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Fabrication and Evaluation of Tansdermal Patches of Primaquine Phosphate

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ABSTRACT

Primaquine phosphate, 8-[(4-amino-1-methyl butyl)amino]-6-metoxyquinoline phosphate, a synthetic compound with potent antimalarial activity and clinical use for treatment of relapses of Plasmodium vivax infections. The drug is usually oral administered for 7-14 consecutive days to achieve radical cure and is known to produce adverse side-effect including gastrointestinal distress, nausea and methaemoglobinemia with cyanosis. In transdermal drug delivery system provide benefits over the oral administration. It is an alternative drug delivery system, in recent years, high patient acceptance in many clinical conditions and the advantages of delivering drugs across the skin for systemic circulation. Fabrication and Evaluation of matrix type Transdermal Patches of Primaquine Phosphate with different ratio of eudragit-L100 and S-100 combination by the solvent evaporation technique. All the patches have been evaluated for their Physiochemical properties was studied by infrared Spectroscopy. Formulation F1 was found best among the all formulation. Hence F1 formulation was used to incorporate penetration enhancer labrafac™ PD, labrefil® 1944CS and lauroglucol™ FCC. By using this three penetration enhancer nine formulation L1 to L9 was fabricated to increasing volume of 0.5ml, 1.0ml, 1.5 ml. All the formulation was tested for thickness, weight variation, folding endurance, surface pH, moisture content and %drug content. L6 was showing maximum drug content and folding endurance. The in-vitro release study was carried out with Shimadzu HPLC system. L6 formulation showed the best release.

Keywords: Primaquine phosphate, Antimalarial, Gastrointestinal distress, Mthaemoglobinemia, Transdermal drug delivery system, penetration enhancer

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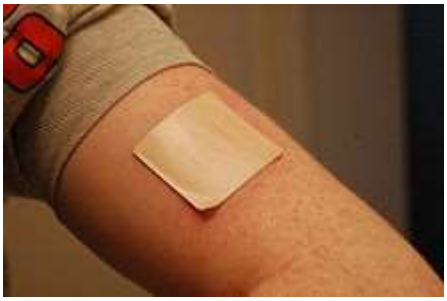
INTRODUCTION

Optimum therapeutic outcomes require not only proper drug selection but also effective drug delivery. The human skin is a readily accessible surface for drug delivery. Over the past three decades, developing controlled drug delivery has become increasingly important in the pharmaceutical industry. The pharmacological response, both the desired therapeutic effect and the undesired adverse effect, of a drug is dependent on the concentration of the drug at the site of action, which in turn depends upon the dosage form and the extent of absorption of the drug at the site of action[1].



A recent approach to drug delivery is to deliver the drug into systemic circulation at pre-determined rate using skin as a site of application. Transdermal patches are delivered the drug through the skin in controlled and predetermined manner in order to increase the therapeutic efficacy of drug and reduced side effect of drug. Controlled drug release can be achieved by transdermal drug delivery systems (TDDS) which can deliver medicines via the skin portal to systemic circulation at a predetermined rate over a prolonged period of time[2].

Delivery of drugs into systemic circulation via skin has generated a lot of interest during the last decade as transdermal drug delivery systems (TDDS) offer many advantages over the conventional dosage forms and oral controlled release delivery systems notably avoidance of hepatic first pass metabolism, decrease in frequency of administration, reduction in gastrointestinal side effects and improves patient compliance[3].

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. It is ideally suited for diseases that demand chronic treatment[4].

Transdermal drug delivery systems (TDDS), also known as transdermal patches, are dosage forms designed to deliver a therapeutically effective amount of drug across a patient's skin. In order to deliver therapeutic agents through the human skin for systemic effects, the comprehensive morphological, biophysical and physicochemical properties of the skin are to be considered.

Transdermal delivery provides a leading edge over injectable and oral routes by increasing patient compliance and avoiding first pass metabolism respectively[5].

Transdermal delivery not only provides controlled, constant administration of the drug, but also allows continuous input of drugs with short biological half-life and eliminates pulsed entry into systemic circulation, which often causes undesirable side effects. Thus various forms of novel drug delivery system such as transdermal drug delivery systems, controlled release systems, transmucosal delivery systems etc., are emerged. Several important advantages of transdermal drug delivery are limitation of hepatic first pass metabolism, enhancement of therapeutic efficiency and maintenance of steady plasma level of the drug. The first transdermal system, transdermal-SCOP was approved by FDA in 1979 for the prevention of nausea and vomiting associated with travel, particularly by sea[6].

Advantages of Transdermal Drug Delivery[7, 8, 9]

Transdermal drug delivery systems offer several important advantages over more traditional approaches, including :-

- Longer duration of action resulting in a reduction in dosing frequency.
- Increased convenience to administer drugs which would otherwise require frequent dosing.
- Improved bioavailability.
- More uniform plasma levels.
- Reduced side effects and improved therapy due to maintenance of plasma levels up to the end of the dosing interval.
- Flexibility of terminating the drug administration by simply removing the patch from the skin.
- Improved patient compliance and comfort via non-invasive, painless and simple application.

Disadvantages of Transdermal Drug Delivery[10]

Some of the greatest disadvantages to transdermal drug delivery are:

- Possibility that a local irritation at the site of application.
- Erythema, itching, and local edema can be caused by the drug, the adhesive, or other excipients in the patch formulation.

Factor Influencing Transdermal Drug Delivery System[10]

Transdermal drug absorption significantly alters drug kinetics. Success depends on a variety of biological physiological, biochemical, and biophysical factors including the following:

- Body site of application.

- Thickness, composition and integrity of the stratum corneum epidermis (a skin layer).
- Size and structure of the molecule (related to molecular weight).
- Permeability of the membrane in the transdermal drug delivery system.
- State of skin hydration.
- pH and other physicochemical drug properties.
- Drug metabolism.
- Lipid solubility.
- Degree of partitioning of the drug and associated components into the skin Depot (reservoir) of/for drug in skin.
- Alteration of blood flow in the skin by additives and body temperature.

Routs Of Transdermal Delivery System[1]

There are two main pathways by which drugs can cross the skin and reach the systemic circulation. The more direct route is known as the transcellular pathway. By this route, drugs cross the skin by directly passing through both the phospholipid membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. Although this is the path of shortest distance, the drugs encounter significant resistance to permeation. This is because the drugs must cross the lipophilic membrane of each cell, then the hydrophilic cellular contents containing keratin, and then the phospholipid bilayer of the cell one more time. This series of steps is repeated numerous times to traverse the full thickness of the stratum corneum. Few drugs have the properties to cross via this method-

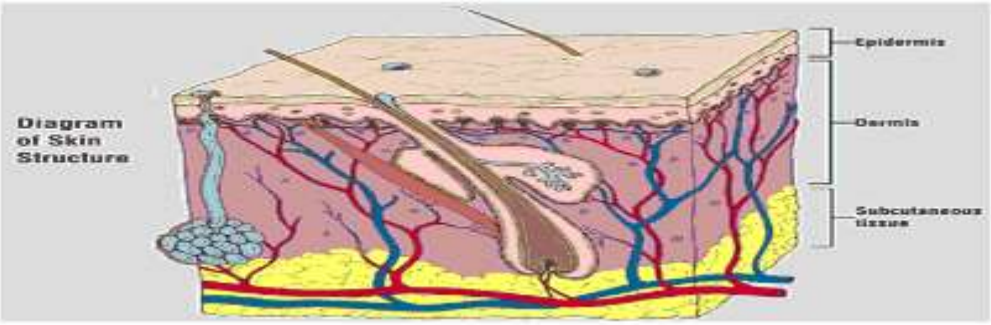


Figure 1: Structure of skin

And second path way- The more common pathway through the skin is via the intercellular route. Drugs crossing the skin by this route must pass through the small spaces between the cells of the skin, making the route more tortuous. Although the thickness of the stratum corneum is only about 20 μm, the actual diffusional path of most molecules crossing the skin is on the order of 400 μm.

The 20-fold increase in the actual path of permeating molecules greatly reduces the rate of drug penetration

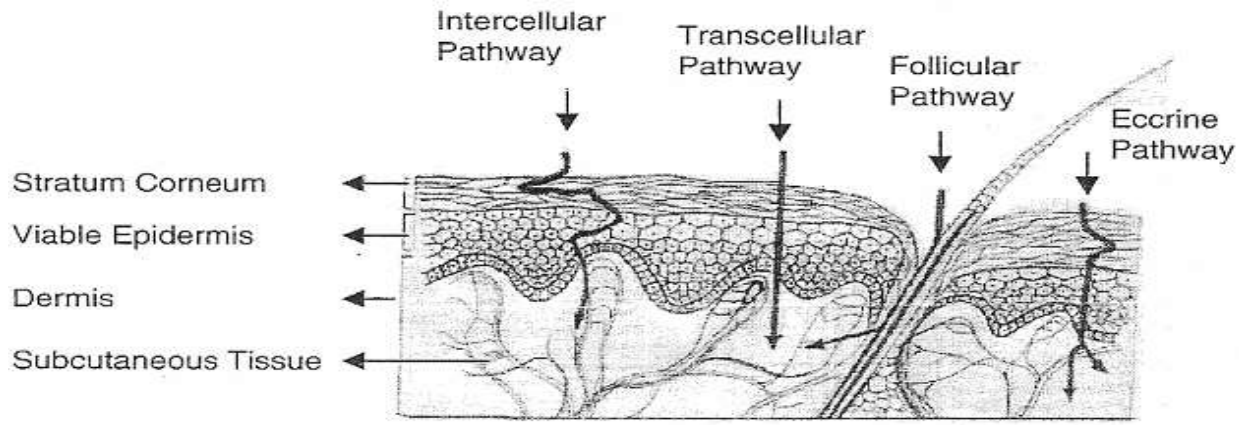


Figure 2: Various routes of drug absorption

MECHANISM

The drug penetration in to systemic circulation-

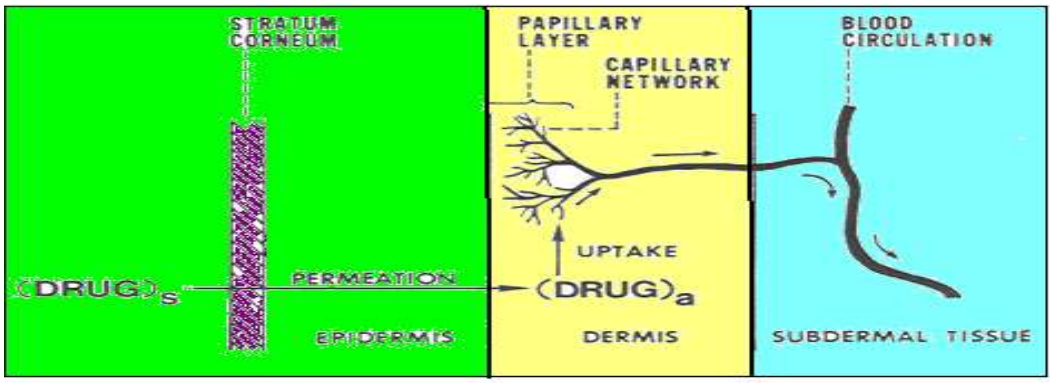


Figure 3: Multilayer skin model showing sequence of transdermal permeation of drug for systemic delivery

Ideal Molecular Properties for Transdermal Drug Delivery [11]

Ideal molecular properties for drug penetration are follows.

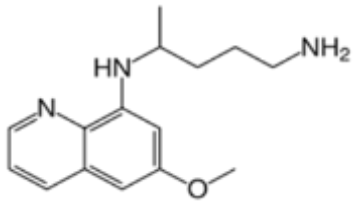
- An adequate solubility in lipid and water is necessary for better penetration of drug (1mg/ml).
- Optimum partition coefficient is required for good therapeutic action.
- Low melting point of drug is desired (<200°C).
- The pH of the saturated solution should be in between 5 to 9.

Drug profile

Primaquine (DrugBank, 2011)

Chemical Characteristics

⇒ **Structure:**



⇒ **IUPAC Name:** N-(5-aminopentan-2-yl)-6-methoxyquinolin-8-amine

⇒ **Category:** Antimalarial and antiprotozoal agent.

⇒ **Chemical formula:** $C_{15}H_{21}N_3O$.

⇒ **Molecular weight:** 259.3467.

⇒ **Log P:** 2.1

⇒ **Melting point:** 195-205^oC

⇒ **Half-life:** 3.4-7.4 Hr.

Pharmacology of Primaquine

Indication: For the treatment of malaria.

Pharmacodynamics:

Primaquine is an antimalarial agent and is the essential drug treating *P. vivax* of malaria. In the blood, malaria parasites break down a part of the red blood cells known as haemoglobin. When this happens haemoglobin is divided into two parts; haem and globin. Haem is toxic to the malaria parasite. To prevent it from being damaged, the malaria parasite produces a chemical which converts the toxic haem into a non-toxic product. Primaquine acts by interfering with a part of the parasite (mitochondria) that is responsible for supplying it with energy. Without energy the parasite dies. This stops the infection from continuing and allows the person to recover. Primaquine kills the intrahepatic form of *Plasmodium vivax* and *Plasmodium ovale*, and thereby prevents the development of the erythrocytic forms that are responsible for relapses (it also kills gametocytes). Primaquine has gametocytocidal activity against all plasmodia, including *P. falciparum*.

Mechanism of action:

Primaquine mechanism of action is not well understood. It may be acting by generating reactive oxygen species or by interfering with the electron transport in the parasite. Also, although its mechanism of action is unclear, primaquine may bind to and alter the properties of protozoal DNA.

Pharmacokinetic

Primaquine is readily absorbed after oral ingestion. It is oxidized in liver with a plasma $t_{1/2}$ of 3.4-7.4 h and excreted in urine within 24 h (Tripathi.K.D., 2008).

MATERIAL AND METHOD

Materials

Primaquine phosphate was received as a gift samples from Merck specialties Pvt Ltd (Mumbai, india).Eudragit L-100and Eudragit S-100 were acquired from Evonik rohm pharma polymer (Germany). Polyethylene glycol (PEG-400) was acquired from Ozone international. Labrafac, Labrifil, Lauroglycol were Gattefosse India Pvt.Ltd (Mumbai, India).Distilled water was used throughout the study.

PRE-FORMULATION STUDIES

Morphological characterization:

Drugs were evaluated on the basis of their appearance like physical form, color, odor, and taste.

Drug identification by IR:

Primaquine Phosphate sample (30mg) was sent to Central Drug Research Institute, Lucknow for IR. The infrared spectrum of the pure Primaquine Phosphate sample was recorded and the spectral analysis was done. The dry sample of drug was directly placed after mixing and triturating with dry potassium bromide.

Drug identification by NMR:

Primaquine Phosphate sample (30mg) was sent to Central Drug Research Institute, Lucknow for NMR.

Drug identification by mass spectroscopy:

Primaquine Phosphate sample (30mg) was sent to Central Drug Research Institute, Lucknow for mass spectroscopy.

Compatibility studies of drug and polymer by DSC

The DSC studies revealed that there was no significant change in the original peak of the drug and the polymers indicating that there is no interaction between drug and polymers [28].

ANALYTICAL METHODS FOR EVALUATION

HPLC analytical method

Chromatographic condition:

HPLC Method was developed by[29] was selected for the analysis. Analysis was performed with shimadzu HPLC system using C_{18} column under reversed phase partition chromatographic condition. Mobile phase consisting of a mixture of A: 0.01%aqueous trifluroacetic acid and B:

acetonitrile in the ratio 75:25(v/v) for isocratic mode with flow rate 1.0ml/min. injection volume was 20µl.Detection was set at 265 and 275nm.Run time was 10 min.

Sample preparation

Preparation of standard solution:

Primaquine phasphate is highly disolve in water so prepare the con. Of the sample in water, so10mg Primaquine Phosphate was accurately weighed into 10 volumetric flask. The volume was produce solution of 1mg/ml.

Preparation of calibration samples and validation samples:

Calibration sample (5µg/ml,10µg/ml,15µg/ml,20µg/ml,25 µg/ml) and validation samples (6,11,16µg/ml).

Preparation of standard calibration curve:

All prepared standard solutions were analyzed and recorded by using the above chromatic condition. Standard calibration curve was prepared by Microsoft excel 2007.Calibration curve was prepared for three days used fresh sample and data recorded.

Methods of Fabrication of transdermal patches:

Matrix type transdermal patches of primaquine phasphate were prepared by the solvent evaporation technique. The polymers namely Eudrgit S-100 and Eudragit L-100 were accurately weighed and dissolved in the solvent system consisting of Methanol: Dist. Water (9:1). Propylene glycol 10 % (w/w of dry polymer composition) were used as plasticizer . Drug and permeation enhancer were added to the polymeric dispersion under constant stirring with a magnetic stirrer and the resultant homogeneous solution was poured into a petridish. Controlled solvent evaporation at room temperature was achieved by inverting a funnel over the petridish to obtain matrix type transdermal patches. The dried patches were wrapped in aluminium foil and kept in dessicator until used.

Table 1: Composition of Transdermal patches

Sr no.	Ingredients	Formulation code				
		M1	M2	M3	M4	M5
1	Drug (mg)	225.52	223.73	225.04	224.77	226.71
2	Polymer(EDT-S100) (mg)	200	267	133	240	160
3	Polymer (EDT-100) (mg)	200	133	267	160	240
4	Propylene glycol(10%)	0.04	0.04	0.04	0.04	0.04
5	Methanol:Water (9:1)ml	10	10	10	10	10

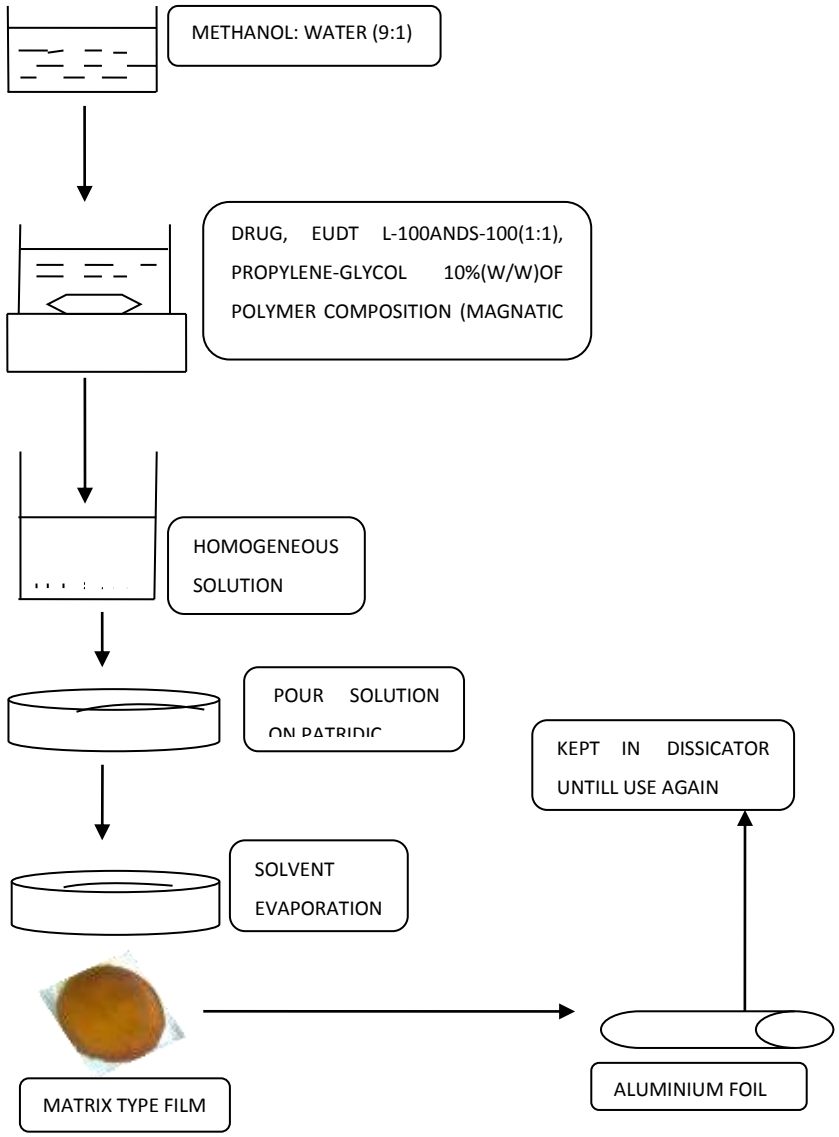


Figure 4: Schematic representation of formulation of transdermal patch

EVALUATION OF TRANSDERMAL PATCHES:

Appearance, Size, Shape and Thickness

The formulated patches were checked for their appearance, shape and thickness. The thickness of patches was determined at five different places using a micrometer screwgauge (Mitutoyo Co., Japan) for each formulation and mean value was calculated[21].

Weight variation

The patches were subjected to mass variation by individually weighing on digital weighing balance (Sartorius,BSA2245-CW) and randomly selected patches. The average of five observations of each formulation was calculated. Such determinations were carried out for each formulation[30].

Folding Endurance

It was determined by repeatedly folding a small strip of the patch (1cm^2) at the same place till it broke. The number of times a patch can be folded at the same place without breaking gave the value of folding endurance. Further, less folding endurance gives more brittle[14].

Surface pH

Surface pH of the patches was determined by the method described by Botten berg et al. The patches were allowed to swell by keeping them in contact with 0.5 ml of double distilled water for 1 hour in glass tubes. The surface pH was then noted by bringing a combined glass electrode near the surface of the patch and allowing it to equilibrate for 1 minute[31].

Percentage of Moisture Content:

The patches of (1cm^2) were weighed individually and kept in a desiccators containing activated silica at room temperature for 24 hours. Individual patch were weighed repeatedly until they showed a constant weight. The percentage of moisture content was calculated as the difference between initial and final weight with respect to initial weight[32].

$$\text{Percentage moisture content} = \frac{\text{Initial weight - Final weight}}{\text{Final weight}} \times 100$$

Percentage of moisture uptake:

The patches were weighed accurately and placed in desiccators containing 200 ml of saturated solution of potassium chloride (84 % relative humidity) at room temperature. After 3 days, the films were taken out and weighed. The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight. The test was repeated in triplet[24].

$$\text{Percentage moisture uptake} = \frac{\text{Initial weight - Final weight}}{\text{Initial weight}} \times 100$$

Drug Content

The patch of specified area (1 cm^2) was cut and added to a volumetric flask containing 100 ml of phosphate buffer pH 7.4. The medium was stirred in a magnetic stirrer for proper dissolution for 6 hours. The contents were filtered using Whatman filter paper and the filtrate was analyzed by HPLC system (Shimadzu Japan UFLC-20AD) at 265 nm. The experiment was performed in triplicate[33].

***In-vitro* skin permeation studies**

Preparation of Skin:

In this study used albino rats (weighing between 200-250 g) were purchased from central drug research institute, Lucknow. Rat was sacrificed by cervical dislocation and skin removed using with the help of scalpel and scissor. Hair was removed with an electric clipper and small hairs were removed with hair removal cream.

Franz diffusion cell:-

The *in-vitro* skin permeation studies were carried out on a Franz diffusion cell with a donar and receptor compartment. capacity of receptor was 10 ml.

Method

- The receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4.
- The excised skin was mounted between the receptor and donor compartment of the diffusion cell. 1 cm diameter of formulated patch was placed in intimate contact with the stratum corneum side of the skin and the donor compartment was kept in contact with the receptor compartment.
- The whole assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was continuously stirred.
- The temperature was maintained at 37±2°C throughout the experiment.
- The samples of 0.3 ml were withdrawn at different time interval of 0,1,2,4,8,10,12,16,20 and 24 hrs. The same volume of phosphate buffer pH 7.4 was added to receptor compartment to maintain sink conditions.
- Analyzed for drug content HPLC method at 265 nm. The cumulative percentages of drug permeated were plotted against time[34.]

Figure 5: Franz diffusion cell

Selection of Best Batch:

On the basis of all evaluated parameter such as, thickness, Folding endurance, Drug content, Moisture content moisture uptake Sucface pH, in-vitro drug release. Best patch was selected for incorporation of penetration enhancer with different concentration(Labrafac PG, Lauroglycol FCC,

Labrafil M 1944).After fabricated of new batch with penetration enhancer all above evaluation parameters were observed for selection of best penetration enhancer.

Table 2: Incorporation of Penetration Enhancer

Penetration Enhancer								
Labrafac PG			**Lauroglycol FCC**			**Labrafil M 1944**		
0.5ml	1ml	1.5ml	0.5ml	1ml	1.5ml	0.5ml	1ml	1.5ml

Skin irritation study

The patches were tested for their potential to cause skin irritation/sensitization in rat. Albino rat of either sex, each weighing 140 to 170 gm (n=3 for each patch) were used in this study. The dorsal surface of the rat was shaved carefully avoiding peripheral damage. Form 0.8% a standard solution of formaline used on the shaved skin of one rat .second one The control patch (without any drug and penetration enhancer) and third one experimental patch (with drug and penetration enhancer) was applied to the shaved skin of rat respectively, with secured using adhesive tape. The animals were observed for any sign of erythema and oedema for a period of 3 days.[35]

Stability studies

Drug decomposition or degradation occurs during storage because of chemical alteration (reaction) of the active ingredients or additives. The knowledge of chemical kinetics is helpful to predict the rate of such reactions and furthermore to evaluate the self life.

Stability is officially defined as the time lapse during which the drug product retains the same properties and characteristics that it proposed at the time of manufacture. the stability of a product is expressed as the expiry period or technically as shelf life. Expiration period is a valuable attribute for all pharmaceutical dosage forms. The mentioning of the expiration date preferably should be accompanied by details of specific storage condition as provided in the pharmacopoeia for this purpose (preservation, packaging, storage and labeling)[36]

FDA Guidelines provide recommendations for

- The design of stability studies to establish appropriate expiration dating periods and product storage requirements.
- The submission of stability information for investigational new drugs, biologicals, new drug applications, and biological product license applications.

Thus, the guidelines represent a framework for the experimental design and data analysis as well as the type of documentation needed to meet regulatory requirements in the drug-development[37.]

To ensure the stability of a pharmaceutical preparation for the period of its intended shelf life, the products must be stored under proper conditions. The labelling of each product includes the

desired conditions of storage. The terms generally employed in such labelling have meanings defined by the USP.

Cold – Any temperature not exceeding 8^0 C ($46\ ^0$F)

Cool- Any temperature between 8^0 and $15\ ^0$C (46^0 and 59^0F)

Room Temperature – The temperature prevailing in a working area. A controlled room temperature encompasses the usual working environment of 20^0C to 25^0 C (68^0 F to 77^OF)

Warm – Any temperature between 30 ^{0C} and 40 ^{0}C (86 ^{0}Fand 104 ^{0}F)

Excessive Heat – Any temperature above 40 ^{0}C (104 ^{0}F) [38, 39]

RESULTS

Fabrication of Primaquine Phosphate is prepared by the combination with eudragitS-100 and eudragit L-100 and Propylene glycol with incorporation of penetration enhancers Lauroglycol, Labrafac, Labrafil and produced smooth, flexible and transparent films. DSC studies indicated there was no interaction between Primaquine Phosphate and polymers used. Primaquine Phosphate patches were fabricated and evaluated with combination of polymers and penetration enhancers. From the results, it was observed that thickness, weight variation, low moisture loss, low moisture absorption, tensile strength were suitable for maximum stability of the prepared formulations. The drug content and in-vitro drug release rate increases with penetration enhancer labrafac 1.5 ml.

CONCLUSION

In conclusion, controlled release TDDS patches of Primaquine Phosphate can be fabricated using the polymer combinations EudragitS-100 andL-100,with penetration enhancer labrafac™ PD, labrefil® 1944CS and lauroglucol™ FCC and PEG-400 as plasticizer. The release rate of drug through patches increased when the concentration of penetration enhancer labrafac was increased. The drug release kinetics formulationL6 showing zero order release kinetics. Further, *in vivo* studies have to be performed to correlate with *in vitro* release data for the development of suitable controlled release patches for Primaquine Phosphate

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