REVIEW ARTICLE

EVALUATION AND CHARACTERIZATION OF TRANSDERMAL THERAPEUTIC SYSTEMS: AN EXHAUSTIVE PICTORIAL AND FIGURATIVE REVIEW

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ABSTRACT

Designing and development of transdermal patches can be described as state of the art. The development of Transdermal drug delivery system is multidisciplinary activity that encompasses fundamental feasibility studies starting from the selection of drug molecule to the demonstration of sufficient drug flux in an ex vivo and in vivo model followed by fabrication of a drug delivery system that meets all the stringent needs that are specific to the drug molecule (physicochemical and stability factors), the patient (comfort and cosmetic appeal), the manufacturer (scale up and manufacturability) and most important the economy. The assessment of the performance of transdermal therapeutic devices designed for controlled drug release may result in a complex analytical issue and multidisciplinary studies focused on the evaluation of physicochemical, morphological and textural properties of the products may be required. This paper presents a review of transdermally applied formulations with emphasis on the evaluation of patches in a wide variety of evaluation parameters such as Physico-chemical evaluation, Adhesive evaluation, Skin irritation test, In-vitro, Ex-vivo, In-vivo techniques using animal models and human skin and Accelerated stability studies.

Keywords: Transdermal patches, Physico-chemical evaluation, Adhesive evaluation, Skin irritation test, In-vitro, Ex-vivo and In-vivo techniques, Accelerated stability studies.

INTRODUCTION

Development of controlled release 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. These studies are predictive of transdermal dosage forms and can be classified into following types:

I. Physico-chemical evaluation
II. Evaluation of Adhesive
III. In-vitro and Ex-vivo drug release evaluation
IV. In-vivo drug release evaluation
V. Skin irritation test
VI. Other evaluation instrumental techniques
VII. Accelerated stability studies

I. Physico-chemical evaluation
A. Drug- Polymer Interaction studies

The drug and the excipients must be compatible with one another to produce a product that is stable. The interaction between drug and excipients affect the bioavailability and stability of the drug. If the excipients are new and have not been used in formulations containing the active substance, the compatibility studies play an important role in formulation development.

Interaction studies were conducted on the medicated TDDS formulations by comparing them with the pure drug and placebo formulations on the basis of Thermal analysis (Differential Scanning Calorimetry), Fourier transform infrared spectroscopy (FTIR), ultra violet (UV) and chromatographic techniques by comparing their physicochemical properties like Assay, Melting point, Wave numbers, and Absorption maxima, RF value etc.

Assay

The patches were dissolved in the respective solvent and the drug content was determined by UV spectrophotometry [1,2].

UV Analysis

The medicated and blank formulations were filtered through Whatman filter paper no. 42 and scanned spectrophotometrically at the range of 200–400 nm [2].

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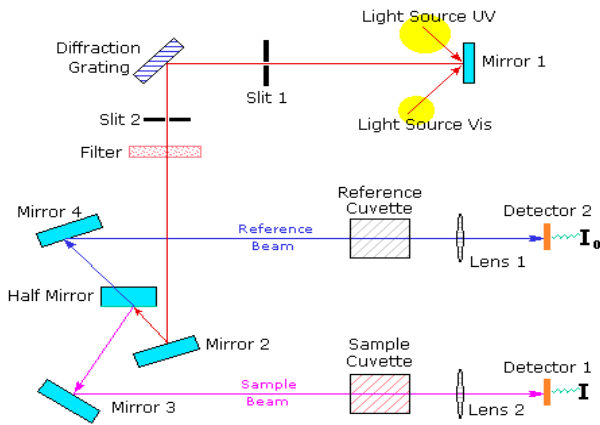*Contact No.:- +91-9754931377*
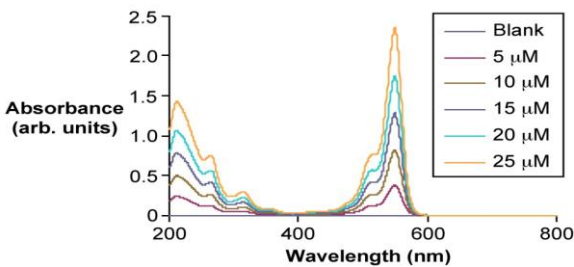
Figure 1: UV-Visible Spectrophotometer

Figure 2: Typical UV-Visible Spectra

Thin Layer Chromatography (TLC)

Compatibility studies are carried out to assess any incompatibility between drug and polymers. The TLC plates having silica gel coating of 0.25 mm thickness were used after activating in an oven for one hour at 120 ^{0}C. A spot of the standard solution was put 2 cm from the bottom. The sample solution was spotted 2cms apart from one another and placed in TLC chamber, which was previously saturated with the solvent system. The solvent was allowed to rise at least 3/4th of the plate and the distance travelled by solvent front was noted. After the migration of the solvent system, the plate was air dried and visualized in CAMAG UV TLC cabinet, whereas; iodine vapor were used as visualizing agent. The Rf value was calculated for the standard and sample solutions using the formula [3]

Rf Value = Distance travelled by the solute from the origin/ Distance travelled by the solvent from the origin
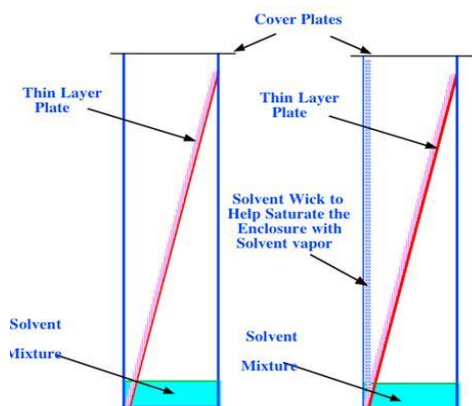

Figure 3: Thin Layer Chromatography

Fourier Transform Infra-Red (FTIR)

The FTIR absorption spectra of the pure, medicated and blank formulations were taken in the range of 400–4000 cm^{-1}using the potassium bromide disc method. IR spectroscopy is one of the important analytical techniques to determine the structure of a compound and predict the presence of certain functional groups which are observed at a definite frequency. A peak-by-peak correlation is excellent evidence for identity [4,5,6].
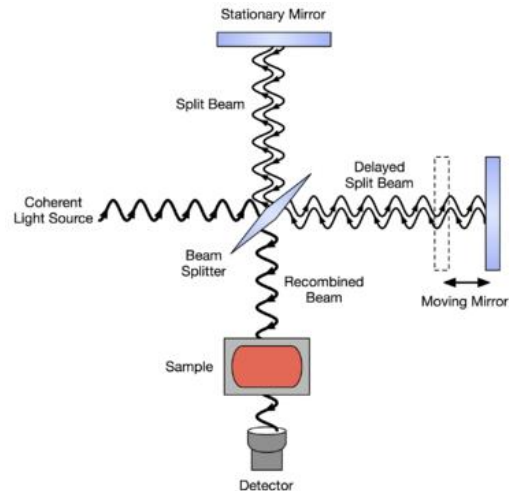

Figure 4: Fourier Transform Infrared Spectrophotometer

Differential Scanning Calorimetry (DSC)

The DSC of the pure drug and drug-polymer blend was studied at a scanning rate of 10°C/ min between 50 to 300°C. The sample was hermetically sealed in an aluminum crucible. Nitrogen gas was purged at the rate of 10 ml/m for maintaining inert atmosphere [4].
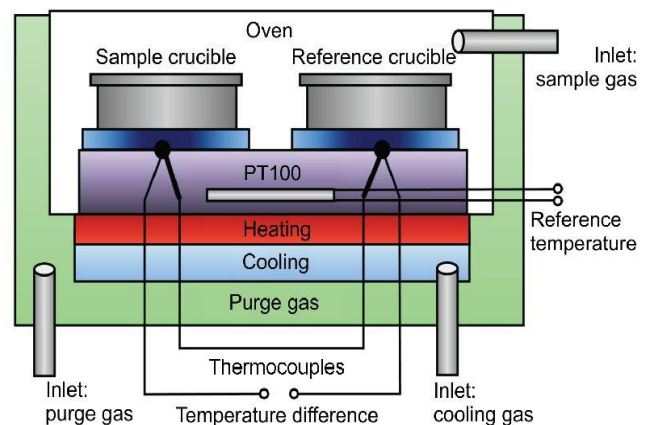

Figure 5: Differential Scanning Calorimeter

B.   Physical appearance:

All the prepared patches were visually inspected for color, clarity, flexibility and smoothness [7,8].

C.   Thickness uniformity

The thickness of transdermal film is determined by travelling microscope, dial gauge, screw gauge or micrometer at different points of the film. The thickness was measured at six different places on each film and the average thickness of the film was taken as the thickness of the film [9,10].

D.   Uniformity of weight

A specified area (1 cm^2) of patch is to be cut in different parts of the patch and is to be dried at 60°c for 4hrs before testing and Weight variation is studied by individually weighing 10 randomly selected patches and calculating the average weight. The individual weight should not deviate significantly from the average weight [11,12].

E.   Drug content determination

An accurately weighed portion of patch is dissolved in 100 ml of suitable solvent in which drug is soluble and then the solution is shaken continuously for 24 hrs in shaker incubator. Then the whole solution is sonicated for complete extraction of drug from the patch. After incubation and subsequent filtration, drug in solution is estimated against the reference solution consisting of placebo films (contains no drug) with the suitable method (UV or HPLC technique) [13,14].

F.   Surface pH

Transdermal films were allowed to swell for 2 h at 37°C on the surface of an agar plate, prepared by dissolving 2% (w/v) agar in worm isotonic phosphate buffer of pH 5.5 under stirring and then pouring the solution into a Petri dish till gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. After 90 s the color developed. The mean of six reading was recorded and tabulated [15].

G.   Folding endurance

Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme conditions of folding. Folding endurance is determined by repeatedly folding the film at the same place until it break; the number of times the films could be folded at the same place without breaking is folding endurance value [16].

Figure 6: Folding Endurance tester

H.   Tensile Strength

Tensile strength of the film was determined with Universal Strength Testing Machine (Hounsfield, Slinfold, Horsham, U.K).The sensitivity of the machine was 1 g. It consisted of two load cell grips. The lower one was fixed and upper one was movable. The test film of size (4×1cm^2) was fixed between these cell grips and force was gradually applied till the film broke. The tensile strength of the patch was taken directly from the dial reading in kg/cm^2. Tensile strength is expressed as follows [17,18,72]:

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross section area}}$$

Figure 7: Tensile Strength Testing Machine

I.   Percentage elongation break test

The percentage elongation break is determined by noting the length just before the break point, the percentage elongation can be determined from the below mentioned formula [19]:

$$\text{Elongation \%} = L_1 - L_2/L_2 \times 100$$

Where, L_1 is the final length of each strip and L_2 is the initial length of each strip.

J.   Flatness

A transdermal patch should possess a smooth surface and should not constrict with time. This can be demonstrated with flatness study. For flatness determination, one strip is cut from the centre and two from each side of patches. The length of each strip is measured and variation in length is measured by determining percent constriction. 0% constriction is equivalent to 100 % flatness [20,75].

$$\text{\% constriction} = L_1 - L_2/L_1 \times 100$$

L2 = Final length of each strip

L1 = Initial length of each strip

K.   Percentage moisture absorption

Initial weight of the patch was taken and noted, then weighed patch are kept in desiccators at room temperature for 24 h. These are then taken out and exposed to 75% relative humidity using saturated solution of sodium chloride in desiccators until a

constant weight is achieved. Final weight of the patch was calculated and percentage moisture uptake is calculated as given below [21].

$$\% \text{ Moisture uptake} = \frac{\text{Final wejght} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Percentage moisture loss

The prepared patch are weighed individually and kept in a desiccators containing fused calcium chloride at room temperature for 24 h. The patch is weighed again after a specified interval until they show a constant weight. The percent moisture content is calculated using following formula [22, 23,24,65]:

$$\% \text{ Moisture Content} = \frac{\text{Initial wejght} - \text{Final weight}}{\text{Final weight}} \times 100$$

L. Water vapor Transmission Rate

For this study, vials of equal diameter were used as transmission cells. These cells were washed thoroughly and dried in an oven, about 1 g of fused calcium chloride was taken in cells and the polymeric patches measuring 1 cm^2 area were fixed over the brim with the help of an adhesive. The cells were weighed accurately and initial weight was recorded, and then kept in a closed desiccator containing saturated solution of potassium chloride to maintain 63 % RH. The cells were taken out and weighed after 72 h. The amount and rate of water vapor transmitted was calculated by the difference in weight using the formula [25,26,70]:

$$\text{WVT rate} = WL/S$$

Where, W is the water vapor transmitted in grams, L is the thickness of the film in cm, and S is the exposed surface area in square cm.

M. Swelling Studies

Weight and area increase due to swelling were measured.

Weight increase due to Swelling

The drug-loaded patch of size 1 x 1 cm^2 was weighed on a pre-weighed cover slip. It was kept in a petridish and 50 ml of phosphate buffer (pH 7.4) solution was added. After every five min, the cover slip was removed, wiped with tissue paper, and weighed upto 30 min. The difference in the weights gives the weight increase due to absorption of water and swelling of patch [27, 28].

Area increase due to Swelling

A drug loaded patch of size 1x1 cm^2 was cut and placed in a petridish containing 50 ml of phosphate buffer (pH 7.4) solution. A graph paper was placed beneath the petridish and was clearly visible, which facilitated the measurement of increase in the area. An increase in the length and breadth of the patch was noted at five min intervals for 60 min and the area was calculated. The percent swelling, %S was calculated using the following equation;

$$\%S = \frac{X_t - X_o}{X_o} \times 100$$

Where Xt is the weight or area of the swollen patch after time t and Xo is the original patch weight or area at zero time.

N. Bursting strength

A test for measuring the resistance of a film to bursting and reported in kilo-Pascal or pounds per square inch or kg/cm^2. The burst strength of all the patches was evaluated by using standard bursting strength tester [29, 30].

Figure 8: Bursting Strength Tester

II. Evaluation of Adhesive

Pressure sensitive adhesives are evaluated for the following properties [27-31,64,68,73,76,84,86].

A. Peel adhesion properties

Peel adhesion is the required to remove an adhesion coating from a test substrate. The important in transdermal devices because the adhesive should provide adequate contact of the device with the skin and should not damage the skin on removal. Peel adhesion properties are affected by the molecular weight of the adhesive polymer, the type & amount of additives and polymer composition. It is tested by measuring the force required to pull a single coated tape applied to a substrate at 180° angle.
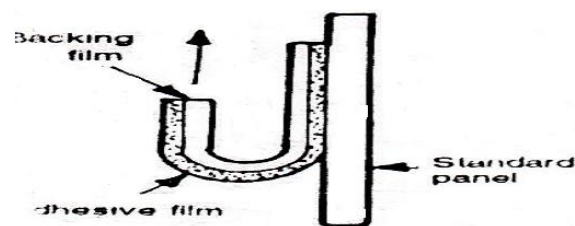

Figure 9: Peel adhesion test for adhesive evaluation

B. Shear strength properties

Shear strength is the measurement of the cohesive strength of an adhesive polymer. Adequate cohesive strength of a device will mean that the device will not slip on application and will leave no residue on removal. It is determined by measuring the time it takes to pull on adhesive coated tape off a stainless steel plate when a specified weight is hung from the tape which pulls the tape in a direction parallel to the plate.
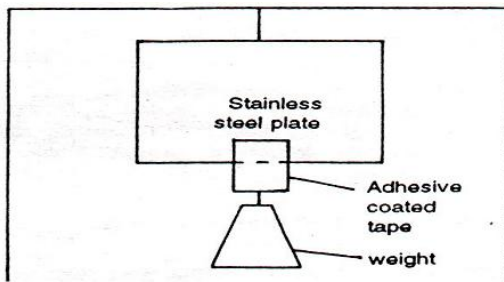
Figure 10: Shear strength test for adhesive evaluation

C. Track properties

Track is the ability of a polymer to adhere to a substrate with little contact pressure.

It is important in transdermal devices which are applied with finger pressure. Track is dependent on the molecular weight and composition of polymer as well as the use of tackifing resins in the polymer. Tests for tack include:

i) Thumb tack test

This is a subjective test in which evaluation in done by pressing the thumbs briefly into the adhesive experience is required for using this test.

ii) Rolling ball tack test

This test involves measurement of the distances that a stainless ball travels along an upward-facing adhesive. The less tacky the adhesive the farther the ball will travel.
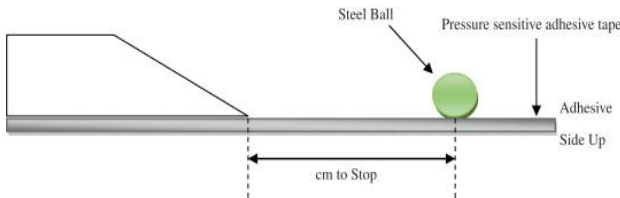

Figure 11: Rolling ball tack test for adhesive evaluation

iii) Quick stick (or peel-tack) test

The peel force required to break the bond between an adhesive and substract is measured bnuy pulling the tape away form the substract at 90° ˢat a speed of 12 inch/min.
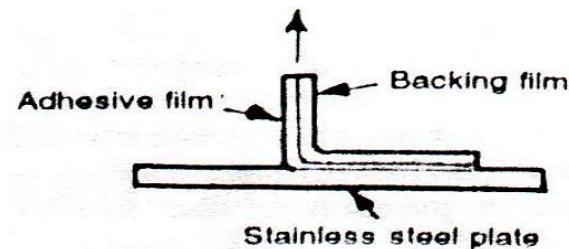

Figure 12: Quick stick test for adhesive evaluation

iv) Probe tack test

The force required to pull a probe away from on adhesive at a fixed rate is recorded at tack.
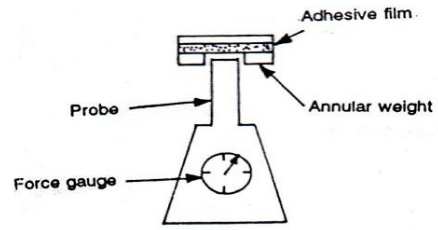

Figure 13: Probe tack test for adhesive evaluation

III. In-vitro and Ex-vivo drug release evaluation
A. In vitro drug release studies

The release rate determination is one of the most important studies to be conducted for all controlled release delivery systems. The dissolution studies of patches are crucial because one needs to maintain the drug concentration on the surface of the stratum corneum consistently and keep it substantially higher than the drug concentration in the body, to achieve a constant rate of drug permeation.[63,66,67] The following apparatus are used for in-vitro drug release studies for transdermal therapeutic systems:-

a. **The Paddle over Disc (USP apparatus V)**
b. **The Cylinder modified USP Basket (USP apparatus VI)**
c. **The Reciprocating disc (USP apparatus VII)**
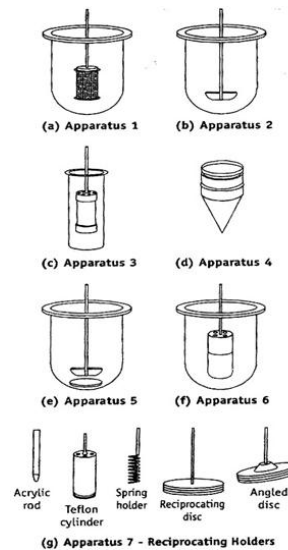
Figure 14: Different Dissolution Apparatus as per USP

a. The Paddle over Disc (USP apparatus V)

The paddle over disc method (USP apparatus V) can be employed for assessment of the release of the drug from the prepared patches. This method is identical to the USP paddle dissolution apparatus, except that the transdermal system is attached to a disc or cell resting at the bottom of the vessel. Dry films of known thickness were cut into definite shape, weighed, and fixed over a glass plate with an adhesive. The glass plate was then placed in a 500 ml of the dissolution medium or phosphate buffer (pH 7.4), and the apparatus was equilibrated to $32 \pm 0.5°C$. The paddle was then set at a distance of 2.5 cm from the glass

plate and operated at a speed of 50 rpm. Samples (5 ml aliquots) can be withdrawn at appropriate time intervals up to 24 h and analyzed by UV spectrophotometer or HPLC. The experiment was performed in triplicate and the mean value calculated [32].

Figure 15: Paddle over Disc (USP apparatus V)

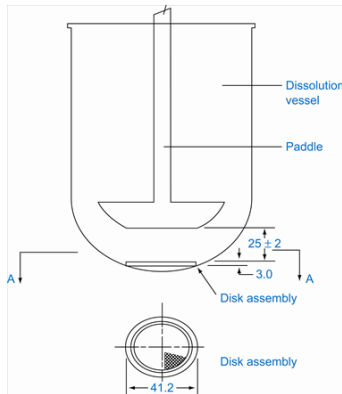**b. The Cylinder modified USP Basket (USP apparatus VI)**

This method is similar to the USP basket type dissolution apparatus, except to replace the basket and shaft with a stainless steel cylinder stirring element. immersed in medium at 32 ±0.5°C [33].
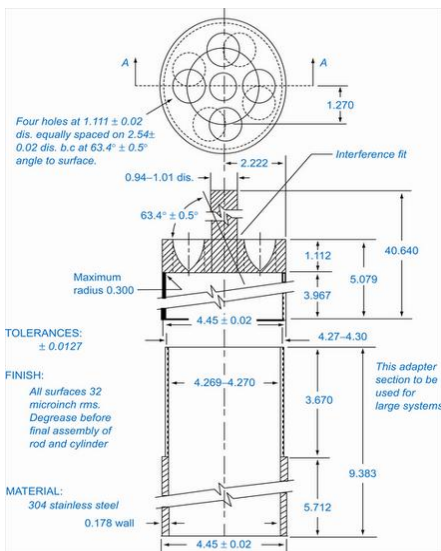

Fig 16: Cylinder modified USP Basket (USP apparatus VI)

c. The reciprocating Disc/Holder apparatus (USP apparatus VII)

Originally introduced in the USP as a small-volume option for small transdermal patches, the reciprocating disk apparatus was later renamed the reciprocating holder apparatus with the adoption of four additional holders for transdermal systems, osmotic pumps, and other low-dose delivery systems. In this method patches attached to holders are oscillated in small volumes of medium, allowing the apparatus to be useful for systems delivering low concentration of drug [34].
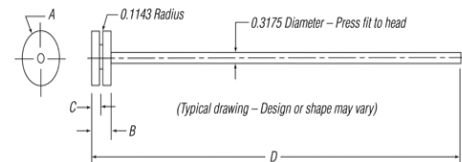
Dimensions are in centimeters

System[a]	HEAD				ROD		O-RING
	A (Diameter)	B	C	Material[b]	D	Material[c]	(not shown)
1.6cm²	1.428	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-113-V884-75
2.5cm²	1.778	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2-016-V884-75
5cm²	2.6924	0.7620	0.3810	SS/VT	8.890	SS/P	Parker 2-022-V884-75
7cm²	3.1750	0.7620	0.3810	SS/VT	30.48	SS/P	Parker 2-124-V884-75
10cm²	5.0292	0.6350	0.3505	SS/VT	31.01	SS/P	Parker 2-225-V884-75

[a] Typical system sizes.
[b] SS/VT=Either stainless steel or virgin Teflon.
[c] SS/P=Either stainless steel or Plexiglas.

Fig 17: Reciprocating disc sample holder

B. Ex vivo skin permeation studies (In vitro skin permeation studies)

The amount of drug available for absorption to the systemic pool is greatly dependent on drug released from the polymeric transdermal films. The drug reached at skin surface is then passed to the dermal microcirculation by penetration through cells of epidermis, between the cells of epidermis through skin appendages.

Skin model used for transdermal drug delivery system

Various skin models used by various researchers are given in Figure. Though there is no rule regarding to selection of the skin model. But generally researchers start with artificial membrane, then in vitro animal skin, then in vitro human skin (cadaver skin), then in vivo animal skin, then finally in vivo human skin 34,35,69,71,74.
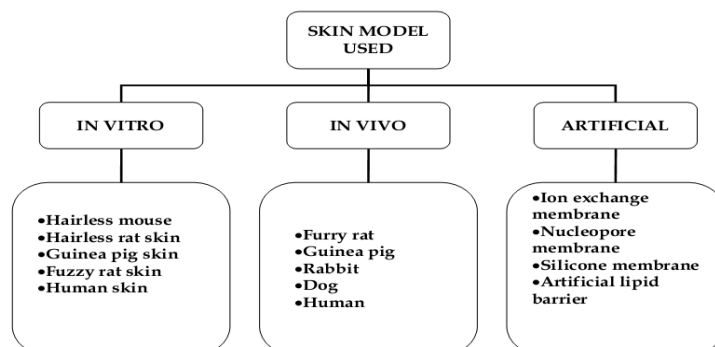

Fig 18: Various skin models for transdermal drug delivery system

Animal Models

Ex vivo penetration and permeation are routinely performed to study percutaneous absorption and transdermal permeation characteristics of drugs and other chemicals. These ex vivo studies allow the determination of drug concentration in the skin (penetration) and rate of transfer across the skin (permeation). Ex vivo experiments are easy to perform and the simplicity of methodology allows flexibility in adapting the model in addressing different aspects involved in preliminary or feasibility studies in the development of skin/transdermal drug delivery systems. It would be advantageous to use human cadaver skin for in vitro permeation studies but, in practice, for most investigators, human cadaver skin is not readily available. Thus animal skin is frequently used for in vitro studies. Weanling pig skin (skin from a pig that has recently been weaned) is recognized as the closest alternative to human cadaver skin in its permeability and lipid composition.

An animal skin membrane that is sufficiently similar or close to human skin is needed to substitute human skin in ex vivo penetration and permeation, and topical bioavailability studies. Human skin is difficult to obtain and uniformity is difficult to maintain, since most of human skin comes from cadavers whose sex, age and genetic history are uncontrolled. Where as, animal skin is easier to obtain and is more uniform. Humans and animals have wide differences in the number of appendageal openings per unit area thickness of skin, structure and porosity of skin, and these factors clearly affect the percutaneous absorption of drugs. Only the progress in in vitro methodology with an appropriate animal model can resolve these limitations.

It would be a simple mathematical exercise to predict the skin permeability in humans from animal experiments. If there is constant ratio between skin permeability in human to animal, independent of the drug under study. If correlation can be established for drugs with different physicochemical properties, experiments using appropriate animal skin will be more reliable in the developmental stage of skin/transdermal drug delivery systems.

It appears that hairless guinea pig and Brattleboro rat are to be good animal models for skin/ transdermal drug delivery systems, where as snake appears not to be a good model to evaluate permeation of drugs across skin. In reviewing the studies comparing transdermal absorption of drugs between animals and humans, care must be taken to ascertain the influences of methodology and model might have on the data, and utmost care must be taken to avoid any misinterpretation or wrong conclusions [35,78-80].

According to the literature available no consensus is available regarding animal models which truly reflect human skin.

Skin Preparation

Respective skin was excised from the animals. The subcutaneous fat, tissue, blood vessel and epidermal hairs were carefully removed. The skin with free of obvious holes or defects were cleaned with normal saline and finally with sterile water. Then it was soaked in phosphate buffer pH 7.4 solutions for 12 hrs refrigeration before use. T o perform ex-vivo skin permeation, the skin was thawed at room temp and used [35,36].

Apparatus for Ex vivo skin permeation studies

Ex vivo skin permeation methods are used in laboratories all over the world with the intention to assess the penetration characteristics of specific substances. A range of different designs have been developed with the general aim to measure the penetration of agents through the skin membrane into a fluid reservoir [77,81].

a. Static Franz diffusion cell

In 1975 Franz developed a static diffusion cell which is now one of the most commonly used in vitro systems in the research of skin penetration. The system has a simple design and is inexpensive to use. Human as well as animal skin can be mounted on the metal grid which divides the donor chamber and the receptor chamber. The skin is set placing the dermis in contact with the receptor fluid below. The skin can be either full-thickness or split-thickness skin. The receptor compartment has a volume of 5 -12ml and effective surface area of 1-5 cm^2. The diffusion buffer is continuously stirred at 600rpm by a magnetic bar. The receptor chamber of the cell is placed in circulation water in a water bath with a temperature of 37 ºC keeping the temperature at the skin surface at 32° C to imitate a real life skin condition as much as possible. The receptor fluid is kept homogenous in concentration and in temperature by a magnetic stirring bar. The fluid in the receptor chamber is manually sampled at predefined time intervals [38].
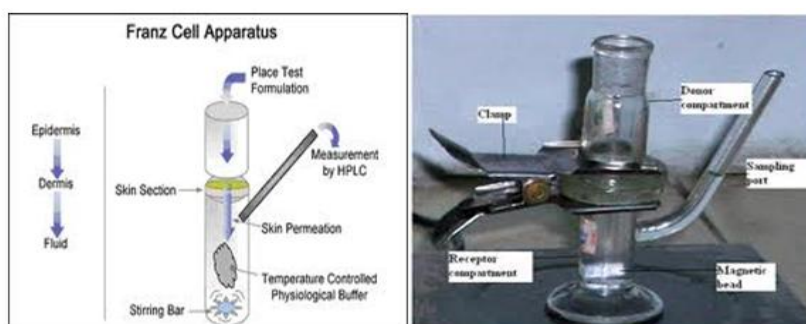

Figure 19: Static Franz diffusion cell

a. Horizontal /Side-by-side diffusion cell

This has been widely used for the evaluation of drug permeation across skin. The cell is divided in receptor and donor compartments with a low solution volume (3.5ml) for each compartment and a small membrane area (0.64cm^2). They are continuously stirred by matched set of star-head magnets, which are rotated at a speed of 600rpm. The system is controlled by thermostated water through a water jacket surrounding the two compartments [39].

Figure 20: Horizontal /Side-by-side diffusion cell

b. Flow-through Franz diffusion cell

Flow through diffusion cells have the advantage that they can be used when the drug has lower solubility in the receptor compartment. This cell can be fully automated and connected directly to HPLC. They have large capacity donor chamber to allow appropriate loading of the applied compound and a low volume (0.3ml) receiving chamber that ensures rapid removal of penetrant at relatively low pumping rates.

The receptor fluid is in the flow-through cells continuously replaced and collected every hour to imitate an in vivo situation where the blood circulation removes the transdermal penetration substances. This has an additional benefit when dealing with substances with low solubility in the receptor medium and the sink conditions are maximized as the fluid is continually replaced. The flow-through system provides an environment similar to real physiological conditions by the continuous replacement of receptor fluid resembling the systemic uptake of the drugs/chemicals in the blood vessels [40,41].
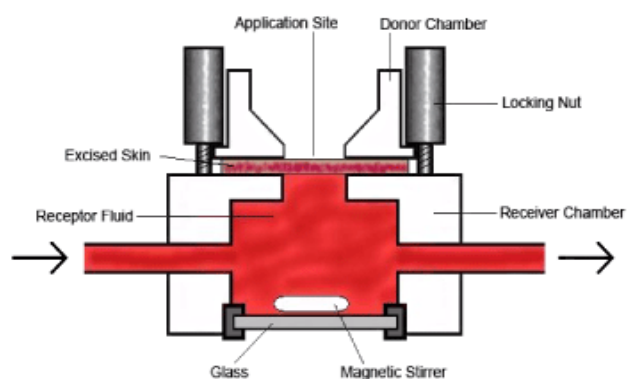

Figure 21: Flow-through Franz diffusion cell

IV. In-Vivo Evaluation

In vivo technique is based on a physiologically and metabolically intact system. There are two kinds of in vivo studies [82, 83, 85, 87]:

1) Animal studies

2) Human studies

1) Animal studies

In vivo animal models are preferred because considerable time and resources are required to carry out studies in humans. Some of the species that have been used for in vivo testing include; mouse, rat, guinea pig, rabbit, hairless mouse, hairless rat, hair less dog, cat, dog, pig, swine, horse, goat, squirrel, monkey, rhesus monkey, chimpanzee, etc. Various experiments have been carried out to determine which of the animal models provide the best prediction of the behavior of the device, being tested, in humans. The most frequently used animal is the rat even though it is well known that rat studies generally overestimate human skin absorption. Other animals like Rhesus monkey is one of the most reliable models for in vivo evaluation and demonstrate a better agreement with human absorption, but the costs of these are considerably higher. Various experiments conducted lead us to a conclusion that hairless animals are preferred over hairy animals in both in vitro and in vivo experiments.

A group of researchers has investigated percutaneous absorption and found a decreasing order of permeation, thus, rabbit>rat> swine>man. Nevertheless, other studies have shown that that data from these lower animals are comparable to that of man even though such studies in animals either in vivo or in vitro can only be useful approximations of activity in man [42-44].

2) Human studies

The last phase in the transdermal absorption studies of a drug is the in vivo clinical studies on humans. The standard principals described were application of the test substance to the skin in proper form and time, taking samples of different body fluids, excreta or tissue at specific intervals, and quantifying the test substance in the samples or the metabolite in the samples by an appropriately sensitive analytical method [45].

The conduct of in vivo studies in volunteers is closely regulated. The study protocol should be approved by an ethical committee and the subjects have to give written informed consent.

Several methods are available for in vivo percutaneous studies. These are discussed below:

a. Traditional in vivo technique (Plasma/ excreta measurement)

The test substance is applied to the skin of healthy humans and blood and/or urine is collected and analysed. The amount of test substance measured in the blood and/or urine gives a good indication of the amount of substance absorbed through the skin into the systemic circulation. In vivo studies, the substance is applied

during a certain period to a specified area of the skin site of volunteers/animal. The percutaneous absorption can be assessed by analyzing the parent chemical and/or its metabolite(s) in the skin layers or in biological media such as plasma, urine or exhaled air. The amount of a chemical measured after dermal exposure is compared to that after a reference exposure with a known dose via, for example, intravenous administration or inhalation.

This in vivo technique has been used before all other techniques were ever considered and is still used where there is no adverse risk to the volunteers participating. Often the test of a substance is intended to disclose different unknown characteristics of the substance signifying that the knowledge of the substance is limited or incomplete and therefore the risk assessment may not be completely explained. This gives rise to ethical concern and limits the use of healthy volunteers [46-48].

b. Microdialysis

Microdialysis is a technique used in the clinic as well as in research for sampling of endogenous and exogenous substances in the extracellular space in the living tissue e.g. the skin. Microdialysis is so far the only technique that provides information from the extracellular space, and it is therefore of great importance in the investigation of pharmacological and biochemical procedures in these tissues. Several results in drug discovery and development are established by measuring serum concentrations of different molecules, even though most drugs exert their effects in the tissues and not in the blood stream. Thus, data on pharmacokinetics at the target site are important, just as determination of pharmacodynamic effects in relation to tissue drug concentrations in the target tissue is a more precise approach to describe exposure effects.
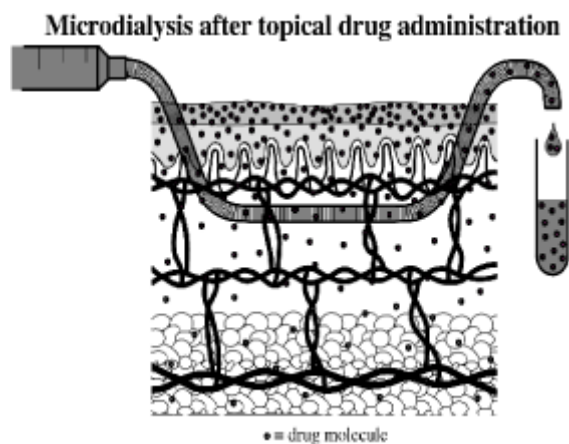

Figure 22: Microdialysis

Microdialysis is currently the most essential tool to estimate active drug profiles at the target site and for providing pharmacokinetic and pharmacodynamic information. Principals in microdialysis are to imitate the function of a small blood vessel in the dermis. A test substance applied to the skin will penetrate the skin surface to the dermis where the artificial blood vessel/probe is placed. The probes consist of a semi-permeable structure which allows molecules to pass into the perfusate inside the probe by passive diffuse. The molecules follow a concentration gradient across the

probe membrane as the perfusate inside the probe passes through, driven at a constant and very accurate pace by a pump. A partial equilibration of molecules across the membrane occurs. The perfusate – now called the dialysate - leaves the probe, holds the test substance and is collected in small vials for analysis. This technique has been used in human volunteers as well as in animals [49, 50].

c. Tape stripping method

Tape stripping is a well known in vivo technique but can also be used in vitro. A test substance (often radioactively labelled) is allowed to penetrate the skin at a predetermined area for a certain time period. Afterwards the skin is gently washed to remove remaining unabsorbed test substance on top of the skin. The method then involves sequentially removing of microscopic layers of the exposed stratum corneum by repeated application and removal of adhesive tape. The amounts of recovered substance in these shedded cells attached to the tape are then analyzed using an appropriate analytical method. It has been reported that the amount of the chemical in these skin layers gave good estimate of the total amount of the chemical absorbed into the systemic circulation. The method is inexpensive, uncomplicated and a minimally invasive method given that only dead cells (corneocytes) embedded in their lipid matrix are removed. Tape stripping is a particularly helpful technique to assess the local bioavailability of drugs whose target site is the stratum corneum itself, like e.g. antiseptics, antifungal drugs [51,52].

Figure 23: Tape stripping method

V. Primary Skin irritation study

The major cutaneous toxicological reaction results from direct toxic injury to cell membrane, cytoplasm or nuclei. This is generally manifested (to show, clearly especially a feeling) by inflammation and itching and can occurs from the drug, vehicle or absorption enhancer. Reversible inflammatory changes occurs due to interaction of chemicals with the sensory receptors in the skin at the site of application. The albino rabbit is the preferred animal species when testing for skin irritation because of its high skin sensitivity and light skin, on which even slight skin irritant effects of a substance usually can be detected. In general, substances or

products are tested for skin irritation using a test design first proposed by Draize et al. in 1944, which based on scoring method. Scores as assigned from 0 to 4 based on the severity of erythema or oedema formation.

The skin irritation test was performed on healthy albino rabbit weighing 1.5 to 2.0 kg and 24 months of age. The animals were singly housed in suspended stainless steel caging with mesh floors in the animal unit under controlled temperature (27 ± 2°C) with 30 - 70 % relative humidity and 12 hours light/dark cycle. They were fed a standard laboratory diet and filtered tap water was provided ad libitum. A minimum of 7 days acclimatization was allowed before the commencement of the study. The animals were divided in 4 groups, 6 animals per group. The dorsal surfaces (50 cm^2) of the rabbits were shaved carefully avoiding peripheral damage. The skin was cleaned with 75% ethanol. Representative patches were placed on the skin and held in place with an adhesive tape. On the previous day of the experiment, the hair on the back side area of rat was removed. The animals of group I were served as control without any treatment. Transdermal systems (blank and drug loaded) were applied onto nude skin of animals of II and III groups respectively. A 0.8 % v/v aqueous solution of formalin was applied as a standard irritant (Group IV).

The reaction at the site of application was assessed and scored according to the following numerical system. A score of 0 indicates no erythema and edema formation. A score of 1 indicates very slight erythema and edema formation (barely perceptible). A score of 2 indicates well defined erythema and edema formation (edges of area well raised). A score of 3 indicates moderate to severe erythema and edema formation (raised approx.1mm). A score of 4 indicates severe erythema and edema formation (raised more than 1mm and extending beyond area of exposure).

Primary dermal irritation (PDI) score is obtained by adding average erythema and average edema scores. Primary dermal irritation index (PDII) is obtained by adding the PDI scores for 1, 24, 48 and 72 hour scoring intervals and dividing by the number of evaluation intervals i.e. 4. PDI score for the given tested substance is 0 for Non-irritant, 0.1-0.4 for Negligible Irritant, 0.41-1.9 for Slight Irritant, 2.0-4.9 for Moderate Irritant and 5.0-8.0 for Severe Irritant. [53-56]

VI. Other evaluation instrumental techniques
a. X-ray diffraction studies

Diffraction pattern of pure drug, placebo films and drug loaded matrix films were scanned over 2θ ranges of 2^0 and 60^0 at a rate of 2^0 per min in 0.02^0 2θ step size by using X-ray diffractometer consisting of a 30KV, 15MA generator with a Cu-Kα radiation anode tube was used..

The X-ray diffraction gives us about the crystal-line and amorphous nature of the drug within in the transdermal films. The sharp peaks present in diffractogram indicates crystalline of the compound which were absent in case of amorphous compounds.

Diffraction pattern of pure drug will show distinctive peaks which shows that drug is in crystalline form. For the placebo films and drug loaded films no sharp peaks were observed indicates that the drug is in amorphous state in the drug loaded patches [57].
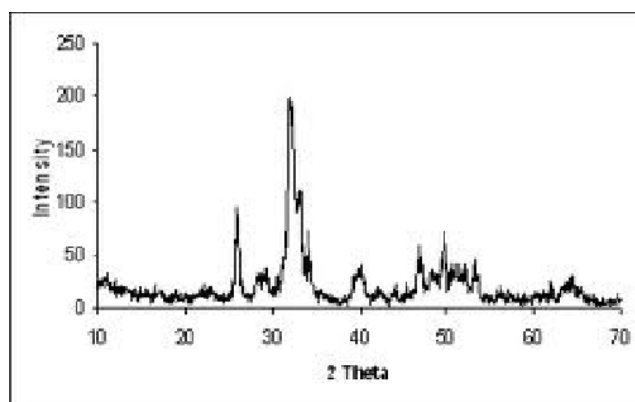

Figure 24: Typical X-Ray Diffraction

b. Scanning electron microscope (SEM) analysis

For the optimized formulation the external morphology of the transdermal patches was analyzed before and after in vitro skin permeation using a scanning electron microscope (SEM). Films were fixed on aluminium studs and coated with gold using a sputter coater SC 502, under vacuum [0.1 mm Hg] at 20 kV.
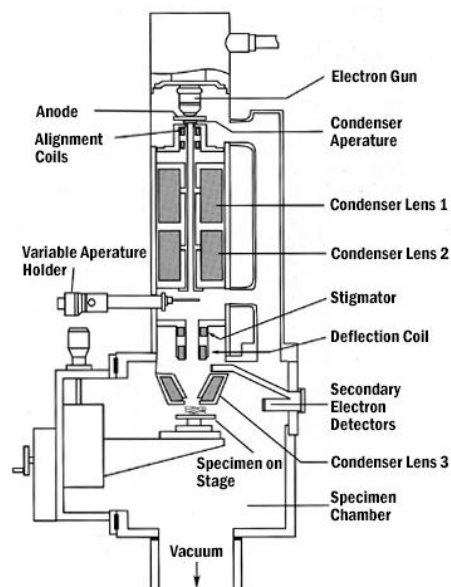
Figure 25: Scanning Electron Microscope

The SEM pictures before in vitro skin permeation exhibits the uniform smooth surface which indicates the homogeneous dispersion of drug in the transdermal films. Uniform distribution of drug in the transdermal films is one of the important characteristics and that also ensures the uniform reproducible sustained release of drug molecules from the films. The SEM pictures after the in vitro skin permeation will show the presence of pores indicating that the drug was released from the films by diffusion [58].

c.   Polariscope examination

This test is to be performed to examine the drug crystals from patch by polariscope. A specific surface area of the piece is to be kept on the object slide and observe for the drugs crystals to distinguish whether the drug is present as crystalline form or amorphous form in the patch [59,60].

VII.   Accelerated stability studies

Stability of a drug has been defined as the stability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications throughout its shelf life. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light, and enables recommended storage conditions, re-test periods and shelf-lives to be established. The International Conference on Harmonization (ICH) Guidelines titled "stability testing of New Drug substances and products" (QIA) describes the stability test requirement for drug registration applications in the European Union, Japan and United Stated of America.

ICH specifies the length of study and storage conditions:

Long Term testing: 25ºC ± 2ºC / 60% RH ± 5% for 12 months

Accelerated Testing: 40ºC ± 2ºC / 75% RH ± 5% for 6 months

The selected formulations were packed in amber colored bottles, which were tightly plugged with cotton and capped with aluminium. They were then stored at 25ºC / 60% RH, 30ºc / 65% RH, & 40ºC / 75% RH for 3 months and evaluated for their drug content and other physicochemical parameters like thickness, flatness, folding endurance, tensile strength, moisture content and moisture uptake, drug content as well as drug release. The logarithms of percent drug remaining were plotted against time in days, which gave almost straight line suggesting that drug degradation followed first order kinetics. The slope of the straight line for each temperature was obtained and the degradation rate constant was calculated using the formula given below:

$$slope = \frac{-K}{2.303}$$

where K is the degradation rate constant

An Arrhenius plot was drawn by plotting logarithm of K values against reciprocals of absolute temperature. The value of K at 25°C (K_{25}) was extrapolated from

the Arrhenius plot and shelf-life of the formulation was calculated by substituting the vales of K_{25} in the following equation [61,62]:

$$t_{0.9} = \frac{0.1054}{K_{25}}$$

Where, $t_{0.9}$ is the time required for 10% degradation of the drug and is referred to as the "Shelf-life"of the product.

CONCLUSION

Transdermal drug delivery systems has been used as safe and effective drug delivery system since 1981.Its potential role in controlled release is being globally exploited by the scientists with high rate of attainment. Drug with right mix of physical chemistry and pharmacology, is remarkably effective in transdermal delivery. Due to large advantages of the TDDS, many new researches are going on in the present day to incorporate newer drugs via the system. A transdermal patch has several basic components like drug reservoirs, liners, adherents, permeation enhancers, backing laminates, plasticizers and solvents, which play a vital role in the release of drug via skin. Transdermal patches are divided into various types like matrix, eservoir, membrane matrix hybrid; micro reservoir type and drug in adhesive type transdermal patches and different methods are used to prepare these patches by using basic components of TDDS. These are after preparation of transdermal patches, evaluated for physicochemical studies, in vitro permeation studies, skin irritation studies, animal studies, human studies and stability studies. All prepared and evaluated transdermal patches must receive approval from FDA before sale. Future developments of TDDSs will likely focus on the increased control of therapeutic regimens and the continuing expansion of drugs available for use. Transdermal dosage forms may provide clinicians an opportunity to offer more therapeutic options to their patients to optimize their care.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

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