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INVESTIGATION OF SKIN PERMEATION OF PHOTOSENSITIVE LIPOSOMES USING TAPE STRIPPING METHOD

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ABSTRACT

The objectives of this study were to perform investigative tape stripping model using liposomal formulations to evaluate the skin permeation of drug using these different liposome compared to free drug. The effect of number of strips, surface charge and incubation time on permeability of drug was estimated. Different gamma oryzanol (OZ) loaded liposomes were formulated using REV method soy PC and Cholesterol. Release of OZ was observed from the liposomes up to 6 h. Photosensitive and conventional liposomes demonstrated significant ($p < 0.05$) permeation into the skin compared to the free drug solution. Blockage of the hair follicles may result in reduction in the extent and intensity of drug permeation through the skin layers. Overall, liposomal formulations proved to be an ideal carrier for this study. Surface charge and incubation time played vital roles in the extent of permeation.

Keywords: Tape stripping, Skin Permeation, Liposomes, Gamma Oryzanol

INTRODUCTION

A liposome is a spherical vesicle usually having multiple layers of phospholipid and it has self-forming nature. They have space within occupy by aqueous compartments surrounded by these single or multiple phospholipid bilayers. Basic component is phospholipids and it is responsible for formation of bilayer. To stabilize these layers second most component, cholesterol is added and acts as fluidity buffer. On the basis of nature of drug, it get placed within aqueous cavity or in bilayer **[1]**.

To perform permeation study across the skin pig ear found to be most suitable surrogate for human skin. Swine skin is quite similar to the human skin. It was observed that both skins have similar structure with small differences. They have difference in SC, it is triple in thickness compared to human one (38-88 μm and 13-28 μm, respectively) and epidermis is found to be 1.5 times thicker (115-198 μm versus 74-148 μm). As we know by experimentation porcine ear skin shows high degree of similarity compared to human skin. Nevertheless, for percutaneous absorption pig ear skin appears to be the best for use as *in vitro* model for human skin study **[2]**. For such kind of *in vivo* study tape stripping technique represents widely used method to find penetration profiles of topically applied drugs. In tape stripping method SC get removed using microscopic layers (typically 0.5–1 mm). This method is performed by applying appropriate adhesive tape-strip with good amount of applied pressure. Pressure ensures good contact followed by removal with sudden upward movement. The first few strips of tape must contain almost pure cell layer of corneocytes. On increasing the frequency of tape stripping number, these corneocytes aggregates reduces become less and less. The procedure is comparatively less painless and noninvasive. Only dead cells that are corneocytes are removed **[3]**. Tape stripping is used in various studies related to skin: for example, for the evaluation of the barrier properties of skin **[4]**, to study pathophysiology of several dermatopathologies such as inflammatory or cancer disease **[5]**, to qualify gene expression **[6]**, to find pH profiles **[7]**, to check possibilities of use of animal skin as a surrogate for human skin **[8]**, to induce expression of some receptor like Toll-like receptor 9 **[9]**. When drug is administered via topical route, tape stripping method is a suitable option to evaluate amount of drug or excipient reaches in the skin for dermatopharmacology. This amount can be determined either in the removed tapes or in the skin that is treated with tape. When SC is treated with drugs like antifungal agents or antiseptics, tape stripping appears as a useful method to determine local bioavailability. It establish that local bioavailability can be evaluated effectively with the use of tape stripping method **[10]**. Hence, for topically applied medicines in order to find local bioavailability and/or bioequivalence tape stripping presents a useful methods.

MATERIAL AND METHODS

Materials

Soya phosphatidylcholine (SPC) and dialysis bags (cellulose tubing 25 m long, 10 mm inflated diameter) were purchased from HiMedia, India. Cholesterol was

purchased from Sisco Research Laboratories Mumbai, India. Ketoprofen was purchased from M P Biochemical, India. Gamma oryzanol (OZ) was purchased from TCI India. Dialysis bags were obtained from Himedia Company and were used after washing with tap water for 3–4 h. All other chemicals and solvents used were purchased from local suppliers and were of analytical grade unless mentioned.

Methods

Preparation of liposomal formulation (Reverse Phase Evaporation (REV) Method [11]

The lipid components (SPC and cholesterol) in the range of 5:1 to 5:5 (molar ratio) were dissolved in minimum volume of a mixture of chloroform: methanol (9:1 v/v) in a 50 ml round bottom flask by gentle swirling. KP and OZ was mixed with the lipid mixture during preparation. Both drugs were passively loaded. The flask was attached to a rotary evaporator (Nutronics, India), and nitrogen gas was used to maintain inert environment. A vacuum of about 700 mm Hg was applied to evaporate the organic solvent until a thin film of lipid/cholesterol mixture was formed on the walls of the flask. Diethyl ether (3 ml per 66 µ mol lipid) was added into the flask and vortexed to dissolve the prepared thin film of PC and cholesterol. PBS, pH 6.4, was then added as the aqueous phase, vortexed, and bath sonicated (EIE Instruments Pvt. Ltd. India) at 4ºC. The sonication was continued till a stable emulsion of the w/o forms (~2-3 min). The flask with the two-phase emulsion was attached to the rotary evaporator and the nitrogen purge was maintained through the flask while stirring. The content was then slowly exposed to low vacuum (~200 mm Hg) to evaporate the ether slowly. The process was continued till a semisolid gel was formed in the flask. The flask was removed and the contents were stirred to break the gel. The vacuum pressure was slightly increased to ~300-350 mm Hg and the level was maintained for about 15 min. As soon as most of the ether was removed the gel converted into a smooth suspension. The flask was removed and the contents were mixed again. The vacuum was further gradually increased to 700 mm Hg, and was maintained for about 30 min to evaporate all the remaining ether. Unencapsulated OZ was removed by centrifugation at 21000 rpm for 90 minutes. The liposomal concentrate was washed twice either with PBS pH 6.4 in the case of OZ. The supernatant was separated; the pellet was re-dispersed in PBS, pH6.4 and stored at 4 °C in air tight glass containers until further testing **[12]**.

Characterization of liposomes

Physicochemical characterization of prepared formulations

Morphology and lamellarity

Morphology and lamellarity of the photosensitive liposomes containing OZ using various molar ratios of SPC and cholesterol were ascertained form photographs taken using an Olympus BX40 microscope. TEM images were taken (JEOL, Japan) using copper grid coated with carbon film and with phosphotungstic acid (PTA, 1% w/v) as a negative stain **Figure 1**.

Mean particle size analysis

Size distribution profile of photosensitive liposomes was determined by dynamic light scattering based on laser diffraction method employing Nanotrac Particle Size Analyzer (Nanotrac NPA250, Microtrac Inc., York, PA, USA). The experiment was performed in triplicate.

Determination of drug entrapment efficiency

The clear supernatant was separated carefully and analyzed by UV spectrophotometer at 327 nm for OZ content after appropriate dilution with phosphate buffer pH 6.4. This indicates the amount of free drug. The liposomal pellet was re-dispersed in phosphate buffer pH 6.4, lysed by the addition of 2% Triton X-100 and sonicated for 10 min. The concentration of OZ was determined after appropriate dilution in phosphate buffer pH 6.4 at 327 nm using a UV visible spectrophotometer **Table 1**. The value of drug entrapment efficiency of OZ in each formulation were calculated as follows:

$$\% \text{ entrapment efficiency (\% EE)} = Q/Q0*100$$

Where, Q is the amount of OZ measured in the liposomes, Q0 is the initial amount of OZ added in liposomes.

Tape striping

The US Food and Drug Administration (FDA) was published guidelines in 1998 regarding procedures for performing tape stripping method to find bioavailability/bioequivalence parameters. guidance proposed were had the following steps **[13]**:

1. Fresh pig ear were kindly provided by slaughterhouse. Ears were stored in fridge and used within 0-7 days after delivery.
2. Ear was taken out 1 hour before experimentation. This was helpful to warm up the ear. They were carefully washed and wiped with paper napkin. The whole ear was fixed on wax plate.
3. 10 mg sample were applied on the marked area and incubated for 1 hour.
4, After tape application movement with roller was made and tape was removed with one quick action.
5. The strips were weighed before and after application to find the weight of SC removed.

The experimental protocol was carefully reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Pharmacy, Nirma University, as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India.

Extraction procedure

OZ was recovered from tissue homogenate by addition of ethyl acetate followed by vortex for 90s and centrifugation of 3 min at 855g. The upper organic layer was added to alkaline buffer. The tube was vortexed and centrifuged similarly. The supernatant was collected and used for drug measurement **[14]**.

RESULTS & DISCUSSION

Preparation and evaluation of liposomal formulations for optimization

In the present study, REV technique was applied as a working method for the preparation of both conventional as well as photosensitive liposomal formulations using KP. The advantage of this method of liposomal preparation is getting a very high encapsulation efficiency up to 50%. Preapred liposomes were clearly spherical. If we see the effect of cholesterol on % EE, we can clearly conclude that as content of cholesterol increases lesser drug get space into lipid bilayer. As cholesterol content increases % EE decreases **[15]**. We have varied cholesterol content from molar ratio of 5:1 to 5:5 compared to PC. As cholesterol content increases vesicle size increases.

Skin penetration study

In the present study skin penetration of OZ from different liposomal formulations were investigated. Stripping method with pig ear as a surrogate for human skin was used for this study. We studied the permeation of OZ into SC from liposomal formulations **Figure 2 A B and C**.

Time-dependent study of skin permeation with formulations

The initial experiments of this study were carried out using conventional (CL) and photosensitive (PSL) liposomes compared to free drug (FD). To study the effect of time on incubation on penetration of OZ, formulations were incubated for different time interval, namely 1, 3 and 6 hours **(Figure 2)**. Permeation from different formulations were observed. An increase in % drug permeated was observed over the 6 hours after that no further increase was observed. The analysis showed that there was increase in the extent of skin permeation of both free drug and liposomes with increase in duration of permeation studies. However, the permeation of PSL was significantly ($p < 0.05$) different compared to CL and FD solution (3.0 and 4 fold more at 6 h, respectively). Further, permeation duration of 6 h resulted in 1.5-

fold more skin permeation of PSL than 3 h compared to free drug. The results have been illustrated in **Figure 3**.

Effect of tape stripping of skin on permeation of formulations

It was observed that with increase in the number of tape stripping % drug permeated decreases. Thickness of SC also decreases with increase in number of strips. 60 tape strippings were made to completely remove the total stratum corneum layer.

In **Figure 3** number of strips were plotted against the % drug permeated. Three different types of samples FD, CL and PSL were tested. It was observed that with increase in the number of tape stripping % drug permeated decreases. Thickness of SC also decreases with increase in number of strips. 60 tape stripping were made to completely remove the total stratum corneum layer. In **Figure 4** number of strips were plotted against the % drug permeated.

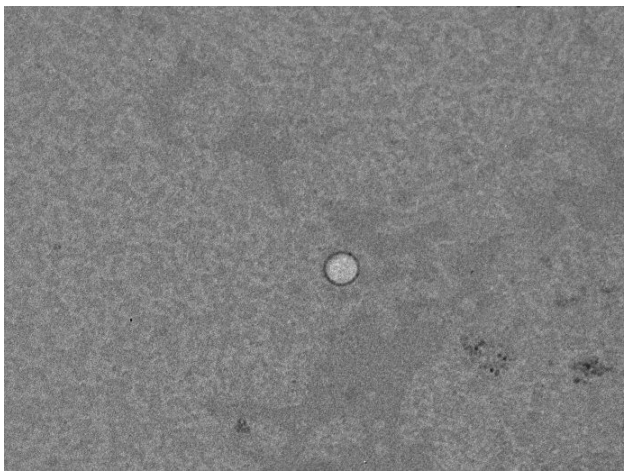

Figure 1: TEM photograph of photosensitive liposome

Table 1: Effect of cholesterol content on photosensitive liposomal formulations

Formulation code	PC	Chol	Mean vesicle size (nm)	% EE
PREV-1	5	1	219.8 ± 4.8	50.5 ±2.1
PREV-2	5	2	251.7± 3.3	46.9±1.6
PREV-3	5	3	255.7±3.3	35.7±2.8
PREV-4	5	4	258.6± 2.5	28.4±1.1
PREV-5	5	5	279.3±3.5	27.2±3.4

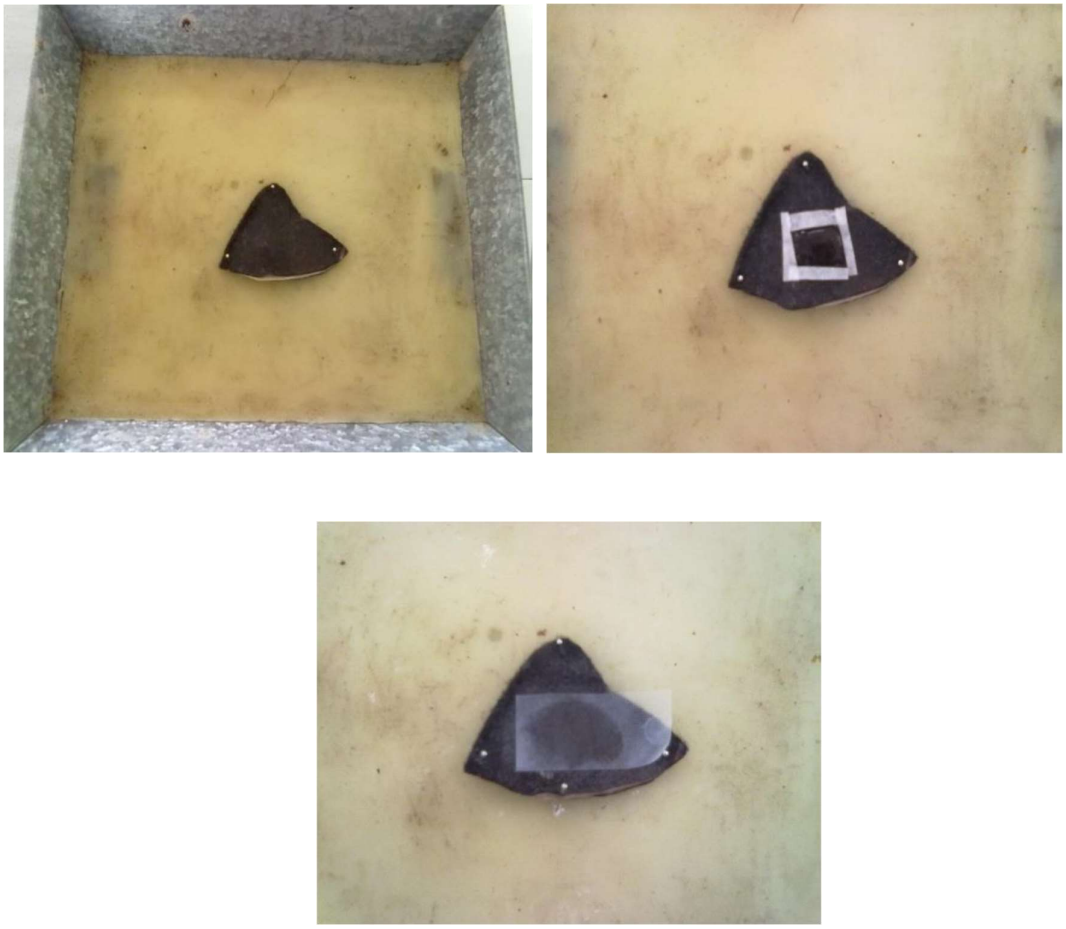

Figure 2: A, B and C: Procedure of tape stripping [16]

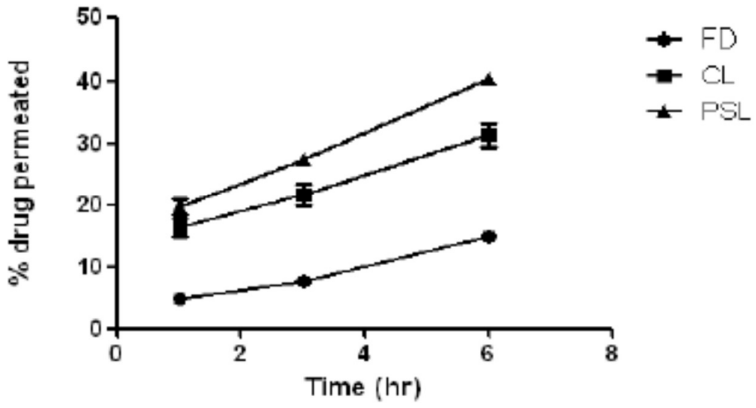

Figure 3: Effect of time of incubation

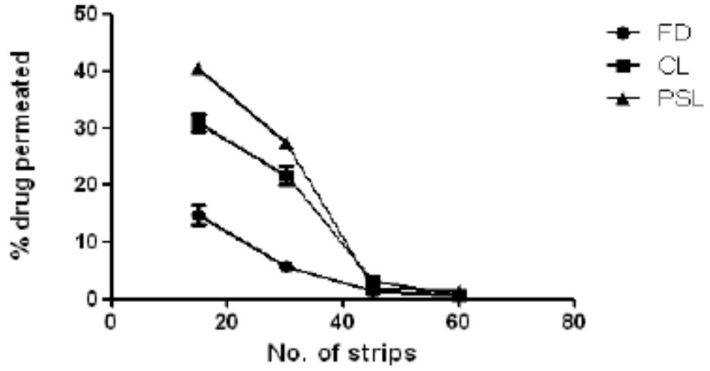

Figure 4: Graph between numbers of strips against % drug permeated

Effect of charge on skin permeation of liposomes

The results showed that the negatively charged PSL permeated into the skin better than the neutral conventional liposomes. PSL permeated deeper into the skin and showed more significant ($p < 0.05$) % drug permeation than both CL and free drug solution.

CONCLUSION

Tape stripping model using liposomal formulations to evaluate the skin permeation compared to free drug was performed. PSL, CL and FD solution were tested. PSL showed highest level of permeation compared to others. The negative charge of PSL supported the permeation process. These PSL can be safely and effectively used in this type of skin permeation studies for different activities.

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