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Tape Stripping Method: A Loom for Quantification of Drug (Lamivudine) in The Different Layers of Skin

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ABSTRACT

Tape stripping method is new loom for the quantification and dermatopharmacokinetic studies of topically-applied drugs. Tape stripping by horizontal sectioning of skin will able to give idea over the drugs penetration capacity to overcome the stratum corneum barrier. Various factors may influences variation in the result of this technique such as numbers of stripping, adhesiveness, size and shape of strips, sources of skin and pressured applied on skin. Currently it was only technique to determine the dermatopharmacokinetics studies are acceptable by FDA. Experiment is carried out for the penetration of water soluble drugs (Lamivudine), which is formulated in ethanolic liposome and applied on human cadaver skin. The adhesive tape of 3M was used and maximum of 20 tape striping had been done to estimate the penetration of plain drug and formulation into skin. Tape striping of skin has done after 8 hrs of the permeation study of plain drug and formulation in franz diffusion cell apparatus. The adhesive tape contains the layers of cells of skin and drug was quantified by RP-HPLC method (Waters HPLC). Results have revealed that 5th striping of skin showed 95.7% and 46.3% amount of lamivudine in both plain drug and formulation respectively. The histosection of the 5th tape striped skin shown major depletion of stratum corneum under microscope. Quantification of lamivudine by tape striping method showed that ethanolic liposome permeates deeper in the different layer of skin compared to plain drug.

Keywords:- Tape striping, Lamivudine, Stratum corneum, ethanolic liposomes

Introduction

The topical application of therapeutic substances to the skin is a concept doubtless as long standing as humanity, the Ebers Papyrus records of ancient Egypt describes a variability of such medication for external use. [1] Assessment of drugs within the skin is vital for topical and transdermal delivery research [2] Stratum corneum (SC) is barrier to topically applied dosage form, modified or novel formulation is required to cross these SC barrier that ensure localization of drug within the skin to enhance the local effect or increase the penetration through the stratum corneum and viable epidermis for systemic effects. [3] Of the several skin layers, it is the stratum corneum that is the rate-limiting barrier to percutaneous drug transport. [4]Stratum corneum ceramides are major determinants of skin barrier function. [5] Ceramides are the major lipid constituent of lamellar sheets present in the intercellular spaces of the stratum corneum. These lamellar sheets are thought to provide the barrier property of the epidermis.[6] Establishing dermal penetration rates is important to better understand the safety of topically applied materials, especially for premature infant skin with compromised skin barrier function.[7] Over the last two decades, horizontal sectioning, consisting of both tape stripping and parallel slicing through the deeper tissues has constituted the traditional investigative technique.[8] Attenuated total reflection by Fourier transform infrared spectroscopic imaging combined with tape-stripping is an advantageous approach to map the depth penetration and lateral distribution of topically applied chemicals in Stratum corneum and the conformational order of SC lipids.[9]

Tape stripping method is useful invasive tool investigation method for dermatopharmacokinetics effect of drugs applied through the skin. Tape stripping of human stratum corneum is widely used as a method for studying the kinetics and penetration depth of drugs. [10,11] Stratum corneum adhesive tape stripping has been utilized in the measurement of stratum corneum mass, barrier function,

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drug reservoir, and percutaneous penetration of topical substances. [12] Prior to tape striping of skin, allow the formulation to penetrate in the different layer of skin. After application of formulation and permeation of formulation, the cell layers of the SC are successively removed from the same skin area using adhesive tape. [13]The critical parameters should be considered in tape striping of skin such as adhesive of tape, amount of pressure applied on tape after applying on skin, size of the tape, velocity of removal of tape from skin. First tape striping removes the first layer of skin, which contains retained formulation and the foremost layer on skin. As tape striping of skin further continues, different layers of skin (SC) will be adhered to tape and detached from skin. **(Figure 1)** Liposomes derivatives (elastic liposomes) have been used to enhance localization and permeation effect of drugs. Ethanolic liposome is a second generation liposomes derivative which has relatively good liquidity and deformability and shown increase penetration of drugs in skin.Ethosomes are bubble-like phospholipid nano-vesicles bearing ethanol which can overcome the SC barrier and enhances the penetration of drug through the skin. Ethosome vesicles enable the amalgamation of both hydrophilic and lipophilic compounds.

Lamivudine is a pyrimidine nucleosides analogues **(Figure 2)** and reverse transcriptase inhibitor (NRTI) with activity against Human Immunodeficiency Virus Type 1 (HIV-1). Lamivudine was chosen as a model hydrophilic drug and which have poor penetration through the skin. Incorporating lamivudine in elastic liposome (ethosomes) enhances the permeation of drug and overcome the SC barrier. Tape striping of the skin (SC) after application of formulation, will give picture of permeation enhancement of drug in skin. The main objective of the study was to evaluate the penetration profile of drug in skin by tape striping method.

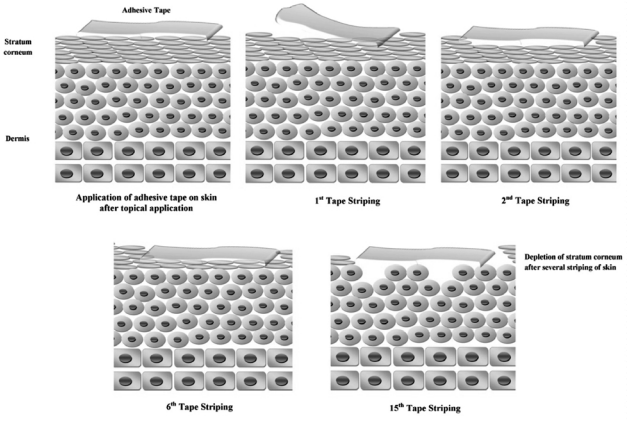

Fig. 1: Graphical representation cross section of skin during tape striping of skin method

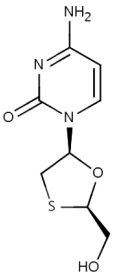

Fig.2: Chemical Structure of Lamivudine

Material and Methods

Lamivudine was procured as a gift sample from Emcure Pharmaceuticals Ltd, Pune; Phospholipid 90H was obtained from Lipoid. 3M adhesive tape, 1,2 Propanediol, ethanol, chloroform, and cholesterol was procured from Sigma, Aldrich, India. Methanol and distilled water are of HPLC grade.

Characterization and Evaluation

Preparation of elastic liposomes

Elastic liposomes (Ethosomes- EL) were prepared according to Touitou*et al*., with certain modification. Phospholipid 90H, drug (Lamivudine), ethanol were mixed in closed vessel with continuous stirring on water bath maintained 60°C, later on propylene glycol is added slowly. Simultaneously distilled water is heated to 60°C and added with fine dispersion into ethanolic dispersion of phospholipid. The mixture is further heated for 30 minutes with continuous stirring. The elastic liposome preparation is passed through polycarbonate membrane for desired size of vesicles. [14,15]

Size, Zeta potential Analysis and entrapment efficiency

Dynamic light scattering (DLS) method was used to determine the zeta potential and size of vesicles. Entrapment efficiency of drug in elastic liposomes was determined by centrifugation method and analysed by HPLC.

HPLC assay

The amount of drug permeated in the receiver compartment of franz diffusion cell during *in vitro* skin permeation experiments and content of tape from skin was determined by HPLC assay using mobile phase consisting of water: acetonitrile (70:20:10 vol/vol) mixture as mobile phase was optimized at a flow rate of 1.0 ml/min (Waters, HPLC). A ten-microliter of sample were injected in C-18 column (15x0.46 cm, analytical column, Sunfire) at room temperature. The column eluent was monitored and analysed at 271 nm, using PDA detector. Similarly HPLC method for the tape striping adhesive and drug mixture is analysed.

Preparation of cadaver skin for permeation study

Cadaver skin from the forearm region of 25-26yr old male acquired from MY hospital, Indore. It was kept in formalin at 4°C and freed from fat. Prior to use of cadaver

skin, the skin was washed with saline to remove trace of formalin and other debris or fats. After washing the cadaver skin is kept in saline for fortnight and cleaned again with fresh double distilled water.

In-vitro Evaluation

The *in-vitro* skin permeation of plain drug (lamivudine) solution and elastic liposomes formulation containing lamivudine were studied using franz diffusion cell. The temperature and stirring was monitored and maintained in receptor cell at 37 ±0.5°C and 120 rpm respectively. Place prepared human cadaver skin between the donor and the receiver compartments. Freshly prepared phosphate buffer pH 7.4 (PBS) is added in receptor compartment. Add the required quantity of formulation in donor compartment; add few drops of saline in donor compartment to mimic the sweat. At regular interval sample are withdrawn from the receptor compartment and replaced with fresh PBS. The samples are analyzed by HPLC(Waters2998)at 271nm using PDA detector. Similarly for plain drug solution the same procedure is carried out.[14-16]

Tape Striping method

Repetitive applications and removal of adhesive tape on the cadaver skin surface, which will remove the sequential layers of SC and it is analyzed for the content it removed from the skin. Tape stripping is an official method for the analysis of the dermatopharmacokinetic study for topically applied substances using adhesive tape films. After application of the formulation on skin which placed on the franz diffusion cell for 8 hours, the tape striping are done repeatedly on skin by applying certain pressure. 3M adhesive tapes (Scotch tapes 810) were used for tape striping. Each tape removes the corneocytes layer of the skin and formulation which applied and analysed by HPLC. After removal of adhesive tapes from skin, it is kept in centrifuge tubes containing ethanol and vortex for 5 minutes at 15000 rpmand it is filtered from 0.2µm filter and analysed by HPLC. The quantity of the substances and the amount of horny layer of stratum corneum depleted with the single tape strip is to be determined for calculation of the penetration profile. [16-19]

Result and Discussion

Repetitive tape striping method is used to study the penetration and pharmacokinetics study of drug in skin. Tape stripping, a relatively older technique, has been extensively used in comparative bioavailabilitystudies of various topical formulations.Tape Stripping is a method used to compute drug concentrations within the SC [18] and is basedon the reservoir principle of the stratum corneum [20]. The SC has the property to act as depot of drugs that are applied to the skindepending on the drug, the formulation, the application procedure and the state of the skin. [21] Histology or transverse section of skin has been employed in the dermatological and pharmaceutical fields to assess the percutaneouspenetration of drugs. **(Figure 3)**

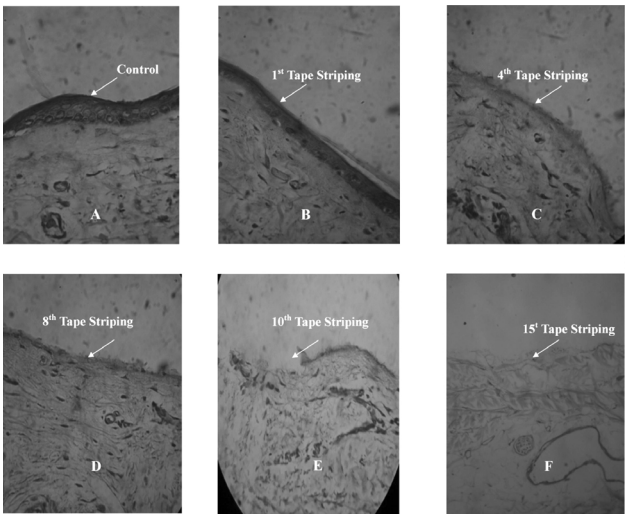

Fig. 3: Cross section of skin after several tape striping of skin after topical application of sample (Elastic Liposome)

Parameters such as applied pressure on adhesive tape during tape striping should be evaluated as it can influence the content and layers removed from skin. Other parameters such as type of tape, size of tape, pressure applied by investigator, duration of application of pressure, drug removal procedure, drug extraction procedure should be considered in the study. The concentration of the drug penetrated and the amount of horny layer of stratum corneum depleted is required, to plot the penetration profilegraph. Penetration profile of drug in skin give information related to permeation of drug to which extent it has penetrated. **(Figure 4)**

Elastic liposomes were prepared and result revealed that entrapment efficiency is 86.4±3.8. (**Table no:-1)** The average size of the EL is 273±17.2 nm and zeta potential is –6.5 ± 0.3 ζmv.(**Table no:-1)**Based on result of *invitro* analysis of EL showed that drug permeates into skin crossing SC barrier. The cumulative percentage of drug release in receiver compartment is 59.23±1.97. **(Figure 5)** Whereas plain drug solution not able to cross the SC barrier to a mark. It has shown only 3.24±0.82 drug release in receiver compartment. **(Figure 6)**In both *invitro*studies, the formulation sample has been retained on the donor compartment. Twenty tape striping of the skin has been done for both formulations. Initially 1st tape striping contains retain formulation on skin and first layer of SC.Results have revealed that cumulative of first 5 striping of skin showed 95.7% and 46.3% amount of lamivudine in both plain drug and formulation respectively. Penetration profile of elastic liposomes and plain drug were shown in **figure4.** In penetration profile of elastic liposomes shows that EL has permeated into deeper layers of skin, even after 20th tape striping of skin a small quantity of drug was estimated, whereas 10th tape striping of skin for plain drug solution has shown no amount of drug in tape stripped. Histology of skin or cross-section of skin for tape striping gives the

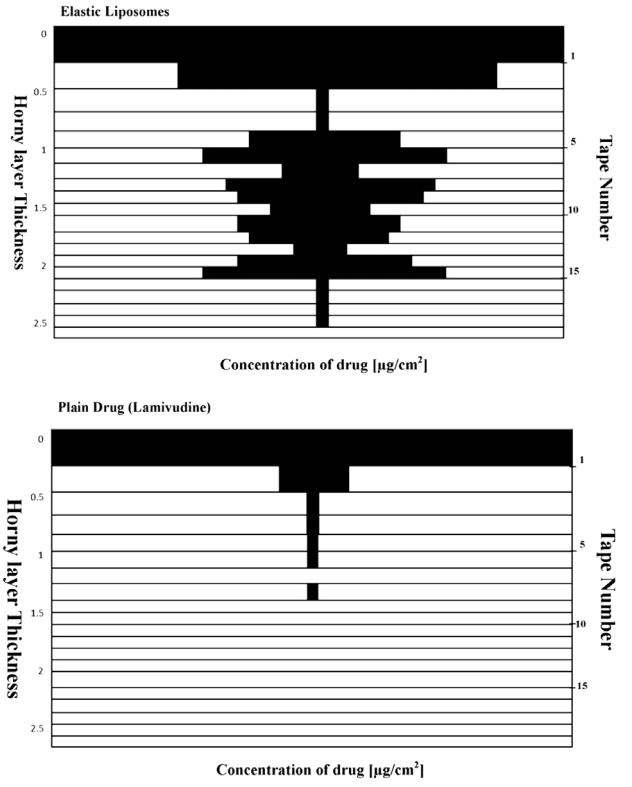

Fig. 4: Penetration profile of Lamivudine in Elastic Liposome and drug solution

number of tapes to be stripped to remove the SC from skin. 15th tape strip mostly deplete the SC from skin as shown in **figure 3(F)**.Five to ten tape strips are sufficient to determine the amount of drug penetrated or localized into the SC. The penetration profile and time-concentration profile graph reveals that EL has shown deeper penetration of lamivudine in the skin and sustains release for 8 hours compare to plain drug solution.

Table 1
Characterization of the elastic liposome for size, entrapment and zeta potential.

Elastic liposomes	
Formulation code	**Elastic liposomes (EL)**
Entrapment Efficiency (%)	86.4±3.8
Vesicle Size (nm)	273±17.2
Zeta Potential (ζmv)	–6.5 ± 0.3

Conclusion

Ethosomes well known for its permeation of drugs into skin, utilizing ethanol as penetration enhancer, it will help in penetration of drugs into deeper layers of skin. Tape striping method showed that ethanolic liposome penetration deeper in the different horny layer of skin compared to plain drug. This experiment of the tape stripping method to enumerate drug permeation through the skin, featuring its versatile application in the field of the topical delivery system

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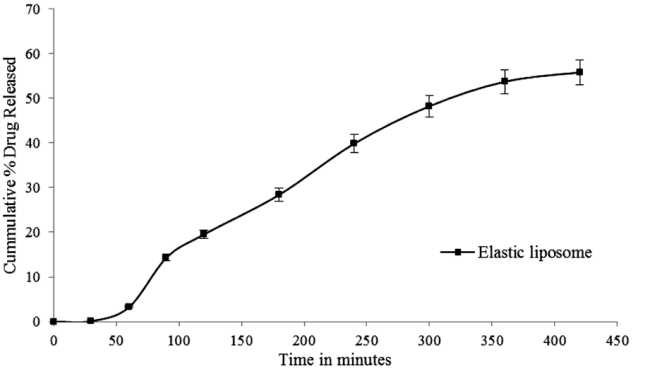

Fig.5 : Invitro release study of elastic liposome bearing lamivudine

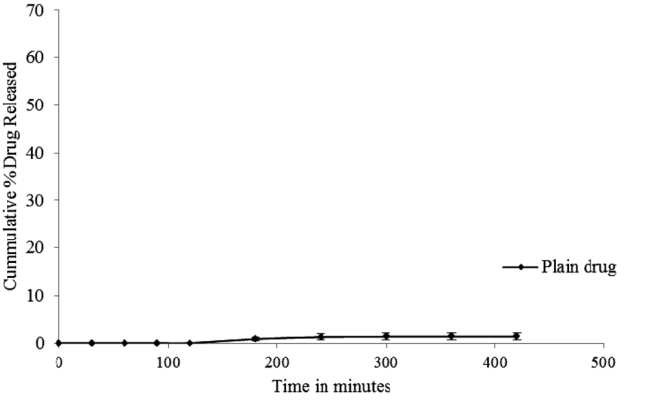

Fig.6: Invitro release study of plain drug solution containing lamivudine

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