percutaneous absorption of drug molecules is of particular importance in the case of transdermal drug delivery systems because the drug has to be absorbed to an adequate extent and rate to achieve and maintain uniform, systemic, therapeutic levels throughout the duration of use. In general, once drug molecules cross the stratum corneal barrier, passage into deeper dermal layers and systemic uptake occurs relatively quickly and easily.[5]

Generally, drug absorption into the skin occurs by passive diffusion. The rate of drug transport across the stratum corneum follows Fick's Law of Diffusion. In other words, the rate of drug transport depends not only on its aqueous solubility, but is also directly proportional to its oil/water partition coefficient, its concentration in the formulation vehicle, and the surface area of the skin to which it is exposed; it is inversely proportional to the thickness of the stratum corneum. The stratum corneum is thickest in the plantar (soles) and palmar regions and thinnest in the postauricular, axillary, and scalp regions of the body. An understanding of the transport behavior of drugs is vital for designing an effective topical or transdermal product, as well as reasonably predicting and comparing drug behavior in various formulations. The latter is of practical importance to the pharmacist who is required to suggest one or more effective drug products out of the many commercial formulations available or to counsel patients on proper use and handling transdermal products.[6-7]

Fick's Law of Diffusion as applied to drug transport across stratum corneum [8]

$$\frac{dM}{dt} = \frac{D.\Delta C.K}{h} \quad \ldots\ldots\ldots (1)$$

Where ***d*M/*d*t** is the steady-state flux across stratum corneum

D is the diffusion coefficient or diffusivity of drug molecules

ΔC is the drug concentration gradient across the stratum corneum

K is the partition coefficient of the drug between skin and formulation medium

h is the thickness of the stratum corneum

TRANSDERMAL DRUG DELIVERY

Transdermal drug delivery systems (patches) are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin[9] also defined as medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the bloodstream. Actually, transdermal drug delivery is a transport process of drugs through a multi-laminar structure, e.g. from the patch to stratum corneum then to the viable epidermis, and finally penetrating into the blood**.**

Transdermal drug delivery means that a pharmaceutical compound is moved across the skin the dermis for subsequent systemic distribution. Hence, strictly semantically this does not only include the more commonly understood "patch", but also traditional subcutaneous administration by means of a hypodermic needle and a syringe. Common to all methods of transdermal drug delivery, by this broad definition, is that the drug is passed through an artificial route into the body. The main advantage of this approach is that the drug is entered into the body undistorted without being passed through the body's various defense systems.

In contrast to oral administration (e.g. swallowing a pill), the most convenient way of drug administration, the transdermal route does not suffer from drug degradation in the gastrointestinal tract and reduced potency through first-pass metabolism (i.e. in the liver). In addition, oral-specific side-effects like liver damages are avoided, which are seen for example with common drugs like estradiol (estrogen) or paracetamol.

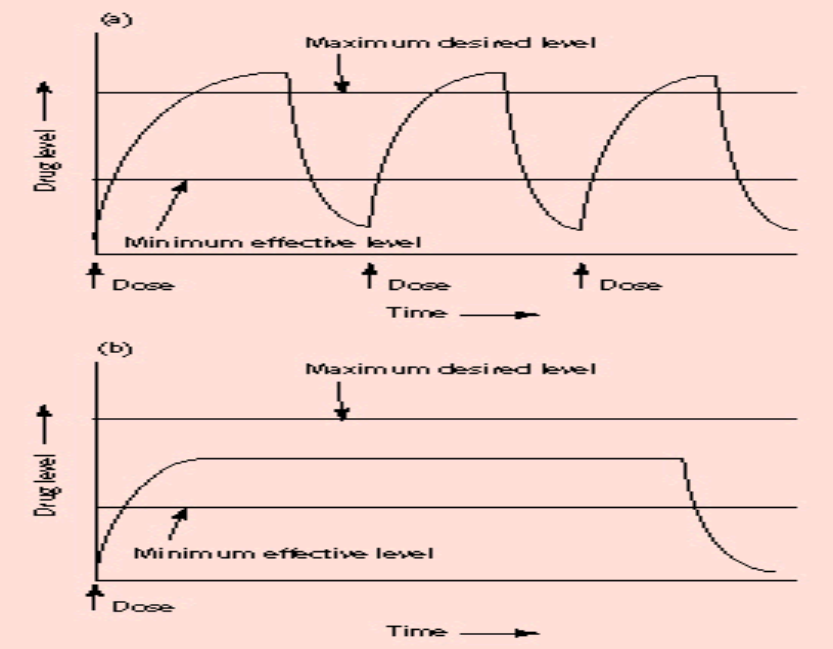

Figure 1: Traditional vs. Transdermal Release.

Transdermal drug delivery systems are topically administered medicaments in the form of patches that deliver drugs for systemic effects at a predetermined and controlled rate. A transdermal drug delivery device, which may be of an active or a passive design, is a device which provides an alternative route for administering medication. These devices allow for pharmaceuticals to be delivered across the skin barrier. In theory, transdermal patches work very simply. A drug is applied in a relatively high dosage to the inside of a patch, which is worn on the skin for an extended period of time. Through a diffusion process, the drug enters the bloodstream directly through the skin. Since there is high concentration on the patch and low concentration in the blood, the drug will keep diffusing into the blood for a long period of time, maintaining the constant concentration of drug in the blood flow.[10]

Pathways for a drug molecule to traverse stratum corneum

The stratum corneum of epidermis is the main barrier for traversing drug molecule from TDDS.

i) Trans appendageal transport (shunt route transport)

In this pathway the appendages (hair follicles, sweat ducts) offer pores that bypass the barrier of the stratum corneum. However, these openings onto the skin surface occupy only around 0.1% of the total skin surface area**.**

The shunt routes may be important for ions and large polar molecules that struggle to cross intact stratum corneum.[11]

ii) Intracellular route (Transcellular)[12]

The pathway is directly across the stratum corneum and the molecule crossing the intact stratum corneum faces numerous repeating hurdles. First, there is partitioning into the keratinocyte, followed by diffusion through the hydrated keratin.

In order to leave the cell, the molecule must partition into the bilayer lipids before diffusing across the lipid bilayer to the next keratinocyte. For highly lipophilic molecules the transcellular route may be predominant.

iii) Intercellular route[12]

In this route the pathway is via lipid matrix between the keratinocytes. It is now accepted that this route provides the principle pathway by which most small, uncharged molecules traverse stratum corneum and therefore many enhancing techniques aim to disrupt or bypass its elegant molecular architecture.

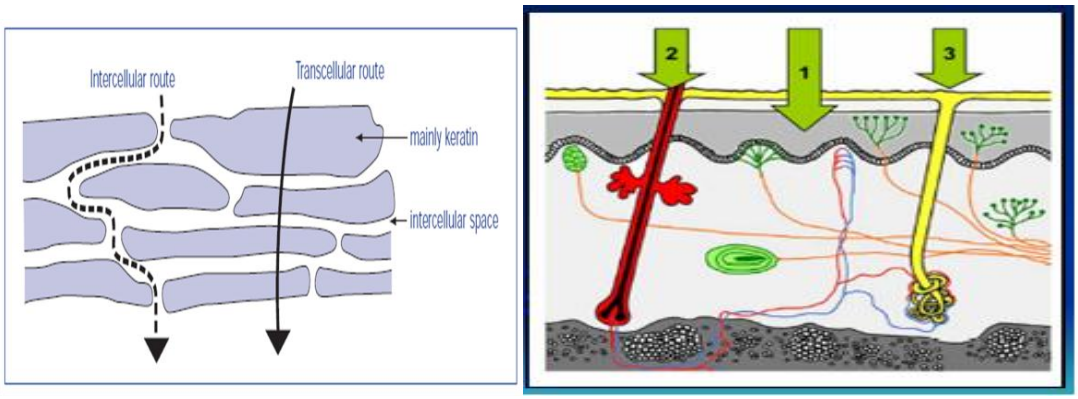

Figure 2: Pathways of transdermal permeation.

A hydrophilic drug permeates by Intercellular pathway and Lipophilic drugs permeates by Intracellular (Transcellular) mechanism**.** Transport of hydrophilic or charged molecules is especially difficult attributable to the lipid-rich nature of the stratum corneum and its low water content; this layer is composed of about 40% lipids, 40% protein, and only 20% water. Transport of liphophilic drug molecules is facilitated by their dissolution into intercellular lipids around the cells of the stratum corneum. Absorption of hydrophilic molecules into skin can occur through ‘pores’ or openings of the hair follicles and sebaceous glands, but the

relative surface area of these openings is barely 1% of the total skin surface. This small surface area limits the amount of drug absorption.

Process of drug permeation in transdermal delivery[13-15]

Transdermal permeation of a drug involves the following steps:

i) Diffusion of drug from drug reservoir to the rate controlling membrane.
ii) Diffusion of drug from rate limiting membrane to stratum corneum.
iii) Sorption by stratum corneum and penetration through viable epidermis.
iv) Uptake of drug by capillary network in the dermal papillary layer.
v) Effect on target organ

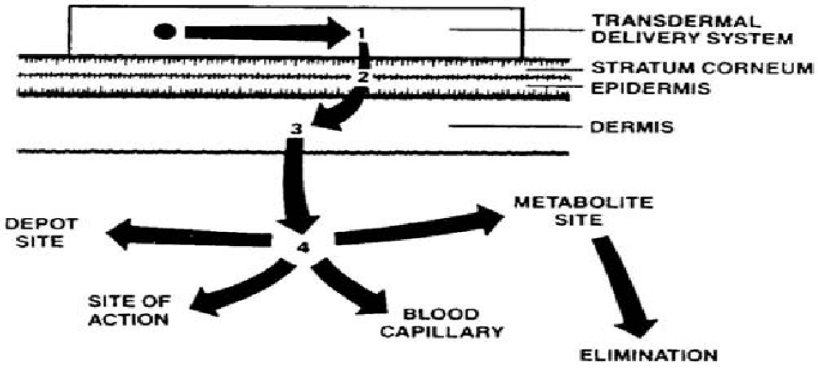

Figure 3: Process of drug permeation.

Kinetics of transdermal permeation

Knowledge of skin permeation kinetics is vital to the successful development of transdermal therapeutic systems. This permeation can be possible only if the drug possesses certain physiochemical properties.

The rate of permeation across the skin is given by

$$dQ/dt = P_s (C_d - C_r) \ldots\ldots\ldots\ldots (2)$$

Where C_d and C_r are the concentrations of the skin penetrant in the donor compartment i.e. on the surface of stratum corneum and in the receptor compartment i.e. body respectively.

P_s is the overall permeability coefficient of the skin tissue to the penetrant. This permeability coefficient is given by the relationship.

$$P_s = K_s . D_{ss}/h_s \ldots\ldots\ldots\ldots (3)$$

Where K_s is the partition coefficient for the interfacial partitioning of the penetrant molecule from a solution medium or a transdermal therapeutic system on to the stratum corneum, D_{ss} is

the apparent diffusivity for the steady state diffusion of the penetrant molecule through a thickness of skin tissues.

$\mathbf{h_s}$ is the overall thickness of skin tissues. As $\mathbf{K_s}$, $\mathbf{D_{ss}}$ and $\mathbf{h_s}$ are constant under given conditions the permeability coefficient $\mathbf{P_s}$ for a skin penetrant can be considered to be constant.

From equation (2) it is clear that a constantrate of drug permeation can be obtained only when $\mathbf{C_d >> C_r}$ i.e. the drug concentration at the surface of the stratum corneum $\mathbf{C_d}$ is consistently and substantially greater than the drug concentration in the body $\mathbf{C_r}$. The equation is

$$\mathbf{dQ/dt = P_s C_d} \quad \mathbf{. . . . . (4)}$$

And the rate of skin permeation is constant provided the magnitude of $\mathbf{C_d}$ remains fairly constant throughout the course of skin permeation.

For keeping $\mathbf{C_d}$ constant the drug should be released from the device at a rate $\mathbf{R_r}$ i.e. either constant or greater than the rate of skin uptake $\mathbf{R_a}$

i.e. $$\mathbf{R_r >> R_a} \quad \mathbf{. . . . . (5)}$$

Since $\mathbf{R_r >> R_a}$, the drug concentration on the skin surface $\mathbf{C_d}$ is maintained at a level equal to or greater than the equilibrium solubility of the drug in the stratum corneum $\mathbf{C_s}$ .i.e. $\mathbf{C_d >> C_s}$. Therefore a maximum rate of skin permeation is obtained and is given by the equation:

$$\mathbf{(dQ/dt)_m = P_s C_s} \quad \mathbf{. . . . . (6)}$$

From the above equation it can be seen that the maximum rate of skin permeation depends upon the skin permeability coefficient $\mathbf{P_s}$ and is equilibrium solubility in the stratum corneum $\mathbf{C_s}$. Thus skin permeation appears to be stratum corneum limited.

Conditions in which Transdermal patches are used[16]

- When the patient has intolerable side effects (including constipation) and who is unable to take oral medication (dysphagia) and is requesting an alternative method of drug delivery.
- Where the pain control might be improved by reliable administration. This might be useful in patients with cognitive impairment or those who for other reasons are not able to self-medicate with their analgesia.

- It can be used in combination with other enhancement strategies to produce synergistic effects.

Conditions in which Transdermal patches are not used[16]

✓ Cure for acute pain is required and where rapid dose titration is required.

✓ Where requirement of dose is equal to or less than 30 mg/24 hrs.

Merits of Transdermal drug delivery[16-17]

This approach to drug delivery offers many advantages over traditional methods.

- Enables the avoidance of gastrointestinal absorption, with its associated pitfalls of enzymatic and pH associated deactivation.
- This method also allows for reduced pharmacological dosaging due to the shortened metabolization pathway of the transdermal route versus the gastrointestinal pathway.
- The patch also permits constant dosing rather than the peaks and valleys in medication level associated with orally administered medications.
- Multi-day therapy with a single application.
- Rapid notifications of medication in the event of emergency, as well as the capacity to terminate drug effects rapidly via patch removal.
- The steady permeation of drug across the skin allows for more consistent serum drug levels, often a goal of therapy.
- Intravenous infusion also achieves consistent plasma levels, but it is more invasive than transdermal drug delivery.
- The lack of peaks in plasma concentration can reduce the risk of side effects.
- Alternative route of administration to accommodate patients who cannot tolerate oral dosage forms.
- It is of great advantage in patients who are nauseated or unconscious.
- First pass metabolism, an additional limitation to oral drug delivery, can be avoided.
- Controlled release over extended period
- Easily terminable means for systemic as well as topical drug delivery
- Improved patient compliance.

Demerits of Transdermal drug delivery[16-17]

However this system has its own limitations

- The drug that requires high blood levels cannot be administered and may even cause irritation or sensitization of the skin.
- The adhesives may not adhere well to all types of skin and may be uncomfortable to wear.
- The high cost of the product is also a major drawback for the wide acceptance of this product.

Permeablity Enhancement

Methods of enhancement[18-19]

- Chemical enhancement
- Physical enhancement
- Biochemical enhancement
- Supersaturation enhancement
- Bioconvertable prodrug

i) Chemical enhancement

The skin permeability of drugs can be greatly improved by treating the stratum corneum surface with an appropriate skin permeation enhancer. Ideally penetrating enhancers reversibly reduce the barrier resistance of the stratum corneum without damaging viable cell.

A synergistic effect in the skin permeation enhancement could be achieved by incorporating two or more enhancers in the adhesive layer. Sometimes chemical penetration enhancers may also provoke unwanted biochemical and metabolic events within skin but this is not their aim.

In the past two decades tremendous amount of work has been done to search specific chemicals that can act as penetration enhancer with ideal properties as follows.

ii) Physical enhancement

Different physical approaches to increase percutaneous absorption have been utilized but the most important approaches are iontophoresis, ultrasound, electroporation and heat. These methods show most promising in the percutaneous delivery of large molecular weight compounds but the major limitation is the input of energy to achieve their effects.

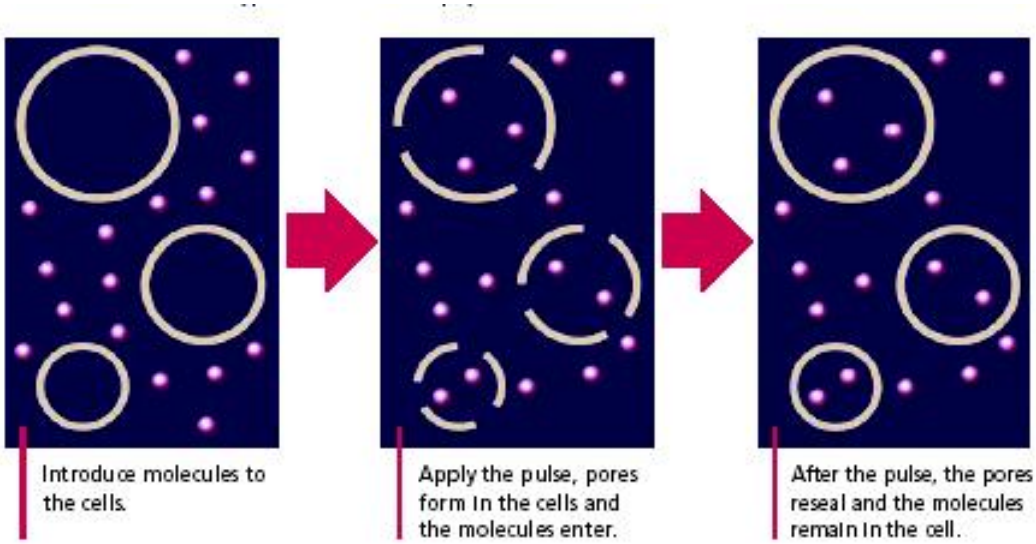

Figure 4: Enhanced permeation by electroporation.

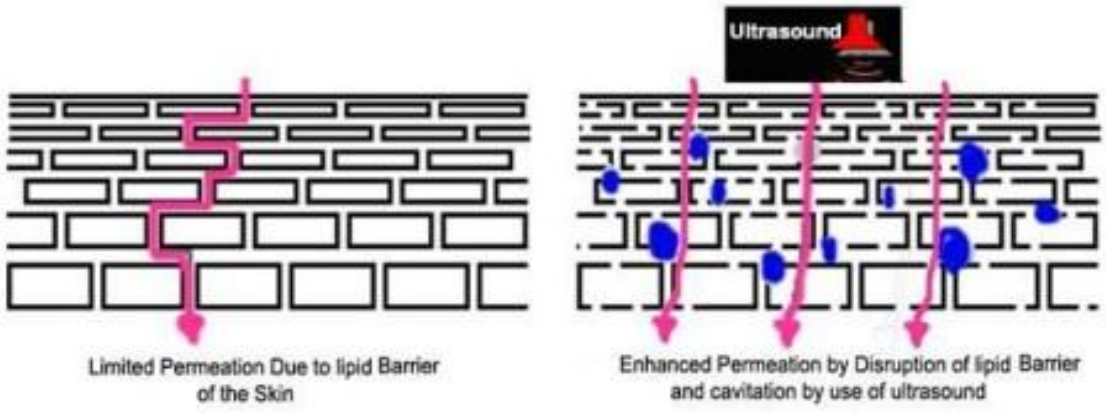

Figure 5: Enhanced permeation by ultrasound.

iii) Biochemical enhancement

This chemical provokes the biological and metabolic events within the skin and significantly increases skin permeability. These types of enhancers reduce barrier properties of the skin either by inhibiting enzymes responsible for synthesis of stratum corneum lipid or by promoting metabolism of existing skin lipids that are responsible for barrier function.

iv) Supersaturation enhancement

The thermodynamic activity of drug can be increased by employing supersatured systems that gives rise to unusually high thermodynamic properties. However topical vehicle relying on supersaturation have the major limitation of formulation instability, both prior to and during application to skin, unless the formulation can be stabilized with antinucleant and anticrystal-growth agents.

v) Bioconvertable prodrug

The prodrug concept can be applied in transdermal controlled drug delivery by altering skin permeability via modification of the physicochemical properties of the drug molecules to enhance its rate of transdermal permeation. One example of this approach is the esterification of less skin permeable estradiol to form lipophilic estradiol ester (like estradiol-17-acetate, estradiol-3, 17-diacetate and estradiol-17-cypiuonate etc.

Chemical penetration enhancers[20]

Chemical substances temporarily diminishing the barrier of the skin and known as accelerants or sorption promoters can enhance drug flux.

Several types are known

i) Sulphoxides and similar chemicals

Dimethyl sulphoxides (DMSO) is one of the earliest and most widely studied penetration enhancers. It is a powerful aprotic solvent which hydrogen bonds with itself rather than with water. It is colourless, odourless and is hydroscopic and is often used in many areas of pharmaceutical sciences as a universal solvent. DMSO alone has been applied topically to treat systemic inflammation. DMSO works rapidly as a penetration enhancer - spillage of the material onto the skin can be tasted in the mouth within a second. Although DMSO is an excellent accelerant, it does create problems.

The effect of the enhancer is concentration-dependent and generally co-solvents containing > 60% DMSO is needed for optimum enhancement efficacy. However, at these relative high concentrations, DMSO can cause erythema and wheal of the stratum corneum. Denaturing of some skin proteins results in erythema, scaling, contact uticaria, stinging and burning sensation. Since DMSO is problematic for use as a penetration enhancer, researchers have investigated a similar chemically-related material as a accelerant. DMAC and DMF are similarly powerful aprotic solvents. However, South well and Barry, showing a 18-fold increase in the flux of caffeine permeating across a DMF treated human skin, concluded that the enhancer caused irreversible membrane damage. DMF irreversibly damages human skin membranes but has been found *in vivo* to promote the bioavailability of betamethasone-17-benzoate as measured by vasoconstrictor assay. DMSO may also extract lipids, making the horny layer more permeable by forming aqueous channels. The mechanism of the sulphoxide penetration enhancers is widely used to denature protein and, on application to human skin, has been shown to change the intercellular keratin conformation, from helical to ß sheet.[21]

ii) Azone

Azone (1-dodecylazacycloheptan-2-one or laurocapran) was the first molecule specifically designed as a skin penetration enhancer. Azone is a colourless, odourless liquid with a melting point of -7ºC and it possesses a smooth, oily but yet non-greasy feel. Azone is a highly lipophilic material with a log p-octanol / water of around 6.2 and it is soluble in and compatible with most organic solvents including alcohol and propylene glycol. Azone enhances the skin transport of a wide variety of drugs including steroids, antibiotics and antiviral agents. Azone is most effective at low concentrations, being employed typically between 0.1- 5% but more often between 1-3%.[21] Azone partitions into a bilayer lipid to disrupt their packing arrangement but integration into the lipid is unlikely to be homogeneous. Azone molecules may exist dispersed within the barrier lipoid or separate domains within the bilayer.[22]

iii) Pyrrolidones

Pyrrolidones have been used as permeation enhancers for numerous molecules including hydrophilic (e.g. mannitol and 5-flurouracil) and lipophilic (progesterone and hydrocortisone) permeants. N-methyl-2-pyrolidone was employed with limited success as a penetration enhancer for captopril when formulated in a matrix-type transdermal patch.[23] The pyrrolidones partition well into human stratum corneum within the tissue and they may act by altering the solvent nature of the membrane. Pyrrolidones have been used to generate reservoirs within the skin membrane. Such a reservoir effect offers a potential for sustained release of a permeant from the stratum corneum over extended time periods.[24]

iv) Fatty acids

Percutaneous drug absorption has been increased by a wide variety of long-chain fatty acids, the most popular of which is oleic acid. It is of interest to note that many penetration enhancers such as azone contain saturated or unsaturated hydrocarbon chains and some structure - activity relationships have been drawn from the extensive studies of Aungst who employed a range of fatty acids, acids, alcohols, sulphoxides, surfactants and amides as enhancers for naloxone**.** Shin et al studied various penetration enhancers like glycols (diethylene glycol and tetraethylene glycol), fatty acids (lauric acid, myristic acid and capric acid) and nonionic surfactant (polyoxyethylene-2-oleyl ether, polyoxy ethylene-2-stearly ether) on the release of triprolidone. Lauric acid in Propylene glycol enhanced the delivery of highly lipophilic antiestrogen. Oleic acid greatly increased the flux of many drugs such as

increasing the flux of salicylic acid 28-fold and 5-flurouracil flux 56-fold through human skin membrane *in vitro*.[25-26]

The enhancer interacts with and modifies the lipid domains of the stratum corneum as would be expected for a long chain fatty acid with cis-configuration.

Apart from different techniques mentioned **natural permeation enhancers (NPEs)** are comparatively new class of penetration enhancers in the pharmaceutical industry. Due to its advantages such as low cost, better safety profile more research need to be focused in this field to develop a stable transdermal formulations containing natural permeation enhancers (NPEs) which can be scale up for commercial transdermal drug product.[27]

Papain

Papain is isolated from *Carica papaya*. It is an endocytic plant cysteine protease enzyme. Papain, which is a proteolytic enzyme, was studied *in vitro* and *in vivo* permeation of low-molecular-weight heparin (LMWH). The combined administration of LMWH and papain was found to be a new approach in improvement in absorption of orally administered heparin and hence its bioavailability.

Piperine

Piperine is obtained from mature fruits of *Piper nigrum* and *Piper longum*. Piperine was investigated for *in vitro* permeation of aceclofenac across human cadaver skin, and Fourier transform infrared technology was used to check the possible mechanism from which the results obtained showed that piperine enhances transdermal permeation of aceclofenac by biphasic mechanism involving partial extraction of SC lipid and interaction with SC keratin.

Capsaicin

Capsaicin is a major alkaloid among capsaicinoids which is produced only in capsicum fruits of the genus capsicum and belonging to the *Solanaceae* family.The permeation enhancing properties of capsaicin were studied for naproxen taking azone as the standard enhancer and capsaicin were compared to it. Onto the skin, different amount of chosen enhancer was applied before experiment. A formulation containing 3% capsaicin and commercially available naproxen gel formulation was also studied, and the results were compared. It was found that penetration increased when the skin was treated with azone and capsaicin and also some alteration by capsaicin was seen in the SC layer. Hence, it was observed that capsaicin

increases the penetration of naproxen through SC, which concludes that capsaicin is quiet capable enhancer of skin like well-known enhancer azone.

Myristica fragrans

M. fragrans was evaluated as a penetration enhancer in a transdermal gel formulation containing diclofenac sodium as the target drug. Methanolic extracts, chloroform extracts, and n-hexane extracts of *M. fragrans* were used as a penetration enhancer in comparison to a synthetic enhancer Triton X. It was found that in both *in vivo* and *in vitro* studies, methanol and chloroform extract showed better percentage cumulative release (%), and hence, better penetration was shown as compared to the synthetic enhancer.

Essential Oils

Essential oil is natural products which are extracted from aromatic plants having a concoction of a number of aromatic smelling volatile compounds, primarily consisting compounds such as terpenes, terpenoids, and phenylpropanoids. They can be accepted as a natural alternative to synthetic skin penetration enhancer due to their promising penetration enhancing activity.

As penetration enhancer, essential oils help in the delivery of drug compounds into the skin by interacting with the intercellular lipids by different physical processes such as increased disorder, phase separation, and fluidization. As they are penetrated easily by the skin, they are easily excreted also by the body with urine and feces. Hence, due to their better safety profile in comparison with other penetration enhancers, their use is increasing.[28]

Essential oil as skin permeation enhancer

Penetration enhancer interacts with the tissue components to lessen the barrier properties by partitioning into the SC but do not cause any damage to the underlying skin cell. Both D-limonene and 1, 8-cineole have shown to modify permeate diffusivity by disruptingSC lipid.[29]

Eucalyptus oil

The oil of eucalyptus can be obtained from a number of species of the Myrtaceae family, such as *Eucalyptus citriodora*, *Eucalyptus dives*, *Eucalyptus globulus*, *Eucalyptus polybractea*, and *Eucalyptus radiata*. By steam distillation of eucalyptus leaves, its oil is extracted. The eucalyptus oil was subjected to permeation studies on full thickness human skin and it was found that the oil enhanced the permeation of chlorhexidine (2% [w/v]) into the dermis and the lower layer of the epidermis when it was combined with 70% (w/v)

isopropyl alcohol and 10% (v/v) eucalyptus oil in comparison to the solution of chlorhexidine/isopropyl alcohol alone.

Niaouli oil

The extraction of niaouli oil can be made by steam distillation of twigs and leaves of *Melaleuca quinquenervia*, of the family *Myrtaceae*. The major constituents of niaouli oil was 55-70% 1,8-cineole (oxide) and limonene (monoterpene), 7-15% a-pinene (monoterpene), 2-6% s-pinene (monoterpene), and 2-6% viridiflorol (sesquiterpene). *In vitro* studies were performed to determine the permeation enhancing the effect of niaouli oil at 10% (w/w) concentration in propylene glycol on estradiol model drug using a hairless mouse skin. It was found that niaouli oil proved to be more effective in transdermal permeation of estradiol than cajput, myrtle, orange, and cardamom essential oil.

Fennel oil

The extraction of fennel oil can be made from the seeds of *Foeniculum vulgare*, of the family *Umbelliferae*. On permeation studies, the percutaneous penetration of trazodone hydrochloride was enhanced by fennel oil followed by eucalyptus oil, citronella oil, and mentha oil. The variable physicochemical properties and molecular weights of phytochemicals present in the different essential oils may be the factors for the differences in the permeation enhancement activity between the oils.

Black cumin oil

The extraction of black cumin oil is made by steam distillation of the seeds of *Cuminum cyminum* by steam distillation. Black cumin oil showed a relatively greater permeating effect for the drug carvedilol when it was compared to clove oil, eucalyptus oil, tulsi oil, oleic acid, Tween 80 and the enhancement factor was found to be 6.40. Furthermore, Fourier transform infrared spectroscopy studies confirmed the alteration caused by black cumin oil on the permeability of the skin by extracting lipids and by hydrogen bonding which affects other hydrogen bonds between the ceramides.

Almond oil

Oil of almond and oleic acid were found as promising carriers/vehicles for enhanced permeability and solubility of aceclofenac. Hence, these oils can be used to develop drug delivery systems for improved bioavailability of aceclofenac.

In the study, topically applied ketoprofen gels and patches were formulated and evaluated, and almond oil was checked as a penetration enhancer for ketoprofen gels and patches through artificial membrane/rabbit skin. It was found that almond oil in different concentration as a penetration enhancer enhances the penetration of drugs from transdermal gels and patches across synthetic membrane/rabbit skin but notably when used in 3% concentration.

Basil oil

Basil oil was studied for its potential as a permeation enhancer for labetalol hydrochloride with respect to camphor, thymol, geraniol, and clove oil. It was suggested that basil oil is having good penetration enhancing property for improved transdermal drug delivery of labetalol.

Basil oil was used for the enhancement in bioavailability of flurbiprofen applied transdermally and it was concluded that the bioavailability of the flurbiprofen applied transdermally using basil oil increased by 2.97, 3.80, and 5.56 times in comparison to the flurbiprofen administered orally in albino rats.

***Alpinia oxyphylla* oil**

A. oxyphylla oil was extracted from *A. oxyphylla* and was divided into a higher polarity fraction and a lower polarity fraction. *In vitro* studies were performed using Franz diffusion cell across the dorsal skin of Wistar rats, the results indicated that the high polarity fraction of *A. oxyphylla* oil was having more efficient permeation enhancing the effect of indomethacin at concentration 3% and 5% then the lower polarity fraction.

Turpentine oil

Turpentine oil on the skin permeation rate of flurbiprofen showed an additive effect when it was added to an optimized cosolvent mixture of propylene glycol and isopropyl alcohol (30-705 [v/v]), and at the concentration of 5% (v/v) of turpentine oil, maximum transdermal penetration rate was obtained. The effectiveness of turpentine oil was investigated for permeation enhancing activity for diclofenac dimethylamine matrix patches across the artificial skin in the Franz diffusion cell. It was found that the oil showed increasing permeation with increasing concentration of turpentine.

Rosemary oil

Rosemary oil is extracted from *Rosmarinus officinalis*. Rosemary oil when investigated for skin permeation enhancing activity for diclofenac sodium topical gel showed enhanced skin absorption at 0.5% and 1% concentration, respectively.

Cardamom oil

Cardamom (*Elettaria cardamomum*) is a common spice of India belonging to the *Zingiberaceae* family. The oil extracted from cardamom has a number of volatile compounds such as monoterpenes including 1,8-cineole and cis-ocimene and sesquiterpene including guanine and nerodilol . Cardamom oil on *in vitro* permeation studies through the rabbit abdominal skin showed an increase in penetration of the drugs indomethacin, diclofenac, and piroxicam.

Terpenes

In transdermal drug delivery studies, terpenes are one of the major choices. This class includes a heterogeneous range of members. The physicochemical properties of a specific terpene play a role in showing the effect on the skin, in particular, its lipophilicity. However, smaller terpenes with nonpolar groups are said to be better skin permeation enhancers. Terpenes are also reported to increase drug partitioning and diffusibility into the skin by disturbing the lipid bilayers of the skin. They are relatively safe as skin penetration enhancers for both hydrophilic and lipophilic drugs.

Farnesol

Farnesol is present in many essential oils, such as citronella, neroli, cyclamen, lemongrass, tuberose, balsam, and tolu. It is a sesquiterpene alcohol. Farnesol (0.25%) was reported to increase the permeation of diclofenac sodium with respect to other terpenes in the following order: Farnesol > carvone > nerolidol > menthone > limonenoxide.

Menthol

Menthol, which is one of the potent penetration enhancers, is obtained from the flowering tops of *Mentha piperita*. Menthol and limonene together can be used as a prototype of terpenes that can be used as permeation enhancer.

Eucalyptol

Eucalyptol is a cyclic ether and a monoterpenoid known by a number of synonyms such as 1, 8-cineole, cajeputol, eucalyptol, and cineole. Eucalyptol is used in cosmetic, fragrances and

flavoring industries because of its spicy aroma and taste. 1, 8-cineole has also been used for the percutaneous absorption of several lipophilic drugs through the hairless mouse skin. [30]

Eugenol

Eugenol was evaluated for permeation enhancing effect for the drug lornoxicam which is a nonsteroidal anti-inflammatory drug of oxicam class. Lornoxicam transdermal patches were formulated which werethen subjected to *in vitro* studies in a Franz diffusion cell using rat skin. *In vitro* studies showed that eugenol does increase the permeation of lornoxicam across rat skin.

Borneol

The transdermal permeation enhancing activity of borneol was investigated on 5 model drugs, namely, 5-fluorouracil, antipyrine, aspirin, salicylic acid, and ibuprofen and it was found that borneol effectively promoted the transdermal permeation of the model drug.

MATERIALS AND METHODS

Materials

Itraconazole were obtained as a gift sample from Hetero Pharma Ltd., Hyderabad .Polymers such as Hydroxy Propyl Methyl Cellulose E 5, Ethyl cellulose (EC) and penetration enhancers such as Pepper oil, Clove oil, Ginger oil were provided by the institute and pressure sensitive adhesive (Eudragit RL-100) were provided by Natco Pharma. Ltd. Other chemicals such as Diethyl phthalate, sodium hydroxide, potassium di hydrogen orthophosphate, Di chloro methane and methanol used in the study were analytical grade.

Methods

Preparation of standard solution

The standard solution was prepared by dissolving 10 mg of Itraconazole in 10 ml of phosphate buffer pH 7.4 and the volume was made up to 100 ml using phosphate buffer pH 7.4.

From this standard solution, a series of dilutions containing 0.1, 0.2, 0.4, 0.6, 0.8, 1,1.2, 1.4, and 1.6,ml were pipetted out and subsequently diluted to10 ml with phosphate buffer pH 7.4 to give 1, 2, 4, 6, 8, 10, 12, 14, 16 µg/ml respectively.

The absorbances of these dilutions were measured using UV spectrophotometer at 262nm using phosphate buffer pH 7.4 as blank solution.

Design and Formulation Development of the Transdermal Patch

The initial design for the transdermal patch was based on the simple DIA design. In order to meet the key properties for a successful topical patch a DIA type transdermal patch was designed as shown in figure 4.

The transdermal patches were prepared by film casting technique using liquid Paraffin as lubricant. Prepared patches composed of four Layers:

- Backing membrane
- Pressure sensitive adhesive membrane

---- (Eudragit RL100 5 % w/v of aqueous solution)

- Drug loaded HPMC film

----- (Itraconazole +PE+HPMC+EC+Plasticizer)

- Release linear (Peeling paper)

This Matrix system patch design is characterized by the inclusion of a semisolid matrix containing a drug solution or suspension which is in direct contact with the release liner. The component responsible for skin adhesion is incorporated in an overlay and forms a concentric configuration around the semisolid matrix.

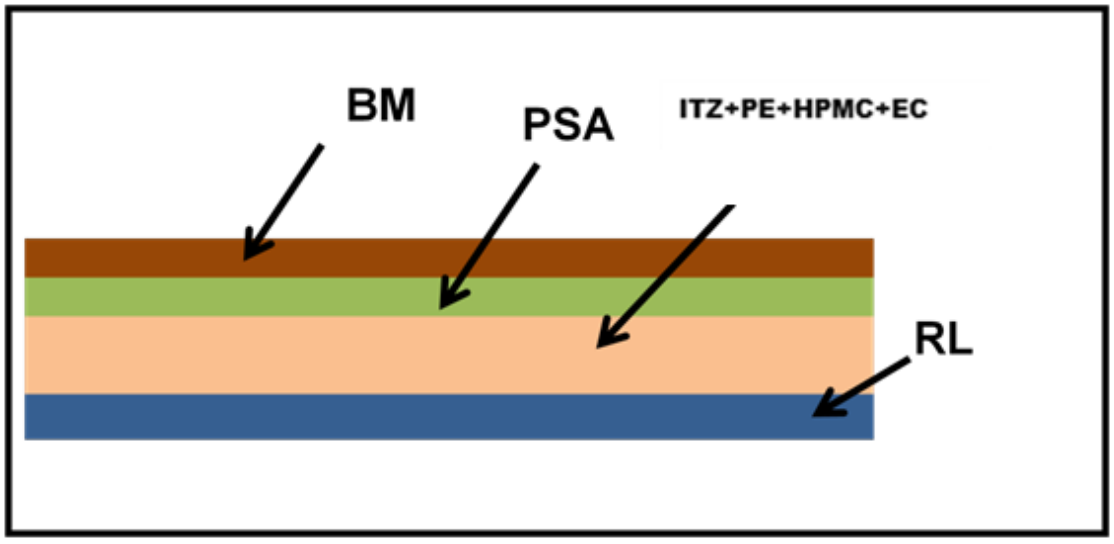

Figure 6: Drug-in-adhesive (DIA) design of the transdermal patch consisting of Backing Membrane (BM), pressure sensitive adhesive (PSA), Drug with PE and polymers (ITZ, HPMC, EC and PE) and Release liner (RL).

Formulation of Itraconazole (Itz) Transdermal Patches

Table 1: Composition of Formulation.

Formulation Code	POTP1	POTP2	COTP1	COTP2	GOTP1	GOTP2
Itraconazole (mg)	200	200	200	200	200	200
HPMC(mg)	800	800	800	800	800	800
Ethyl cellulose(mg)	200	200	200	200	200	200
Eudragit RL 100 (%W/V)	5	5	5	5	5	5
Pepper oil (%)	4	6	---	---	---	---
Clove oil (%)	---	---	4	6	---	---
Ginger oil (%)	---	---	---	---	4	6
Diethyl phthalate (ml)	5	5	5	5	5	5
DCM: Methanol(ml)	15	15	15	15	15	15

***Each patch contains 200 mg of drug in 15.896 sq.cm area.**

***Each Sq.cm contains 12.5 mg of Itraconazole.**

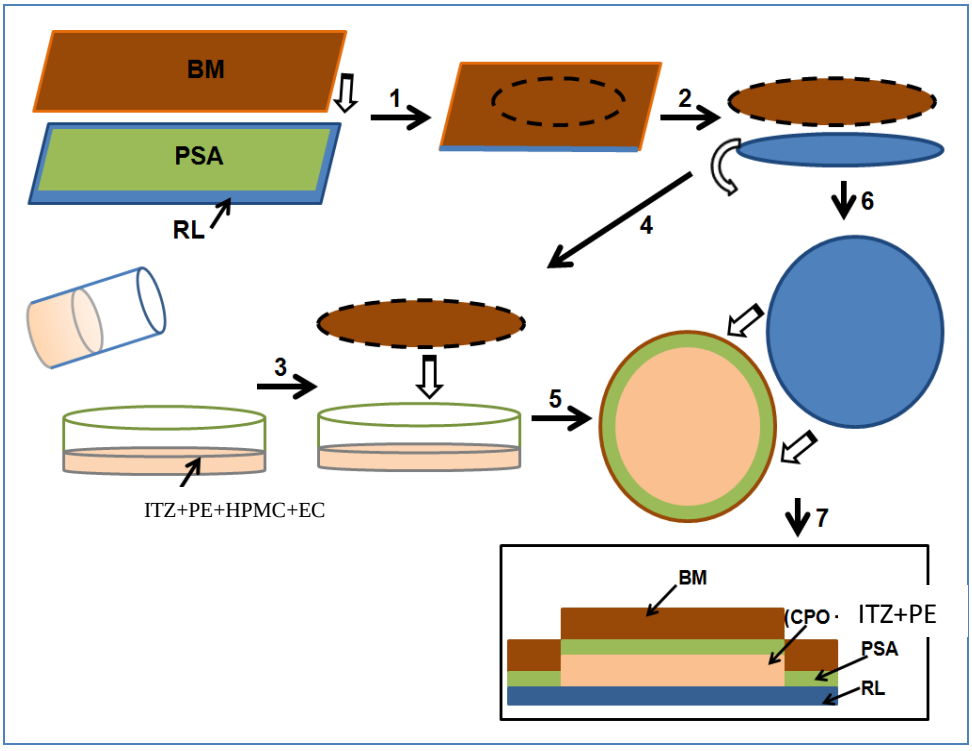

Figure 7: ***Fabrication of novel transdermal patch.***

BM-Backing Membrane, PSA-Pressure Sensitive Adhesive, RL-Release Linear, ITZ-Itraconazole

PE-Permeation enhancer, HPMC-Hydroxy Propyl Methyl Cellulose, EC-Ethyl cellulose

Evaluation of Transdermal Patches

Development of transdermal dosage form is a complex process involving extensive research. Transdermal patches have been developed to improve clinical efficacy of the drug and to

enhance patient compliance by delivering smaller amount of drug at a predetermined rate. This makes evaluation studies even more important in order to ensure their desired performance and reproducibility under the specified environmental conditions.

Physical Appearance

All the transdermal systems were visually inspected for colour, transparency, clarity, flexibility and smoothness.

Patch Weight Uniformity

Weight variation is studied by individually weighing randomly selected patches and calculating the average weight.

The individual weight should not deviate significantly from the average weight.

Patch Thickness Uniformity: The thickness of the formulated patch was measured at 3 different points using a digital caliper and average thickness of three reading was calculated.

Folding Endurance: The folding endurance was measured manually for the prepared patches. A patch was repeatedly folded at the same place till it broke. The number of times the patch could be folded at the same place without breaking/cracking give the value of folding endurance. Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme conditions of folding. This is important to check the ability of sample to withstand folding. This also gives an indication of brittleness.

Percentage Moisture Absorption: The patches were weighed accurately and placed in the desiccators containing 100 ml of saturated solution of potassium chloride, which maintains 80-90% RH. After 3 days, the patches were taken out and weighed. The study was performed at room temperature.

The percentage moisture absorption was calculated using the formula:

$$\text{Percentage moisture absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

Percentage Moisture Loss: The patches were weighed accurately and kept in a desicator containing anhydrous calcium chloride. After 3 days, the patches were taken out and weighed. The moisture loss was calculated using the formula:

$$\text{Percentage moisture absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

Drug content uniformity

Amount of drug entrapped in a patch was determined by completely dissolving an accurately weighed portion of patch (about 100 mg) in 100ml phosphate buffer solution (pH 7.4). Complete dissolution was achieved by placing the solution containing patch on shaker for about 24 hrs. Solution was then filtered and drug content was estimated spectro photometrically at 262nm by appropriate dilution.

Invitro Transdermal Permeation Studies

Transdermal permeation study through Franz diffusion cell

The skin permeation study was performed in a modified Franz- diffusion cell. The drug permeation study was performed using goat skin obtained from local slaughter house. The skin was store at 4 to 5 ^{0}C in saline solution until usage. The dermatome skin (thickness 140 µm) was washed with soap solution, followed by washing with distilled water. The isolated goat skin was mounted between the donor and receptor compartment of the diffusion cell. The dermal side of skin was facing receptor compartment and patch was affixed on the skin so that drug matrix was toward skin. The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4. The temperature of diffusion medium was maintained at 37 ± 0.5oC by circulating water jacket. This whole assembly was kept on a magnetic stirrer and solution in the receiver compartment was constantly and continuously stirred during the whole experiment using magnetic bead. The samples were withdrawn (2 ml, each time) at different time interval and phosphate buffer pH 7.4 was replaced each time. Absorbance of the sample was read spectrophotometrically at 262 nm taking phosphate buffer pH 7.4 solution, as a blank. The amount of drug permeated per square centimetre at each time interval was calculated and plotted against time.

The receiver compartment was maintained at body temperature and was continuously stirred with the help of magnetic stirrer.

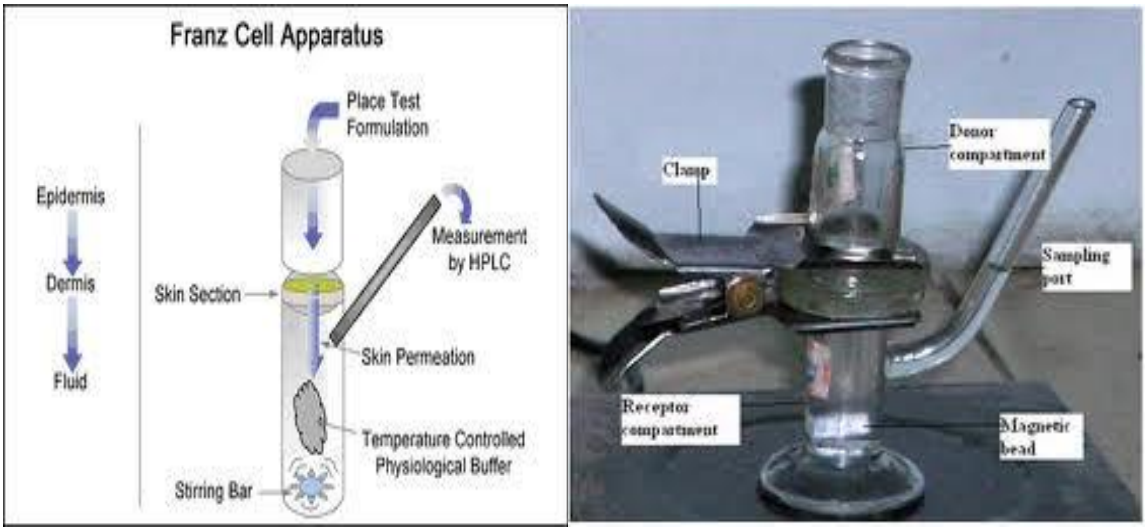

Figure 8: Franz diffusion cell.

The pH of the dissolution medium ideally should be adjusted to pH 5 to 8, reflecting physiological skin conditions. For the same reason, the test temperature is typically set at 37°C (even though the temperature may be higher when skin is covered).

Ph Eur considers 100 rpm a typical agitation rate and also allows for testing an aliquot patch section. The agitation speed and temperature are kept constant.

This may be an appropriate means of attaining sink conditions, provided that cutting a piece of the patch is validated to have no impact on the release mechanism.

Design of system, patch size, surface area of skin, thickness of skin and temperature etc. are some variables that may affect the release of drug.

RESULT AND DISCUSSION

The results are arranged in the order of the experimental methods performed.

Design and Formulation Development of the Transdermal Patch

The matrix-type dermal patches of Itraconazole were prepared by film-casting technique using natural permeation enhancers such as Pepper oil, Clove oil and Ginger oil individually in different ratios. Formulations containing higher proportion of Ginger oil were sticky and were difficult to cast on to the Petri plate.

Air entrapment in the polymeric solutions created a problem in casting patches. Swelling of polymers required time.

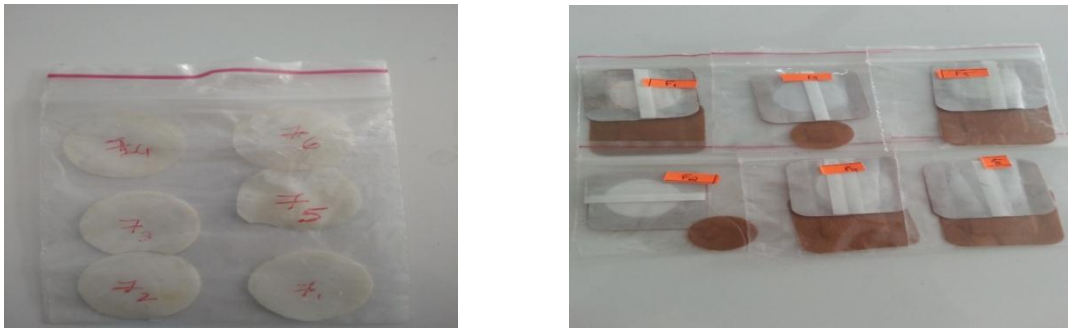

Figure 9: Prepared and Fabricated Itraconazole Transdermal Patches.

Physicochemical evaluation

Physical appearance: The physical appearances of all the formulations were observed visually and the results are tabulated as follows.

Table 2: Physical appearance of Patches.

Formulation Code	Physical appearance
F1	Thin ,Transparent, flexible, Smooth
F2	Thin, Transparent, Smooth, uniform, soft
F3	Thin, Transparent Smooth, uniform
F4	Thin, Transparent, Smooth, uniform, flexible
F5	Smooth, uniform, Thick
F6	Smooth , Sticky ,Thick

The results of remaining physico-chemical evaluations are tabulated as follows

Table 3: Data obtained from physico-chemical evaluation

Formulation code	Weight uniformity (mg)	Thickness uniformity (mm)	Drug content uniformity (%)	Moisture Gain (%)	Moisture Loss (%)	Folding Endurance
POTP1	295	0.19	97.91	2.62	1.20	309
POTP2	299	0.18	97.86	2.82	1.10	316
COTP1	297	0.18	97.21	2.26	1.46	293
COTP2	298	0.20	98.95	2.19	1.30	297
GOTP1	297	0.20	97.42	2.93	1.56	250
GOTP2	299	0.21	98.85	2.23	1.36	257

ii) *In-vitro* drug permeation

The release of Itraconazole through the goat skin from its various transdermal patch formulations are represented as following.

Table 4: Cumulative % drug release of all the formulations

Time in hours	FORMULATION CODE					
	POTP1	POTP2	COTP1	COTP2	GOTP1	GOTP2
0	0	**0**	0	0	0	0
1	12.633	13.778	13.612	15.789	11.088	13.451
2	19.577	23.558	20.817	26.802	15.817	17.644
4	24.706	34.673	33.368	39.159	21.341	22.794
6	36.727	44.856	40.557	46.239	28.398	34.987
8	42.976	56.567	53.782	58.404	36.938	45.425
10	56.053	63.893	63.322	66.292	53.004	56.127
12	64.916	72.065	70.186	74.719	62.863	67.131

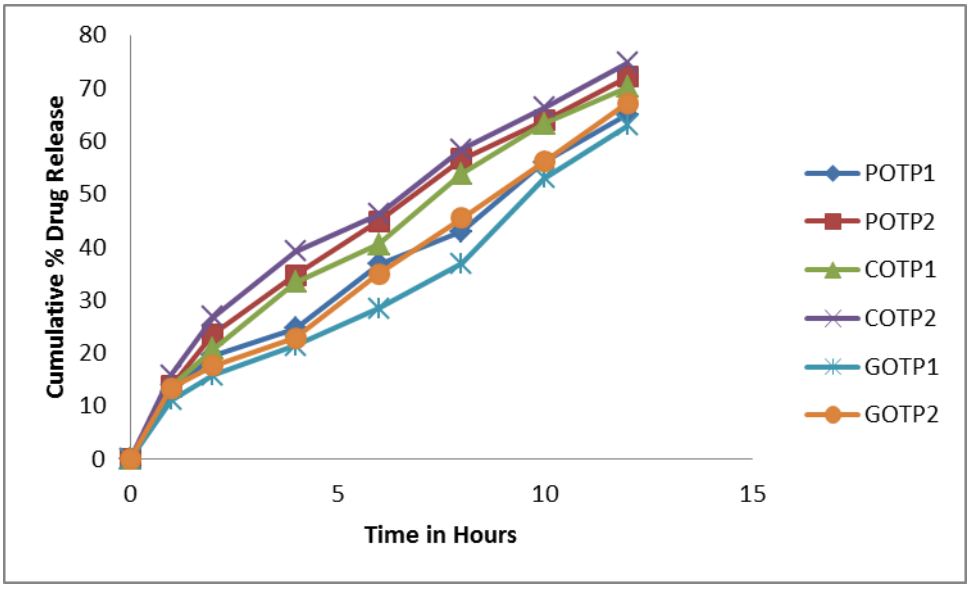

Figure 10: Plot of Cumulative % drug release of all the formulations.

The release of the drug from its transdermal patch formulations can be ranked in the following descending order:

COTP2>POTP2>COTP1>GOTP2>POTP1>GOTP1

DISCUSSION

In the present research work, Itraconazole transdermal patches were prepared by using different natural permeation enhancers such as Pepper oil, Clove oil and Ginger oil where HPMC E5 and Ethyl Cellulose used as polymers. The prepared Transdermal patches were evaluated for parameters like thickness, drug content, *in-vitro* drug release etc.

Physicochemical evaluation

i) Physical appearance

The transdermal patches of formulations that prepared by using different natural permeation enhancers such as Pepper oil, Clove oil and Ginger oil are thin, transparent, flexible, smooth and uniform where as formulations prepared by Ginger oil yielded thickly patches. The transparent nature of patches can see more prominently in formulations prepared by Pepper oil. The flexibility can be due to HPMC.

ii) Patch weight uniformity

The weights of all transdermal patches were found to be uniform. The weight of formulations was determined by digital electronic balance and results are given in table 3. The weights of formulations were found to be in the range of 0.295gm. to 0.299 gm. This indicates that there is no significant weight variation in all formulations.

iii) Thickness uniformity

Thicknesses of formulations were measured using digital callipers, and results are given in table-3. Thickness of transdermal patches was found to be in the range of 0.18 mm to 0.21 mm. The results showed that thickness was uniform for all the prepared transdermal patches.

iv) Drug content uniformity

Homogeneous uniform drug distribution is one of the important characteristic of transdermal patches that ensures the uniform reproducible sustained release of the drug from the patch. The drug content uniformity of all the formulations was determined. Estimation of drug content indicated that the drug is uniformly distributed throughout the patches. The results of the drug content in all the formulations were found to be in the range of 97.21 % to 98.95 %. (Table 3)

v) Moisture Gain: Among the formulations, **GOTP1** showed maximum moisture uptake i.e. 2.93 % and **COTP2** showed minimum moisture uptake i.e. 2.19 %. The percentage moisture uptake results are shown in table -3

vi) Moisture Loss: Among the formulations, **GOTP1** showed maximum moisture uptake i.e. 1.56 % and POTP2 showed minimum moisture loss i.e.1.10 %. The percentage moisture loss results are shown in table- 3

vii) Folding Endurance

The folding endurance measures the ability of patch to withstand rupture. It was found to be satisfactory.

The results indicated that the patch would not break and would maintain their integrity with general folding when used. The patches were quite flexible as shown by the measurement of folding endurance (Table-3). All the transdermal patches were evaluated for folding endurance by folding the patch at the same place till it breaks. The folding endurance values were found in the range of 250 to 316 times. The formulation **POTP2** was found to have lowest folding endurance, whereas formulation **GOTP1** was found to have highest folding endurance. The folding endurance of patches increases with increase in the concentration of penetration enhancers.

viii) In-vitro drug permeation

The drug permeability depends on the permeation enhancer concentration and the crosslink density of patches. Release of the drug from transdermal patches is controlled by the chemical properties of the drug and delivery form, as well as physicochemical properties of the dialysis membrane. The process of drug release in most controlled release devices is governed by diffusion and the polymer matrix has strong influence on the diffusivity as the motion of a small molecule is restricted by the three dimensional network of polymer chains.

In the present study different permeation enhancers was used to prepare patches. In order to overcome the barrier properties of skin, permeation enhancers were employed in this study.

The *in-vitro* diffusion study was performed and data obtained from different formulations of Itraconazole transdermal patches are shown in table 4

The release of the drug from its transdermal patch formulations can be ranked in the following descending order:

COTP2>POTP2>COTP1>GOTP2>POTP1>GOTP1

Where the amounts of the drug release of formulations POTP1, POTP2, COTP1, COTP2, GOTP1 and GOTP2 after 12 hours were found to be 64.916%, 72.065%, 70.186%, 74.719%, 64.916%, 67.131% respectively. COTP2 achieved a high cumulative amount of drug permeation at the end of 12 hours. The highest % release from COTP2 formulation may be

due to the presence of high concentration of permeation enhancer clove oil. The permeability of Itraconazole with clove oil (6%) was found highest in our study.

ACKNOWLEDGEMENTS

The authors wish to thank: Itraconazole was kindly gifted from Hetero Pharma Ltd, Hyderabad, India. And also thankful to management of Marri Laxman Reddy Institute of Pharmacy, Hyderabad for providing the all facilities for carried out this research work.

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