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PERMEATION STUDY THROUGH BACTERIAL CELLULOSE MEMBRANE

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Abstract: The objective of this study was to fabricate topical formulations of diclofenac diethylamine (DD) using isopropyl myristate (IPM) and isopropyl palmitate (IPP) as permeation enhancers. Franz cell and bacterial cellulose were used as analytical instrument and diffusion membrane, respectively. Permeation enhancers exhibited significant effect on the permeation characteristics of DD. It was concluded from the results that improved permeation of DD was observed when IPP was used as enhancer.

Keywords: bacterial cellulose, permeation, transdermal drug delivery

Transdermal drug delivery systems (TDDS) are widely being studied as an excellent substitute to deliver drugs with enhanced bioavailability (1). However, large number of active pharmaceutical substances faces trouble during crossing the intact skin (2). Thus, it is the need of time to focus our attention for overcoming diminished drug permeability *via* skin (3). There are two decisive factors in the development of TDDS including the achievement of sufficient flux athwart the skin and the reduction in lag time during skin permeation. Various approached have been introduced to overcome these issues like the addition of chemical skin enhancers into the formulation (4). Some prominently used enhancers are propylene glycol (5), isopropyl alcohol (IPA) (6), isopropyl myristate (IPM) (7) and isopropyl palmitate (IPP) (8).

It has been studied that a drug molecule passes through many barriers during its traveling from skin surface to systemic circulation. These different barriers are stratum corneum, viable epidermis and dermis. The skin is the largest body organ and is rich in blood capillaries (blood flow rate of 0.05 mL/min/cm of skin). However, skin temperature is needed to be controlled to deliver drug molecules through skin and the removal of waste products. Sink conditions are provided by this blood pool in the proximity of skin for the diffusion of drug substances during percutaneous absorption (9, 10).

Diclofenac diethylamine (DD) being an excellent non-steroidal anti-inflammatory drug is preferred for using in the treatment of painful circumstances (11). Besides, its use in the development of experimental and clinical medicines is very limited up to now, particularly in the fabrication of topical formulations. However, no study is available in the literature showing its permeation studies across bacterial cellulose based artificial skin.

Bacterial cellulose, an extremely uncontaminated cellulose substrate, is developed as a distended membrane by numerous bacteria, particularly from the *Gluconacetobacter* genera. It possesses many novel physico-mechanical characteristics depending upon its structure and elevated limpidness, which is responsible for the initiation of its use in the tissue engineering to regenerate the

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injured tissues like skin. Hovewer, its use in drug delivery system development is very limited. Based on its many versatile properties resembling to skin, it can act as an artificial skin and can perform excellent membrane in the permeation studies using Franz cells (12).

Thus, present study was performed to investigate the usefulness of such formulations, having different permeation enhancers, for transdermal delivery across bacterial cellulose. The effect of incorporation of skin permeation enhancers like IPM and IPP on the *in vitro* permeation was investigated.

Materials and Methods

Materials

Diclofenac diethylamine (DD) was gifted by Abbott Pharmaceuticals, Karachi, Pakistan. All analytical grade chemicals were purchased through local sources from Merck, Germany.

Formulation development

To fabricate matrix based topical formulations, eudragit RL-100 (3 g) and PVP K-30 (20 g) was separately dissolved in ethanol (16.25 g), and then both the solutions are mixed followed by the incorporation of DD (5 g) (drug solution). Another solution of ethanol (16.25 g) in WFI (water for injection, 22 g) was prepared following by the mixing of HPMC (40 g). The resulting solution was then mixed with drug solution. In this way, six different formulations were designed, each containing single enhancer in a quantity specified in the brackets, i.e., F1 (IPM 1.5 g), F2 (IPP 1.5 g), F3 (IPM 3 g), F4 (IPP 3 g), F5 (IPM 4.5 g) and F6 (IPP 4.5 g). A control for-

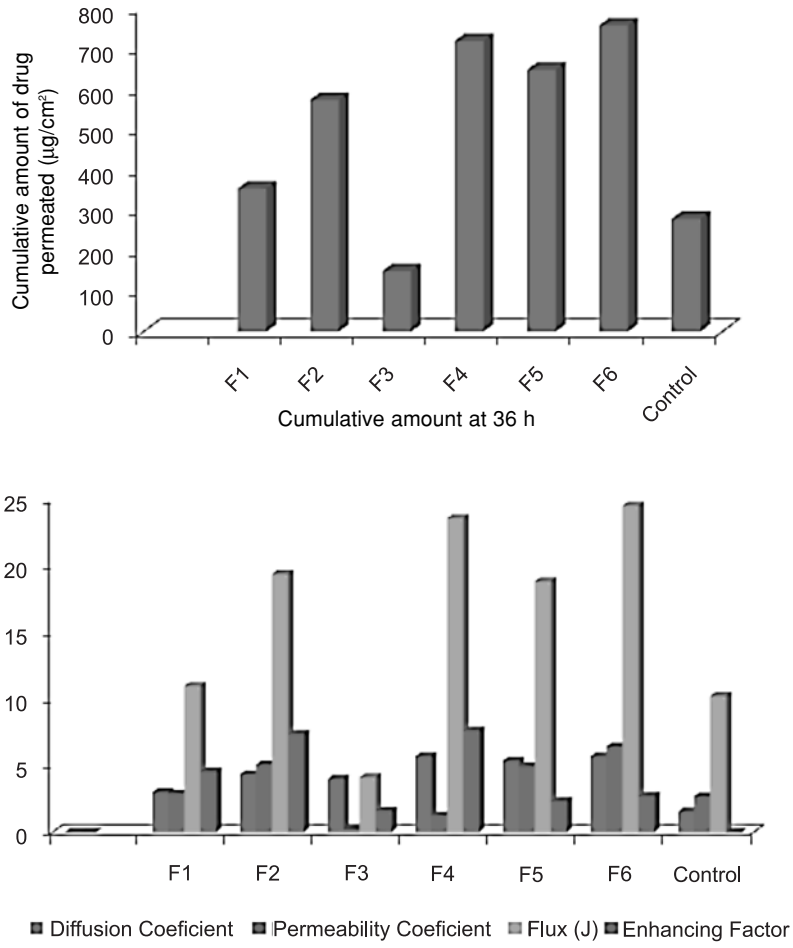

Figure 1. Permeation parameters of diclofenac diethylamine across bacterial cellulose from matrix formulations

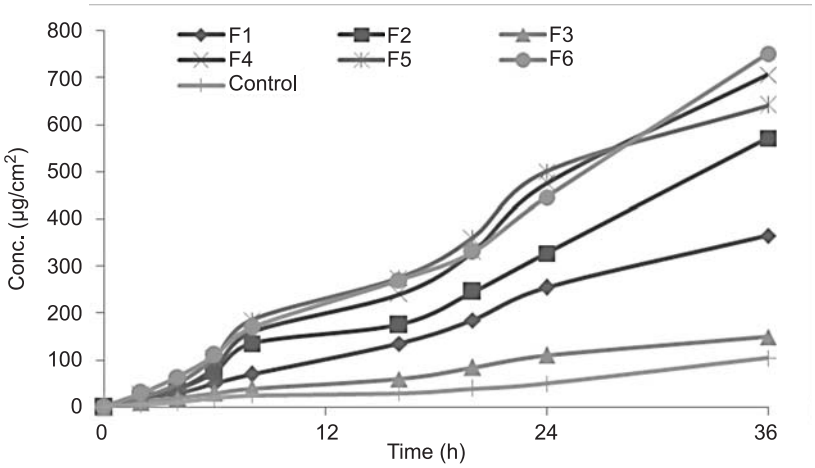

Figure 2. Influence of permeation enhancers (IPM and IPP) on the permeation of diclofenac diethylamine from various matrix formulations across artificial skin as compared to the control formulation

mulation was also formulated using no permeation enhancer.

Permeation studies and calculation of permeation parameters

Permeation studies were conducted using bacterial cellulose (0.81 cm^2) mounted on Franz cells (Emmay, Pakistan). Two grams of each formulation (equivalent to 20, 100 and 200 mg of DD) was placed on a side exposed to donor booth. The receptor compartment contained normal saline at 37 ± 0.5°C stirred continuously using a thermostatically controlled shaker. Samples were taken at predetermined time points and were replaced with the fresh medium. After filtration using disposable filters (Millipore, USA), the withdrawn samples were then analyzed for DD using UV spectrophotometer (UV 1601, Shimadzu-Japan) at 263 nm (13). A perfect sink condition was maintained throughout the experiment.

To draw permeation curve, cumulative amount (µg/cm^2) of drug permeated through membrane was plotted *versus* time. Straight line slope of the permeation curve and its corresponding *x*-intercept was used to evaluate the steady state flux (J_{ss}) and lag time (t_L) of DD, respectively. The permeability coefficients (K_p) and diffusion coefficients (D) were determined as (14):

$$D = h^2/6\ t_L \quad (1)$$

$$K_p = J_{ss}/C_s \quad (2)$$

where, h and C_s represent bacterial cellulose membrane thickness (0.81 cm) and the initial drug concentration in the donor compartment, respectively.

Enhancing factor (EF) was calculated by the following way (14):

$$EE = CP / CU \quad (3)$$

where, CP and CU are cumulative permeated amount of DD of a formulation and cumulative permeated amount of DD of control formulation, respectively.

The enhancing ratio (ER_{flux}) was assessed by the following way (14, 15):

$$ER_{flux} = SS / SP \quad (4)$$

where, SS and SP are steady-state permeation rate of a formulation and steady state permeation rate of control formulation, respectively.

RESULTS AND DISCUSSION

Drug permeation data from various formulations as compared to the control (having no permeation enhancer) are shown in Figures 1 and 2. After 36 h study, there was a higher cumulative amount of permeated drug from all the prepared formulations containing enhancers (except F3) as compared to that of the control (Fig. 1). A previous study (4) has presented that the cumulative amount of permeated diclofenac diethylamine *via* bacterial cellulose was 7.40% and 6.71% after 23 h from gelly microemulsion and liquid microemulsion, respectively as compared to 7.46% from F5 after a permeation study of 24 h. The steady-state flux was 23.59 ± 0.12 µg/cm^2 h and 6.09 ± 0.03 µg/cm^2 h for F5 and F3, respectively. The previous study (14) has presented that the gel containing IPM with carbopol 900 base permeated a flux of 9.30 ± 0.49 µg/cm^2 h. Another

study (16) reported that the microemulsion allowed a flux of 117.89 μg/cm^2 h for diclofenac diethylamine *via* regenerated cellulose membrane.

Figures 1 and 2 show the influence of addition of enhancers, i.e., IPM and IPP, on the DD permeation from the prepared matrix formulations as compared to the control. After a permeation study of 36 h, the permeated cumulative amounts of DD were 717.73 ± 55 μg/cm^2 and 149.78 ± 8.02 μg/cm^2 for F5 and F3 matrix formulation, respectively. It has been stated in a previous study that IPP, which is a fatty acid ester type enhancers, interrelate essentially with the lipids present in cells. The increased permeated cumulative amount of drug could be due to the increase in the breakdown of lipid bilayer of stratum corneum (17).

The F4 formulation (EF value of 7.59) exhibited the highest increase in DD permeation followed by the formulation F2 (EF value 7.40) in comparison to the control formulation (EF value 0.40). It has been proposed that permeation of many drugs through stratum corneum is an essential pathway for drug transport *via* intercellular route (18). The present study also showed that the rate of DD permeation (ER_{flux}) from all the matrix formulations prepared with permeation enhancers was elevated as compared to the control formulation. It has been stated in the previous study that the value of ER_{flux} was higher (7.53) from F4 in comparison to 4.01 ± 2.604 for 5% limonene in horses (18). The obtained value of diffusion coefficients for F2, F4, F6 and control were 4.34 ± 0.39 cm^2/s × 10^{-4}, 5.69 ± 0.39 cm^2/s × 10^{-4}, 5.88 ± 0.43 cm^2/s × 10^{-4} and 1.99 ± 0.40 cm^2/s × 10^{-4}, correspondingly. This elevation in the permeation can be attributed to the jumping of drug molecules into the lipid bilayer resulting in its rotation, vibration and translocation, which cause the development of microcavities. It ultimately increases the free volume vacant for drug dispersion, which is minimal along with the interface of lipid bilayer membrane when no permeation enhancer is used (19). This whole study and discussion explored that the permeation enhancers are crucial for the improvement of drug permeation rate. These results are finally supported by the value of RPR > 1 for all fabricated formulations that permeation enhancers are crucial for efficient drug transportation through bacterial cellulose membrane.

CONCLUSION

The results elaborate that the addition of skin penetration enhancer into the formulations elevated the permeation rate of the drug in comparison to the control formulation. The F6 was found as the most efficient formulation based on its higher steady state flux, permeability coefficient and diffusion coefficient with a decrease in lag time of DD permeation in comparison to the control formulation.

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